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Behavioural, neural and genetic patterns related to age or lifespan in companion dogs

Életkorral, élethosszal összefüggő viselkedési,
agyi és genetikai mintázatok kutyákban

Enikő Kubinyi

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Contents

“I have sometimes thought of the final cause of dogs having such short lives and I am quite satisfied it is in compassion to the human race; for if we suffer so much in losing a dog after an acquaintance of ten or twelve years, what would it be if they were to live double that time?” Sir Walter Scott

I. General introduction

Aging is a naturally occurring complex biological process, and it is one of the most relevant problems to understand how active and healthy aging can be achieved. Currently, 8-10% of the human population is over the age of 60 years, but in 2050 it is expected to be 22%, resulting in a significant challenge for health care (Bloom et al., 2015). With the rapidly aging population, related research is a priority.

The study of dog aging is significant from at least two perspectives. First, dogs’ increased lifespan is a direct consequence of sharing their life with humans. Roughly, one-third of all family households maintain one or more pet dogs around the world, with many reporting that their dog is considered as part of their family. Despite the growing number of aged dogs in present day populations, very little is known about the actual prevalence and risk factors of age-related changes in dogs (Azkona et al., 2009; Neilson, Hart, Cliff, & Ruehl, 2001; Osella et al., 2007; Salvin, McGreevy, Sachdev, & Valenzuela, 2010). The relatively extended lifespan in dogs artificially enhances the proportion of dogs with cognitive decline in the population which is a serious welfare concern. Research could facilitate the early recognition and treatment of certain conditions, as well as provide a way (e.g. feeding, physical and mental exercises) for a preventive and predictive approach.

Second, according to several authors (e.g. Chapagain et al. 2018; Cotman and Head 2008; Gilmore and Greer 2015; Head 2013; Milgram et al. 1999; Overall 2000; Szabó, Gee, and Miklósi 2016; Waters 2011) dogs provide a good model for human aging which has unprecedented advantages in terms of general validity to the human case. Pet dogs share our environment more than any other species and develop homologues of the most prevalent age-related human diseases. As companion animals, the same environmental factors affect them as people (chemicals, air pollution, noise pollution, lack of exercise, etc.) which are suspected risk factors of cognitive decline in humans. Pet dogs also excel in displaying socio-cognitive skills in interaction with humans compared to other animals.

To achieve the goal to increase the population of healthy agers, our research aims to describe the causal factors contributing to the emergence of cognitive decline and to develop standardized behaviour tests for early detection. This could reveal differences between healthy and pathological cognitive aging in dogs. Based on this we could suggest protective measures that improve the welfare of old dogs and such insights would also support human aging research.

1 Four questions about aging

Biological aging is an age-related decline in physiological function, leading to a decrease in survival and reproductive rate, i.e. fitness (Aunan, Watson, Hagland, & Søreide, 2016; Bousquet et al., 2015; Collier & Coleman, 1991; Flatt, 2012; López-Otín, Blasco, Partridge, Serrano, & Kroemer, 2013; Szabó & Kubinyi, 2019). Biological aging affects almost every organism, although in significantly various forms, and phylogenetically is an ancient process (Bonduriansky, Maklakov, Zajitschek, & Brooks, 2008), already present in the single cell bacteria *Escherichia coli* (Nyström, 2007). Below I use the "Four questions" framework of Nikolaas Tinbergen, one of the founders of ethology (Tinbergen, 1963), to investigate what advantages aging may have, how it evolved, how it works, and how it progresses.

1.1 Function. Is aging an adaptive process?

"If organisms can function well in youth, why can they not continue to do so in old age?" – asks Partridge and Barton in their influential review (1993, p. 305). Several theories propose answers (Milewski, 2010). The key conceptual insight of all theories is that the old count less than the young: with age even immortal organisms have impaired fertility and die from random injuries and disease. By the time genes supporting later life would take effect, most of the carriers have already died or infertile and harmful mutations already have been passed on to the offspring of the individuals bearing them. Therefore, natural selection becomes less efficient at old age, i.e. there could be only a weak selection against aging.

According to the *antagonistic pleiotropy hypothesis* (Williams, 1957) a gene with a benefit on early life but detrimental effect later has a net positive effect and will be favoured by natural selection. Taking this idea a step further, the aging process can be a genetically programmed, adaptive trait, because it prevents overcrowding, accelerates the turnover of generations and may even favour closely related individuals (Longo, Mitteldorf, & Skulachev, 2005). For example, the popular *grandmother-hypothesis* claims that menopause evolved in social species because old females with a long post-reproduction lifespan increase their inclusive fitness by investing in their grandoffsprings. Indeed, in addition to humans, in killer whales (*Orcinus orca*) the presence of post-fertile grandmothers increases the reproductive success of daughters (Brent et al., 2015). Note, however, that young, fertile Asian elephant grandmothers also increase the survival of grandcalves and decrease their daughters' inter-birth intervals in contrast to the hypothesis prediction (Lahdenperä, Mar, & Lummaa, 2016). Most probably, menopause, when the oocyte number falls below the threshold required for ovarian function, is an age-related decline and not beneficial. The positive effect of grandmothers is irrespective of their reproductive status.

Another influential hypothesis, the *disposable soma theory* proposes that organisms adjust their investments into either maintenance or reproduction to maximize fitness (Abrams & Ludwig, 1995; Kirkwood, 1977). Aging emerges when an organism allocates resources from anti-aging repairs to other needs. Thus aging is due to an energy-saving strategy, i.e. an evolutionary trade-off between gains during early life (growth, reproduction) and maintenance. The rate of aging depends on the allocation of resources.

The competing *mutation accumulation theory* rejects that aging could be adaptive. It assumes that aging is purely maladaptive since it is due to the build-up of random deleterious mutations that are only expressed beyond a certain age. Natural selection cannot eliminate these deleterious genes, because organisms usually die due to unavoidable environmental risks before reaching the age these genes would be expressed (see above). Therefore, aging-related genes can accumulate over successive generations even in potentially immortal populations, because selection does not oppose the spread of deleterious mutations in the relative lack of old individuals.

The assumption that aging is beneficial for the species relies on group selection. However, individual selection is much stronger than group selection, as the cost of death exceeds the benefit to the group or species. Notably, the extensive search for genes that contribute to increased lifespan has not yet found any that stops aging altogether. Recent studies have found more support for the non-programmed aging theories (Kowald & Kirkwood, 2016) and suggest that aging has no function, it is simply the by-product of development.

1.2 Evolution: How does mortality change across species?

The rate and onset of aging show large variation between species (Nussey, Froy, Lemaitre, Gaillard, & Austad, 2013). Mortality may increase, is constant or decrease with age, linked to energy allocation characteristic to a species, but little is known about what constraints favour a life trajectory (Baudisch & Vaupel, 2012).

In some species the rate of mortality from aging is stable, i.e. they are immortal. Basal metazoans, such as the immortal jellyfish (*Turritopsis dohrnii*) and early bilaterians, e.g. a planarian flatworm (*Schmidtea mediterranea*) are “immortal”. Throughout animal evolution, there is a gradual decline in the abundance of stem cells which are cardinal for regeneration. Higher bilaterians, including humans, opted for greater complexity but less abundant stem cells and consequently lost immortality (Petralia, Mattson, & Yao, 2014).

Among species with a pattern of aging with increasing mortality and decreasing fertility, life-span is generally inversely correlated with metabolic rates. If aging is due to a gradual accumulation of damage from metabolic by-products, species with slower metabolisms and higher weight live longer (Sanz et al 2006). However, there are several exceptions. Birds outlive mammals of comparable size which clearly shows that organisms vary in the extent to which they combat the proximate causes of aging. Mice and rats have a 2-3 years lifespan, but the naked mole rat (*Heterocephalus glaber*) lives up to 30 years, due to several factors such as enhanced antioxidant defence, lower insulin levels, and fewer aberrant proteins (Kim et al., 2011). The *evolutionary theory of aging* predicts delayed aging in species with reduced vulnerability to environmental hazards. For example, flying may have a protective effect against predators, and this could explain why birds and bats live considerably longer than expected based on their metabolic rate (Austad & Fischer, 1991).

1.3 Mechanism: What is the cause of aging?

Hallmarks of aging form three main groups: (1) damage to cellular functions: genomic instability, telomere attrition, epigenetic alterations, and loss of proteostasis; (2) antagonistic responses: deregulated nutrient sensing, altered mitochondrial function, and cellular senescence; (3) integrative hallmarks: stem cell exhaustion and altered intercellular communication (Aunan et al., 2016; López-Otín et al., 2013; Sándor & Kubinyi, 2019). Stem cells avoid apoptosis, i.e. they are immortal (Dunham, Neumann, Fasching, & Reddel, 2000). Less abundant stem cells mean that an organism's renewal capacity is limited. Differentiated cells of more complex animals age and die, eventually leading to the death of the animal. Cancer cells that express a telomere-lengthening enzyme are also immortal (Dunham et al., 2000). In a way, cancer is related to embryogenesis, just like stem cells. The main mammalian tumour-suppressor mechanisms evolved from ancient mechanisms that act to regulate embryogenesis/developmental maintenance but now contribute to aging (i.e. by inducing cell death, Campisi, 2003).

The conserved role of signalling pathways is well-established. For example, activation of the nutrient-sensing TOR (Target of Rapamycin) drives growth and, when growth is completed, TOR enhances the aging process (see the section on the Function of aging above, Blagosklonny, 2010). This is probably the reason on a cellular level, why women outlive men. Men are larger and stronger. Hyperactive mTOR contributes to the physical robustness of young males at the cost of accelerated aging (Blagosklonny, 2010).

During aging, cells cease to divide, and the number of senescent cells in tissues rises which may impair renewal, homeostasis, and decrease organ function. In general, old cells are characterized by bigger size, more diverse morphotypes, increased beta-galactosidase activity (a lysosomal hydrolase), more chromosomes (i.e. three or more in humans instead of two), shortened telomeres (non-coding regions at the tips of chromosomes), and changes in several genes' expression levels (Rodier & Campisi, 2011). Biological mechanisms ultimately contribute to the clinical effects of aging as seen in organ decline and therefore reduced function. In humans, the mechanisms behind the decline of brain functions include a decrease in grey matter volume (after age 20), especially in the frontal cortex. White matter volume also decreases and its function declines (for a brief review see Harada et al. 2013). The rate of such physiological changes can often be accounted for by certain genetic variants, for example, the *beta-2-adrenergic receptor* (*ADRB2*) gene was shown to affect white matter integrity and cognitive ability in old age (Penke et al., 2010).

1.4 Development: The process of becoming older

In healthy educated humans, age-related decline of brain functions begins in the mid-twenties (Salthouse, 2009). Human cognitive abilities can be divided into several domains and they may change differently with age. For example, vocabulary and general knowledge ('crystallized abilities') remain stable or gradually improve up to 60-70 years of age. In contrast, attentional abilities, memory functions, performance on verbal tasks, problem-solving, processing and learning new information, and attending to the environment ('fluid abilities') peak in the third decade of life and then decline steadily (Harada et al., 2013).

2 Healthy, typical and pathological aging

The focus of aging research is usually on losses. However, not every old individual shows a deficit in functioning. Rowe and Kahn were the first to popularize the heterogeneity of health trajectories in later life (1987). They differentiated between "usual" or typical aging, i.e.

functioning well, but with a high risk for disease and disability, and "successful" or healthy aging, i.e. demonstrating a high level of functioning across several domains. Importantly, successful aging is a multidimensional concept. Depending on the definitions utilised, 1-90% of study participants are categorized as successfully aging. The high differences indicate categorization is not easy. The majority of definitions include physiological constructs (e.g. physical functioning), engagement constructs (e.g. involvement in voluntary work, and well-being constructs (e.g. life-satisfaction) (Cosco, Prina, Perales, Stephan, & Brayne, 2014). Cognitive health, the main topic of this thesis, is an inherent part of healthy or successful aging in humans, characterised by social activity and independent life until death (Rowe & Kahn, 1987; Rowe & Kahn, 2015). Mild changes in cognition might be a typical part of aging, but deficits are a sign of pathology. Nearly half of people over 85 years of age have dementia, caused by brain diseases or injuries, which impairs daily functioning and results in an inability to lead an independent life (Bishop, Lu, & Yankner, 2010). As life expectancy rises globally due to better nutrition, health care, sanitation, and economic well-being, the growing number of elderly people with dementia poses an increased burden on health care and pension systems. Therefore, aging research is focusing on increasing the health span, i.e. the length of time that the person is healthy. Animal models can help in understanding the cellular mechanisms and the intrinsic and extrinsic factors that contribute to aging.

3 Conventional model organisms of aging[1]

By examining animal species with extreme longevity or immortality such as the *Hydra* genus (Martínez & Bridge, 2012), the naked mole rat (*Heterocephalus glaber,* >30 years, Buffenstein, 2005), the bivalve (*Arctica islandica,* >500 years, Ungvari *et al.*, 2011, 2013), and the bowhead whale (*Balaena mysticetus,* >200 years, (Keane et al., 2015)) researchers might uncover the genetic elements behind a long (and healthy) life. However, these animals are usually difficult to study in the laboratory. Short lived organisms are more efficient experimental models. Nematode worms (*Caenorhabditis elegans)*, fruit fly (*Drosophila melanogaster),* mice (*Mus musculus*), turquoise killifish (*Nothobranchius furzeri*), and the unicellular yeast (*Saccharomyces cerevisiae)* with a lifespan from a few days to a few years have shed light on many regulatory mechanisms behind aging (D'Mello et al., 1994; Fabrizio, Pozza, Pletcher, Gendron, & Longo, 2001; Hu & Brunet, 2018; Juhász, Csikós, Sinka, Erdélyi, & Sass, 2003; Kenyon, Chang, Gensch, Rudner, & Tabtiang, 1993; Pelicci et al., 1999; Sun, Kale, Childress, Pinswasdi, & Jazwinski, 1994; Tatar et al., 2001). Based on these studies, it was found that genes related to aging are components of essential metabolic and signalling pathways, such as autophagic activity and cellular metabolism (Sándor & Kubinyi, 2019).

3.1 Validity of laboratory findings

Although laboratory model animals are useful in uncovering evolutionary conserved mechanisms, they do not necessarily reflect the variance found in natural populations, or the interaction between the cellular mechanisms of aging and complex extrinsic factors. Genetic

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[1] Based on Sándor, S., & Kubinyi, E. (2019). Genetic pathways of aging and their relevance in the dog as a natural model of human aging. *Frontiers in Genetics*, *10,* 948.

polymorphisms can have different effects on aging in different environmental contexts (Ukraintseva et al., 2016), therefore even valid findings in model laboratory animals might not directly correspond to genetic polymorphisms linked to health- and lifespan in humans living in a very different environment compared to laboratories. Moreover, some genes linked to the development of the central nervous system are unique to the primate lineage or can be found only in humans (Bitar & Barry, 2017).

3.2 Where many models fail: cognitive aging

Cognitive aging refers to age related decline in cognitive functioning, which is experienced by almost all older people in several cognitive abilities such as memory, processing speed, and conceptual reasoning (Salthouse, 2004). In humans, together with the occurrence of age related neurodegenerative diseases, non-pathological variation in cognitive aging has also been well documented in the scientific literature. Although normal cognitive aging can influence the day-to-day life of the elderly, the most common health issues posing a great burden on the healthcare systems of developed countries are related to pathological cognitive aging. Over 85 years of age 25-45% of individuals suffer from dementia, a severe cognitive decline (Bird, 2008). Identifying the genetic and environmental factors that influence the development of impaired cognition among the elderly has been a major quest for gerontology research. As it seems, the genetic background of age-related cognitive decline, even that of specific neurodegenerative states, is very complex (Hardy, Lewis, Revesz, Lees, & Paisan-Ruiz, 2009; Karch & Goate, 2015; Mostafavi et al., 2017; Pan & Chen, 2013), and further research is needed to reveal the interactions between genetic variants with each having subtle effects. Translational studies have many limitations in this regard, as many genes linked to the development of the human central nervous system are unique to the primate lineage or can be found only in humans (Bitar & Barry, 2017). Furthermore, rodents and non-mammalian animals do not develop age-related neurodegenerative disorders by nature. Although this limitation of worms, flies, mice and other organisms has been overcome by different techniques used to induce neurodegenerative processes in the central nervous system, the findings of such studies may not be easily implemented in humans. This is because causes of neurodegeneration can be many and are influenced by cognitive and environmental factors that cannot be assessed in laboratory animals because of their limited cognitive and social capacities. Dementia can be the result of multiple small strokes in the brain, certain diseases (AIDS, Huntington’s disease), and Alzheimer’s disease (AD). AD is characterised by amyloid-beta and tau protein pathology inside the brain (Khan & Bloom, 2016). Rodents and non-mammalian animals do not naturally develop age-related neurodegenerative disorders, including AD. Although transgenic mouse models have amyloid beta plaque formation in the brain, they naturally have resistance to amyloid beta pathology, and therefore do not show an extensive neuronal loss. Importantly, the brain of rodents is lissencephalic, i.e. lacking surface convolutions. Unsurprisingly, the findings from spontaneous AD people were not consistent with those in transgenic AD mouse models (Ambrosini et al., 2019).

Humans are also unique with their highly extended post-fertility lifespan (PFLS, see *Function* above). Some researchers argue that the high prevalence of Alzheimer’s disease (AD) is linked to PFLS (Gunn-Moore, Kaidanovich-Beilin, Gallego Iradi, Gunn-Moore, & Lovestone, 2018). Menopause in women starts at the age of 45 while the lifespan of humans goes beyond 110. Thus, PFLS as a percentage of maximum lifespan is 40.9 %. Organisms with similarly long post-fertility lifespan are at a high risk of AD because of the link between longevity and the malfunction of the insulin signaling pathway (which in turn is linked to AD, see above). The killer whale has a similarly long PFLS (48.7%) to humans. Although orcas with dementia have not been reported, studies found evidence of both amyloid deposits and tau pathology in related cetaceans, three species of dolphins (Gunn-Moore et al., 2018). The PFLS of chimpanzees (*Pan troglodytes*) is 18.8, the domestic dog (beagle breed, *Canis familiaris*) 23.3, and the domestic cat (*Felis silvestris catus*) is 38.0. Chimpanzees were found to have amyloid plaques, but not neurofibrillary tangles, dogs have amyloid but not tau pathology (but this is probably breed-specific and the literature is not consistent about it, see below *Age–related cognitive pathology in dogs*), and cats have the full extent of tau pathology. Thus, humans, sea mammals, cats and maybe some dog breeds with especially long PFLS might have AD-like neurodegeneration (Gunn-Moore et al., 2018). However, there are serious ethical reasons against keeping these animals in large numbers for research purposes and especially against invasive experimental interventions. Besides, characterising longevity phenotypes would be almost as challenging in long-living primates and orcas as in humans. The limitation that traditional laboratory animals do not develop dementia has been overcome by transgenic and gene-edited animals. However, the long history of failed AD trials challenges the validity of these models (Götz, Bodea, & Goedert, 2018). Consequently, there are still many unanswered questions about the biology of cognitive aging which cannot be properly addressed in current laboratory animal models.

4 Canine aging[2]

Dogs stand out from the animal kingdom in many aspects. Firstly, they represent the oldest domesticated species, living beside humans for 35,000-15,000 years depending on different approaches to study the origin of dogs (MacHugh, Larson, & Orlando, 2017). As this period is rather long even in the evolutionary time scale, it is not surprising that dogs developed social abilities and morphological characteristics that differentiate them from their closest relatives, the wolves (Kubinyi et al., 2006). Furthermore, the history of dogs living with humans under different circumstances, in different environments, and being used for many purposes, has created an almost unmatched variability in morphology and behavior among breeds. This also resulted in an extension of linkage disequilibrium regions in the genomes of modern dog breeds, as breeding strategies in the last 200 years have tended to expose dog populations to strict selection criteria and bottleneck effects. This renders modern breeds excellent candidates for genetic association mapping. Dogs also develop age-related cognitive decline that shows many similarities with human neurodegenerative diseases and dementia (see details below).

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[2] Partly based on Sándor, S., & Kubinyi, E. (2019). Genetic pathways of aging and their relevance in the dog as a natural model of human aging. *Frontiers in Genetics*, *10*, 948 ; Szabó, D., Miklósi, Á., Kubinyi, E. (2018). Owner reported sensory impairments affect behavioural signs associated with cognitive decline in dogs. *Behavioural Processes*, *157*, 354-360 ;Wallis, L. J., Szabó, D., & Kubinyi, E. (2019). Cross-sectional age differences in canine personality traits; influence of breed, sex, previous trauma, and dog obedience tasks. *Submitted.*

Importantly, companion dogs are exposed to the same environmental factors as their owners, and the consequences of urban lifestyle and westernized diet can be easily detected in pets. Taken together, our canine friends possess high potentials to help us unravel the mechanisms that influence aging and age-related diseases in natural populations.

On the other hand, characterizing the aging process of dogs may benefit humans not only by augmenting human gerontology research, but also by making it possible to increase the healthy lifespan of companion and service animals. Among all domesticated species, the dog is unique in its wide range of functionality. Owning a guide dog or service dog can lead to great improvements in the quality of life of disabled people. Also, service dogs may facilitate human-human interactions and contribute to the socio-emotional well-being of their owners. Caron-Lormier et al. (2016) reported that most guide dogs were retired due to age related diseases or simply old age, after an average of 8.5 years of service. Increasing the lifespan and health span of working dogs could be emotionally beneficial for their owners, and also could be financially beneficial for human societies, as the training of these animals is time consuming and expensive. Furthermore, providing simple pet dogs an elongated health span may also benefit their owners. Several studies have reported a positive correlation between dog walking, physical activity and health variables in owners, although results are often controversial, suggesting the need for further research on this topic (Brown & Rhodes, 2006; Christian et al., 2016; Lentino, Visek, McDonnell, & DiPietro, 2012). In some cases, improvements were most pronounced in older cohorts (Curl, Bibbo, & Johnson, 2016; Garcia et al., 2015; Thorpe et al., 2006; Toohey, McCormack, Doyle-Baker, Adams, & Rock, 2013). Thus, providing a long and healthy life for these animals may benefit the health and welfare of their owners as well.

Despite the clear benefits, there are still many unanswered questions regarding the natural aging process in family dogs. The nature and dynamics of the cognitive and physical declines is still very much under debate. So far, there is no agreement as to what age dogs start to show symptoms of aging, since average life span varies greatly among dog breeds, and so does the time they start aging.

4.1 The diversity of lifespan in wolves and dogs

Wild wolves’ mean expected lifespan is between 5-7 years of age (e.g. see Mech, 2006). The majority of dogs existing today are free ranging (Corrieri, Adda, Miklósi, & Kubinyi, 2018), with general short lifespan. For example, in Zimbawean communal lands the mean age of dogs was 2 years, with a range from one week to 16 years. 40.8% of the population was younger than 1 year, and the mean life expectancy in the population was 1.1 years. Mortality rates of the puppies were very high with 71.8% of dogs dying in their first year of life. These dogs are largely unsupervised, unrestricted, their reproduction is uncontrolled, but they are fed regularly by people who can be regarded as their owners. In a Central American indigenous community, the average age of death was 3.7 (±2.2) years for hunting dogs (Koster and Tankersley, 2012). Some dogs were malnourished, which may contribute to the high mortality rate of puppies. The leading sources of mortality for adult dogs were attacks by jaguars and snakebites.

In captivity, at the W.O.L.F. Sanctuary (http://wolfsanctuary.co/faqs/, accessed 29. 03. 2019) an individual wolf was reported to die at 21 years of age and many others lived up to 15-17 years. This surpasses the life expectancy of similar sized (approx. 40 kg) dogs. Similar to captive wolves, “captive” dogs (i.e. dogs kept in kennels and companion dogs) live considerably longer than their unrestricted counterparts. The protective human environment at least doubled the expected lifespan of pet dogs compared to street/village dogs. Mixed-breed dogs' mean lifespan is 13.1 years. Purebred pet dogs' mean lifespan may range from 5.5 to 14.5 years, depending on size and breed-associated health-problems (Michell, 1999; O'Neill, Church, McGreevy, Thomson, & Brodbelt, 2013). In the past three decades the longevity of companion dogs has extended considerably in developed countries. For example, it increased 1.67 fold from 8.6 years to 13.7 years due to the increased provision of veterinary care and the assumed improved nutrition (Inoue, Kwan, and Sugiura 2018). In different databases the oldest dogs are around 22-24 years old (Adams, Evans, Sampson, & Wood, 2010; Michell, 1999; O'Neill et al., 2013). In a Japan pet cemetery data 23 dogs out of 12,039 (0,2%) lived beyond the age of 22, and only one dog lived up to 25 years (Inoue et al., 2018).

4.2 At what age is a dog considered as old?

Due to the highly variable expected lifespan of dog breeds, one could ask, at what age a dogs is considered as old? Authors use different threshold for canine aging, mainly because they investigate different breeds (Azkona et al., 2009; Fast, Schütt, Toft, Møller, & Berendt, 2013; Golini, Colangeli, Tranquillo, & Mariscoli, 2009; Neilson, Hart, Cliff, & Ruehl, 2001; Salvin et al., 2010; Studzinski et al., 2006). In the beagle (~12 kg) the median age at death is estimated at 13.3 years (Michell, 1999). One study (Studzinski et al., 2006) introduced five periods to classify the different life stages of adult dogs: young adult (1-3 years), adult (3-6), middle aged (6-8), old (8-10), and senior (11+) individuals.

The beagle's lifespan seems to correspond well to the 11-12 years of age which was calculated as an overall mean lifespan for all pet dogs by Michell (1999), however only 36% of the dog breeds listed in O'Neill et al. (2013) reach this median age. To solve this problem several authors suggested to use the mean/median lifespan for each breed as a reference and divide the actual age of the dog by this value. In this case 0.5 means that the dog's current age is half of the expected lifespan of its breed, while a relative age of 1.1 means that the dog's current age is 10% beyond that expected on average for the breed. For the means/medians of individual breeds Michell (1999) or O'Neill et al. (2013) can be used as a source or it can be calculated by using the equation provided by Greer et al. (2007): $Lifespan(years) = 13.62 + height(cm) * 0.0702 - weight(kg) * 0.0538$. Using the relative age of individuals allows researcher to put various breeds and cross-breeds in the same data set when investigating life-long changes of different phenotypic parameters. Note that this method assumes a linear relationship between all life stages in dog breeds, which may not be the case according to Kraus et al. (2013). For a more accurate calculation one would need the breed specific age period spans calculated from actual data (Szabó et al., 2016).

4.3 Main factors influencing the longevity of dogs

Size

Companion dogs' lifespan mainly depends on body size, both regarding lifespans of breeds and mixed-breed individuals (Selman, Nussey, & Monaghan, 2011; Urfer, Wang, Yang, Lund, & Lefebvre, 2019). Large dogs die younger: 70-80 kg dogs live an average of 7-8 years, 6 years less than 10-20 kg dogs do (but see below Inoue, Kwan and Sugiura, 2018 for different results).

While the positive correlation between body size and longevity exists for great taxonomic clades (see above *Evolution*), within species smaller individuals live longer. Not only in dogs but also in domesticated horses (Wolf, 2010), laboratory mice (Rollo, 2002), and humans (Samaras, Elrick, & Storms, 2003). Shorter mean lifespan of large individuals can be explained by different mechanisms including earlier onset of aging and increased rate of biological aging (Galis, Sluijs, Dooren, Metz, & Nussbaumer, 2006; Kraus et al., 2013). According to one hypothesis, faster aging is the main reason for the relative short lifespan in large dogs (Kraus et al., 2013). This means that these breeds are characterised by an abnormally shortened old and senior period. Most researchers believe that IGF-1, an insulin-like growth factor plays a crucial role in this interaction, as there is a positive association between *IGF-1* concentration and size/weight (Greer et al., 2007). *IGF-1* alleles may explain the large percentage of size variation in dog breeds (Sutter et al., 2007). Thus it seems that selection for smaller size in dogs at early state of domestication increased the lifespan, then selection for greater size at their later stage of domestication (using smaller breeds as the starting population) involved heavily the *IGF-1* pathway, which, apart from allowing for rapid early growth, had many side-effects which led to truncated lifespan.

Sex and neutering

It is widely accepted that female mammals generally live longer than males. However, in captive rodents, free from sex-specific extrinsic factors, there is no consistent differences in longevity between the sexes (Hoffman et al., 2018). Among companion dogs, females outlive males by about half a year. However, this result was confounded by the impact of neutering which had a greater effect on lifespan than sex. Females were more likely to be neutered than males and this resulted in their longer lifespan. Indeed, neutered females were longer-lived than any other group (maybe because they cannot develop pyometria or malignant mammary tumours). Regarding intact individuals, males were slightly longer-lived than intact females. Therefore, the majority of apparent sex differences may be due to the effects of neutering. On the other hand, intact individuals are more likely to reach the oldest age (Hoffman et al., 2018).

Breed

During domestication, it seems that lifespan of the dogs has been affected by often opposing selective factors, e.g. decreasing size and increasing docility during early domestication and new selection for large body size during breed formation. Life expectancy at breed level might be independent from the size, because certain diseases frequently affect particular breeds and this could result in early death. Although it was expected that small dogs live longer than middle sized dogs, in Tokyo the small French bulldog, Pug, Chihuahua, and Cavalier King Charles spaniel had low life expectancy (10.2-13.1 years, 1-10 kg), while the larger Shiba had the highest median age of death (15.5 years, body weight ~10 kg), and Labrador retrievers also lived long (14.1 years, ~30kg, Inoue, Kwan and Sugiura, 2018). Among dogs of the same body size, mongrels live longer (Patronek, Waters, & Glickman, 1997) which also supports that inherited diseases in breeds has a large negative effect.

Breed-typical behaviour was also found to be correlated to longevity. More obedient (or docile, shy) breeds live longer than bold ones (Careau, Réale, Humphries, & Thomas, 2010), in harmony with the “pace of life syndrome“ predicting that less reactive animals live slow and die old. It can be argued, however, that not docility itself, but the reduced stress to anthropogenic factors plays the key role.

Environmental factors

Dogs living in smoking homes are more likely to suffer from DNA damage and show signs of premature aging than those living in non-smoking homes (Hutchinson, 2017). Obesity can have detrimental effects on health and longevity. Overweight dogs are at risk of developing diabetes mellitus, osteoarthritis and urinary incontinence, as well as altered respiratory function (German, 2016). They also have elevated levels of inflammatory markers (TNF-alpha and C-reactive protein) (German et al., 2009).

The environment, in which the dog is kept, and the management choices of the owner (such as how much time they spend with the dog) can also influence healthspan and wellbeing. Shared activities between dogs and owners decrease with the dog's age, reducing the quality of the dog-owner relationship (Bennett & Rohlf, 2007; Marinelli, Adamelli, Normando, & Bono, 2007). Chronic stress can also have negative effects on health and lifespan in the domestic dog (Dreschel, 2010).

4.4 Age-related changes in personality and social life

Aging, including decreasing adjustability (Rose et al., 2012), affects every dog above a certain age. Personality is defined as "behavioural differences that are stable across time and situations". However, there is cross-sectional evidence for mean personality trait change across the lifespan in humans (Roberts, Walton, & Viechtbauer, 2006) and in dogs (Jones and Gosling 2005) and studies rarely take into account lifestyle demographic factors, which may influence results (Mirkó, Kubinyi, Gácsi, & Miklósi, 2012; Szabó et al., 2016). Younger dogs show higher boldness (Starling, Branson, Thomson, & McGreevy, 2013), sociability (Kubinyi, Turcsán, & Miklósi, 2009b), companionability, energy, excitability, playfulness, active engagement, (Henriksson, 2016), extraversion (Ley et al., 2009), and attentiveness (Vas et al., 2007; Wallis et al., 2014). The literature is contradictory about anxiety; while older dogs show higher calmness (Kubinyi et al., 2009) and lower anxious/destructive behaviour than younger dogs (Bennett & Rohlf, 2007), neuroticism (a general measurement of fearfulness) was found to correlate positively with age (Temesi, Turcsán, & Miklósi, 2014)). Touch sensitivity, fear of handling, fear of noises (Blackwell, Bradshaw, & Casey, 2013; Henriksson, 2016), human and object fear (Lofgren et al., 2014), aggression towards dogs, and owner directed aggression (Henriksson, 2016; Hsu & Sun, 2010) also increase with age.

Inconsistencies may be due to the fact that different methods were used to obtain the trait scores, including one-word adjectives, and complete sentence descriptions (with examples to set the trait in context), and/or different age groups and age ranges were examined. In addition, nearly all studies reported only linear age relationships, and many had only small effect sizes.

Dominance describes long-term dominant-subordinate social relationships within a dyad or group, therefore, it is not a personality trait. Personality is largely independent of context and it is stable over time (Jones & Gosling, 2005) while dominance status depends on the interacting partners.

The dog is a social species, and owners keep several individuals in the same household. The existence and validity of linear dominance hierarchies in companion dogs is highly debated

(Bradshaw, Blackwell, & Casey, 2016, 2009b; P. D. McGreevy, Starling, Branson, Cobb, & Calnon, 2012; Overall, 2016; Schilder, Vinke, & van der Borg, 2014; J. A. M. Van Der Borg, Schilder, Vinke, De Vries, & Petit, 2015; van Kerkhove, 2004; Westgarth, 2016). Dominant individuals usually have priority access to key resources such as food and reproductive partners, but companion dogs usually do not need to compete for resources and have no access to sexual partners (Clutton-Brock, Albon, Gibson, & Guinness, 1979; Drews, 1993).

When hierarchy was detected, older dogs were found to be more often dominant than young individuals (Bonanni et al., 2017; Bonanni, Cafazzo, Valsecchi, & Natoli, 2010b; Cafazzo, Valsecchi, Bonanni, & Natoli, 2010; Mech, 1999; Trisko & Smuts, 2015) but it is yet unknown how age is related to leadership and personality.

4.5 Functional declines during aging

Even "successful" aging in dogs is associated with a decline in physiological, perceptual and cognitive functions (Adams, Chan, Callahan, & Milgram, 2000; Beth Adams, Chan, Callahan, Siwak, et al., 2000; Bellows et al., 2015; Salvin, McGreevy, Sachdev, & Valenzuela, 2011; Salvin et al., 2012; Wallis et al., 2014, 2016). Diminished performance of (healthy) older dogs compared to young ones have been found related to memory (Piotti et al 2018), attention (Wallis et al. 2014), problem solving (González-Martínez et al., 2013) and reversal learning (Mongillo, Araujo, et al., 2013). Decline in the spatial function (i.e. the ability to perceive, remember, and manipulate information within a spatial context (Dwight Tapp, Siwak, Estrada, Holowachuk, & Milgram, 2003) and learning is also a part of the normal aging process (Cotman & Head, 2008). Impairment in the spatial function is particularly of interest because it may be detected before other cognitive deficits emerge (e.g. dog: Head et al., 1995; Piotti et al., 2017; Studzinski et al., 2006; Studzinski et al., 2006; Piotti et al 2017; human: Becker, Huff, Nebes, Holland, & Boller, 1988).

However, the rate of deterioration should not affect the individual's day-to-day functioning; otherwise, this might indicate a pathological problem (Salvin et al., 2011a). Despite the growing number of aged dogs, very little is known about the actual prevalence and risk factors of age-related changes in the general population of dogs (Neilson, Hart, Cliff, et al. 2001), especially regarding the baseline occurrence of cognitive decline associated behaviours.

Decreased cognitive performance of successfully aging older dogs compared to young ones have been described in several studies (Szabó et al., 2016). Almost one third of 11-12-year-old dogs and 70% of 15-16-year-old dogs show cognitive disturbances corresponding to human senile dementia: spatial disorientation, social behaviour disorders (e.g. problems with recognizing family members), repetitive (stereotypic) behaviour, apathy, increased irritability, sleep-wake cycle disruption, incontinence, and reduced ability to accomplish tasks (Neilson et al., 2001). However, differentiating between dogs showing signs of normal aging, signd of other medical problems or early signs of cognitive dysfunction based on direct behavioural measures has proven to be a challenging task (Rosado et al., 2012). Cognitive dysfunction syndrome is described as a progressive neurodegenerative disorder, in which the diagnosis of pathological brain aging is achieved by evaluating the associated behavioural signs and excluding other medical conditions (Landsberg et al., 2011). Several publications have described cognitive dysfunction in aged dogs and provided specific questionnaires for clinicians and owners, in order to assess the prevalence, progression and risk factors of cognitive dysfunction in the aging dog population (Azkona et al., 2009; Landsberg et al., 2011; Madari et al., 2015; Salvin, McGreevy, Sachdev, & Valenzuela, 2012). The different scales being currently used in parallel in the literature (e.g. Madari et al., 2015; Salvin et al., 2011) show huge variation in their estimation of the proportion of affected dogs (ranging from 14 % to 68 %, depending on the scale and the senior dog population), with age being the greatest known risk factor (Azkona et al., 2009; Neilson et al., 2001).

Pathological cognitive decline, which is usually referred to as "Canine Cognitive Dysfunction Syndrome" (CCD, Cummings et al. 1996; Landsberg, Nichol, and Araujo 2012, Chapagain et al. 2018; Szabó et al. 2016) is associated with amyloid-beta accumulation in the prefrontal cortex (also occurs in the walls of brain vessels, similarly to humans), noradrenergic neuron loss in the locus coeruleus (Insua, Suárez, Santamarina, Sarasa, & Pesini, 2010) and with the formation of tau tangles in neurons and astrocytes in the cerebral cortex and hippocampus (F. Schmidt et al., 2015; Smolek et al., 2016), which can all be seen in humans in early stages of neurodegenerative diseases.

Whether changes regarding the prevalence of cognitive dysfunction associated behaviours are detectable before 8 years of age has not been investigated. Findings regarding other risk factors such as body size, sex, and neuter status have been contradictory (Azkona et al., 2009; Fast et al., 2013; Hart, 2001).

4.6 The dog as a model for human aging

What constitutes a good cognitive aging model? Much depends on the exact question being asked. Aging has conserved pathways at the cellular level across species, but some biochemical and histological changes behind cognitive impairment in humans are hard to reproduce in animals. Thus, an ideal model animal for cognitive aging should be closely related to humans both in evolutionary terms and, due to the influence of environmental effects, in ecological terms. In addition, it is advantageous if the animal has a relatively short lifespan compared to humans, has high fecundity, is available at low cost, and can be easily manipulated experimentally. On this basis, the companion dog could hold translational promise, except that it cannot be exposed to invasive experimental manipulations (Waters, 2011). The wide but shorter range of lifespan, the environmental similarities, the availability and the vast knowledge about the behaviour, physiology and genetics of the species may promote the dog as a natural model for cognitive aging research and may hold prospects unimaginable in the case of other model organisms (Creevy, Austad, Hoffman, O'Neill, & Promislow, 2016; Gilmore & Greer, 2015; Hoffman, Creevy, Franks, O'Neill, & Promislow, 2018; Kaeberlein, Creevy, & Promislow, 2016; Mazzatenta, Giulio, Robbe, Carluccio, & Cellerino, 2017).

General validity

1. *Cognitive similarities:* In the last two decades several studies have supported the notion that dogs possess cognitive abilities that are similar to human social skills in communication and learning (Bensky, Gosling, & Sinn, 2013; Feuerbacher & Wynne, 2011; Hare, Call, & Tomasello, 1998; Miklósi, 2014; Miklósi, Polgárdi, Topál, & Csányi, 1998; Topál, Miklósi, & Csányi, 1997; Topál et al., 2009). The dog as a species has several unique characteristics for comparative cognition research (Miklósi and Kubinyi, 2016). The divergence of the dog from the wolf is assumed to be similar to the evolution of humans, i.e. the evolution of dogs

mirrors some aspects of human evolution, consequently dogs possess a set of functionally analogous skills corresponding to that of humans (convergent evolution, Topál et al., 2009). Due to their increased sociality, cooperativity, and communicability, companion dogs offer an unprecedented animal model for studying socio-cognitive aging.

2. *Genetic similarities:* The sequencing of the dog genome has offered specific tools for understanding the functioning of neural and mental mechanisms (Wayne & Ostrander, 2007). Dogs share more ancestral DNA sequence with humans than rodents do (Lindblad-Toh et al., 2005).
3. *Physiological similarities:* Dogs share several metabolic and physiological features with us, some of which are a clear sign and consequence of domestication, such as increased capability of starch digestion (Axelsson et al., 2013). Dog diet resemble more to that of humans than rodent diet, consequently human and dog microbial composition (affecting brain functions) is also more alike (Ambrosini et al., 2019). The intestinal absorption profiles of many supplements, including the ones used in aging intervention studies, are very similar in dogs and humans (Roudebush et al., 2005). The dog's convoluted brain is a good model for of a 100-million-year-old carnivore mammal, the common ancestor of man and dog (Springer, Murphy, Eizirik, & O'Brien, 2003), and certainly better than the lissencephalic brain of rodents. Physiological similarities support the validity of findings in cognitive aging research.

Specific validity

4. *Natural model for human dementia:* In the widely used transgenic mouse models the onset of neurodegenerative changes is premature (artificially induced), and the amyloid-beta and tau pathology are considerably different, therefore the dog most probably has higher translational relevance to neurodegenerative diseases (Ambrosini et al., 2019). *C*ognitive decline in dogs was associated with similar beta-amyloid accumulation in the prefrontal cortex, noradrenergic neuron loss in the locus coeruleus (Insua et al., 2010) and, lately, with the formation of Tau tangles (F. Schmidt et al., 2015; Smolek et al., 2016), which can all be seen in humans in early stages of neurodegenerative diseases. These findings could make our best friend a promising model of early stage AD, especially for further investigations in the molecular events involved in the beta-amyloid related pathology (Head, 2013; Schütt et al., 2016).
5. *Natural experiments:* The domestication history of dogs enables researchers to investigate how microevolution affects cognitive aging as representative populations of closely related species (wolves, dingoes, feral dogs, etc.) and dog breeds are available for comparative research to understand this process (Miklósi, 2014). Their unique population structure provides extensive opportunities for gene identification, including the genetic background of complex phenomena, such as cognitive aging. Selective breeding resulted in a great variety in dogs in terms of their appearance, behaviour, health- and lifespan. Most current breeds can be correctly recognized on the basis of their genotype (Heidi G Parker et al., 2004; Sundqvist et al., 2010). Thus breeds are inbred, genetically isolated units, with reduced genetic heterogeneity and particular morphological and behavioural features (Lindblad-Toh *et al.*, 2005; Parker and Ostrander, 2005). Contrary to humans, linkage disequilibrium regions can be extensive within dog breeds, making it easier to pinpoint

phenotype–marker associations (Boyko, 2011; Hayward et al., 2016; Schoenebeck & Ostrander, 2014; Vaysse et al., 2011). Differences between the aging curves of specific breeds could be telling about specific human populations. Dogs also have wide variety of relationships with humans from a very intimate bond to feral and laboratory dogs, which might keep their distance from people even if they depend on humans for their food (Miklósi, 2014), therefore developmental social effects on aging can be easily studied.

Feasibility

6. *Practical and ethical aspects:* The major difficulty of tracking cognitive changes in humans is that funding terms do not support longitudinal measures. Dogs' lifespan is shorter than humans, therefore longitudinal aging studies are much easier to conduct in dogs. Life expectancy of humans in the European Union is 82 years (https://data.worldbank.org/indicator/SP.DYN.LE00.IN), of dogs is around 13 years (Inoue et al., 2018; Michell, 1999; O'Neill et al., 2013), which means humans live six times longer than man as $\frac{82}{13} = 6$. Interestingly, the shift number is only 4 if we compare the longest living dog and human being. The Guinness world record holder among dogs is an Australian cattle dog, Bluey, who died in 1939 at the age of 29.5 years (http://www.guinnessworldrecords.com/world-records/oldest-dog, retrieved 29. 03. 2019), and Jeanne Calment among humans, with the age of 122 (http://www.guinnessworldrecords.com/world-records/oldest-person, retrieved 29. 03. 2019, but challenged by Zak (2019), who considers that Calment's daughter may have assumed Calment's identity). In contrast to human age cohorts, young and old dogs have similar life experiences, therefore cohort differences do not bias cross-sectional invatigations. Companion and feral dogs, as large-bodied mammals are available for non-invasive research almost everywhere at very little cost, unlike apes for example. Owners very often are motivated to participate with their dogs with no financial incentive (or citizen science). This offers the possibility to replicate experimental results, increase reliability, and collect large data sets using citizen science methods (Hecht & Cooper, 2014; Stewart et al., 2015). The fact that owners keep and observe their dogs for years, usually from puppyhood to death, allows researchers to collect longitudinal data from owners. Long-term approaches to collect behavioural, medical and lifestyle data about companion dogs, together with providing the opportunity for owners to donate their dogs' bodies for research purposes to biobanks (i.e. brain and tissue banks) under appropriate ethical considerations, could hold fruitful prospects for the future in canine genomics and aging research (Sándor, Czeibert, & Kubinyi, 2019).

4.7 Studying canine aging for its own sake

Given the number and role of dogs in human societies, any knowledge gained in order to increase the healthspan of dogs can have important practical implications.

1. *Financial, emotional, social burdens:* The toll of taking care of a chronic or terminally ill dog is often overooked. However, compared to owners with healthy pets caregiving is a greater level of burden, higher level of stress, depressive symptoms, and a lower quality of life coupled with high veterinary cost (Spitznagel, Jacobson, Cox, & Carlson, 2017). In

many ways this similar to what we see in a person caring for a family member with, for example, dementia. However, owners often meet scorn rather than sympathy. The breeding and training of working dogs (police, custom, guide, etc.) is especially costly, therefore increasing the working period of these dog and decreasing veterinary expenses is a priority.

2. *Thanatology:* As death is the unevitable end of old age, owners have to face with the loss of their pet. The loss of a dog is associated with stress for the bereaved owner and reduced physical, psychological, and relationship quality of life. Again, lack of social support in the case of death of a companion animal has a strong negative effect on owners' grief reactions (Tzivian, Frigera, & Kushnir, 2015).
3. *Welfare:* The increased rate of dogs with physical, sensory and mental impairements coupled with chronic pain is a serious welfare concern. Behavioural performance is often affected by social stress, too. A threatening approach by a human experimenter resulted in significant elevation of salivary cortisol among old police dogs but not among young ones (Horváth, Igyártó, Magyar, & Miklósi, 2007), suggesting that aged dogs have a lower tolerance for social stress similarly to humans (Lazarus & Folkman, 1984). We need to recognize and treat these conditions as soon as possible and suggest interventions. Some studies revealed that extrinsic factors may protect against rapid cognitive decline, and thus can have the potential to improve the mental welfare of dogs (Milgram et al., 2005; Milgram, 2003). In case of aged laboratory beagles, groups undergoing an enrichment program (including increased exercise, environmental enrichment and cognitive enrichment) performed better in a discrimination task than the control group. This effect was even superior to the application of an antioxidant fortified food. In a similar experiment, (Araujo, Studzinski, Head, Cotman, & Milgram, 2005) found that dogs receiving an antioxidant and mitochondrial cofactor combination diet for two years performed better in a delayed non-matching to sample task than the control dogs, even without the positive effect of additional enrichment. Milgram (2003) provided some evidence that younger dogs benefit more from previous, related cognitive experience in a size discrimination paradigm than older animals. Similar studies on companion dogs will help early detection and interventions can extend the healthspan of aging dogs.
4. *Indirect effect:* Awareness about the aging process in dogs would also raise the owners' awareness on the risk factors and preventive methods about their own aging. Such a knowledge might result in healthier lifestyle and more succesful aging – a step towards healthy aging in human communities.

4.8 Disadvantage of the dog model in cognitive aging research

The advantage of being variable and living in a variable environment could be also considered as the main limitation of the companion dog model. Therefore, detailed information collection is needed about the developmental and environmental factors, including diet, medical history, and lifestyle about a large sample of individuals.

Invasive methods are not applicable in the case of companion dogs. This means that, for example, brain banks have to count on the donations of dog owners, with often insufficient information. Ghi et al. (2009) investigated the brains of dogs that had been donated by owners following medically advised euthanasia. Because the donated dogs have not participated in behavioural assessment previously, the researchers were unable to connect behavioural and molecular data. Behaviourally tested dogs were unlikely to be euthanized and donated within the time-frame of the study. There is also less opportunity for genetic manipulations than in other animal models due to ethical reasons. Targeted genetic manipulations in laboratory dogs can create new strains for animals with favourable traits (Zou et al., 2015). Importantly, therapeutic applications of gene-editing have recently been applied on pet dogs suffering from Duchenne muscular atrophy, with promising results (Amoasii et al., 2018). Hence, it is likely that this line of canine genetics and medical research will continue to unfold its potentials after addressing ethical concerns (Sándor & Kubinyi, 2019).

Taken together, the protective human environment extended the lifespan of companion dogs and artificially increased the proportion of dogs with age-related neurodegenerative pathologies. This means that old age-classes and impaired individuals, suffering from decrease in sensory and mental performance are able to survive and available for research. Although this is an unfortunate situation from a welfare perspective, it paves the way for cognitive aging research in dogs with the aim of increasing the population of healthy agers and supporting human aging research with a unique animal model. Applying companion dogs helps solving some methodological challenges that afflict the study of brain aging. Shorter life of companion dogs enables us to detect valid changes of human-like abilities within a much shorter time. Cross-sectional design is not affected by cohort differences, because young and old age cohorts have similar life experiences. Studying aging in dogs may bring forward results that eventually benefit humans and the companion dogs as well.

5 Aims

Above I have examined the function, evolution, mechanism and development of aging in general, and introduced the dog as an emerging model species not only for human cognition, physiology and disease, but also for aging research. In order to study aging in dogs I have established the Senior Family Dog Project at the Department of Ethology, Eötvös Loránd University in 2016. The group explores the cognitive aging of family dogs using an interdisciplinary approach, integrating methods of ethology, neuroscience and genetics/molecular biology (Figure 1). Overall, our main aim is (1) to provide empirical evidence that the dog model holds unmatched potentials for aging research which is still debated regarding translational aspects and (2) to promote healthy aging for the benefit of both owners and dogs. More specifically, we aim to characterise the canine aging phenotype with cross-sectional and longitudinal investigations, and identify the underlying processes. We use large scale surveys and develop sensitive and standardized behaviour tests to enable veterinarians, dog trainers, and behaviour counsellors to assess dogs' cognitive abilities, document changes occurring over time and detect early signs of pathological cognitive decline. We also aim to identify neural markers of aging with non-invasive neuroimaging techniques and uncover the genetic background of extremely long life by sequencing the genome of methuselah dogs. Our recently established Canine Brain and Tissue Bank provides samples for both gross anatomical evaluation and also for tracing cellular changes (e.g. by RNA-analysis, immunohistochemistry, and proteomics). The database of the Bank includes both molecular and behavioural data from individuals and offers a unique opportunity for obtaining specific canine brain tissues. The results are expected to aid our knowledge about the rate of successful and pathological aging, help canine welfare initiatives through guidelines for a healthy lifestyle toward successful aging, and the understanding of the biology of human cognitive aging.

In the thesis I present published research on age-related differences in the demographics and health, cognition, emotional and face processing, personality, and intraspecific relationships in companion dogs and our recent initiatives about studying brain activity during sleep and the genetic background of individuals with an exceptionally long lifespan. Ongoing research is presented in the Perspectives chapter.

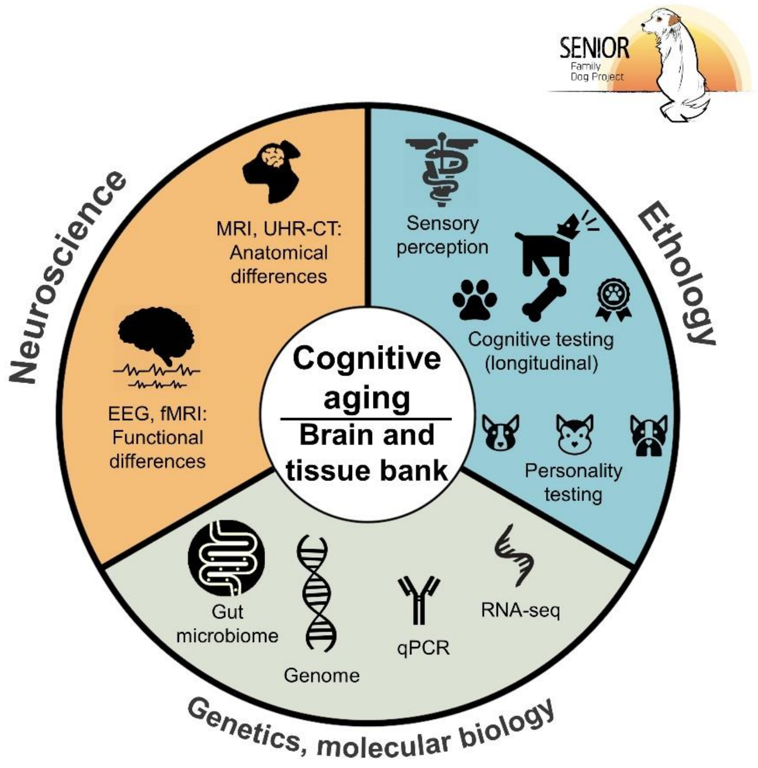

Figure 1. Visual abstract of the Senior Family Dog Project. ((f)MRI (functional) magnetic resonance imaging, UHR-CT: ultrahigh resolution computertomography, EEG: electroencepalography, qPCR: quantitative polymerase chain reaction, RNA-seq: ribonucleic acid sequencing).

Specific questions

I. Demography and health

Chapter 7: The prevalence of age-related cognitive decline in companion dogs across the entire adult lifespan

Before conducting any study about dog aging, we have to estimate the prevalence of cognitive decline. However, differentiating between dogs showing signs of typical aging or early signs of cognitive dysfunction based on direct behavioural measures has proven to be a challenging task. Specific questionnaires have been developed for veterinarians and owners, but these show huge variation in their estimation of the proportion of affected dogs (ranging from 14 to 68%, depending on the measurement tool and the population, Szabó et al., 2016). Whether changes regarding the prevalence of these behaviours are detectable before 8 years of age has not been investigated. Findings regarding other risk factors such as sensory impairment, body size, sex, and neuter status have been also contradictory. Our goal is to investigate the impact of these factors (sensory deficits, sex, neutered status, training) on the occurrence of behavioural signs associated with cognitive decline, taking into account the differences in the expected lifespan of small and large dogs. (The gathered information will be reflective about the relationship between the putative behavioural signs of cognitive decline and relative age, sensory impairments and certain demographic factors across the whole adult lifespan. Diagnosis of cognitive dysfunction needs veterinary examination). We expect that beside relative age (mean lifespan for each breed/ actual age of the dog), impairments in every sensory domain and training history are associated with behavioural problems reflecting cognitive decline.

Chapter 8: Demographics of companion dogs across age groups and identifying the key variables associated with health status

Dogs go through similar stages of development as humans, and their living conditions and nutritional requirements can change considerably as they age. However, many owners do not consider their dog’s life stage when selecting a diet and are unable to recognize if their pets are overweight or obese (Davies, 2012; Holmes, Morris, Abdulla, Hackett, & Rawlings, 2007). Therefore, e.g. in the UK, up to 60% of dogs are now classified as overweight or obese (Courcier, Thomson, Mellor, & Yam, 2010; Holmes et al., 2007; YouGov, 2017). We utilize an on-line questionnaire to examine the link between the age and health of the dog, and owner and dog demographics in a cross-sectional sample. We expect to identify key variables associated with health status. Since this study is exploratory, we include a total of 27 dog and owner demographic factors in our analysis.

II. Cognition, emotion and face processing

Chapter 9: Developing a behaviour test for assessing discrimination and reversal learning

A large body of evidence indicates that functional decline in cognitive domains, such as learning, memory, executive function, and spatial function, occurs similarly in dogs and humans as they age. However, several tests involve laboratory dogs, large apparatus and prolonged training. The aim of this study is to develop a reversal learning task which could detect age-related changes in the learning abilities of companion dogs without overt medical problems in a short time-frame (about 1 hour). We design a simple and reproducible version of a reversal learning task, which does not require large or complex equipment or several weeks of training, in contrast to previous tests. We also aim at developing tests that could be repeated over time, to monitor the progress of the condition. Therefore, we test the dogs with different stimuli, either with the location or the physical attributes of the rewarded object, in order to explore whether these would affect their performance.

Chapter 10: Positivity effect in dogs: do old dogs experience less negative emotions?
Age-related changes in the brain can alter how emotions and the social environment are processed. In humans, attention and memory shift from negative to positive stimuli with increasing age, i.e. older people experience fewer negative emotions, which is known as the “positivity effect”. We measure how fast young and old dogs react to human vocalisations, with positive, negative, and neutral emotional valence, during a sound playback test. We hypothesise that old dogs are less responsive and react slower to negative vocalisations than young dogs, suggesting that dogs have the same positivity effects as humans.

Chapter 11: Age-related effects in looking at faces of humans and conspecifics
The gaze of others is informative for both humans and dogs. In humans, responsiveness to the eyes and gaze change with age. We show pictures of conspecific and human faces to dogs and expect that older dogs orient less towards them due to decreased perception. It is also possible that they react differently to the pictures (e.g. orient more towards the human face but less towards the dog head) than young dogs, due to altered processing of social stimuli. We also investigate the effect of head shape and breed function on the looking behaviour.

III. Personality and intraspecific relationships

Chapter 12: Age-related changes in human-based personality traits and associations with owner and dog demographics
Numerous cross-sectional studies in humans have reported mean-level changes in personality traits across the lifespan, and that significant life events and educational experiences can influence personality traits. People tend to show increased self-confidence, warmth, self-control, and emotional stability with age. The dog has been suggested as a possible model for human personality development. We adapt a human personality questionnaire, and analyse the relationship between canine personality traits, age and other variables on a large sample with more than 10,000 adult individuals.

Chapter 13. Interventions to increase play and training motivation may alleviate the negative effects of aging
The literature is contradictory regarding the number and definitions of canine personality traits. Inconsistencies may be due to the fact that different methods were used to obtain the trait scores, and/or different age groups and age ranges were examined. The Dog Personality Questionnaire has been shown to demonstrate reliability and validity, and has been used in numerous studies to measure personality in dogs via owner report. The aim of this study is to investigate the effects of age on dog personality on a different sample with a different questionnaire than in our previous study. We predict a strong influence of dog age on dog personality and confirm our previous results with an independent survey.

Chapter 14. The relationship between age, personality, dominance and leadership in a group of dogs

Movement interactions and the underlying social structure in groups have relevance across many social-living species. Decisions about which individual leads a group can be based on an 'egalitarian' system, but in practice it is often influenced by underlying social network structures and by individual characteristics such as older individuals being dominant and/or leaders of the group. Our goal is to identify how a group of family dogs decides the direction of their movements and whether there is a leader, or is decision-making based on an egalitarian system. We collect GPS trajectory data from an owner and a group of dogs during several walks. We assume that younger individuals will follow the older, more experienced ones. We also examine the role of personality and dominance in connection with leadership.

Chapter 15. Dominance status and age in companion dogs sharing the same household

Dominance describes long-term dominant-subordinate social relationships within a dyad or group. An age-related hypothesis has been suggested to explain dominance in dogs. According to this, a simple rule of thumb could explain formal dominance in dogs: young dogs perform affiliative behaviours towards older group members. However, the literature does not always support correlations of dominance rank with age, and it remains unexplored whether the age related hypothesis is a better predictor of formal dominance than dominance displays. We launch a questionnaire study to investigate owner-derived estimates of dominance in dog dyads sharing the same household. We suppose that that dog age does not explain the occurrence of dominance-related behaviours over the owners' estimate of dominance status.

IV. Steps towards understanding the mechanisms of aging

Chapter 16. Age related differences in the spindling activity of the sleeping brain

The dog brain is still relatively unknown compared to the brains of other model animals. Mechanistic and correlational evidence from humans and rats support sleep spindle involvement in memory consolidation. Spindle amplitude and occurrence are lower in the elderly, while frequency increases. If findings on humans generalize to dog sleep spindles (0.5-5 seconds long, 9-16 Hz oscillations, observed mainly in the non-REM EEG signal), it would strengthen the argument that dogs are good model animals. We investigate a large sample of intact and neutered family dogs of both sexes, varying in breed and age, searching for sleep spindles in segments of non-REM sleep. We predict a similar pattern for spindle occurrence as in humans.

Chapter 17. The genetic background of longevity based on whole-genome sequence data of two methuselah dogs

The genetic research of aging is generally concerned with mutations found to increase longevity. The oldest known dog (†29) lived 130% longer than the average lifespan of dogs (13), in contrast to humans where centenarians live at most 50% longer compared to the average human lifespan. By studying methuselah dogs (individuals with an exceptionally long lifespan), we aim to extend our understanding on extreme longevity. We use the whole-genome sequence of two extremely old dogs, which lived 22 and 27 years (90-135% longer than the average lifespan of dogs) to investigate the genetic background of longevity and determine why these dogs were successful in aging. We also compare the results to that of human centenarians. We expect to detect rare genetic variants linked to canine longevity, which could be prime candidates for age-related research in the future.

II. Experimental studies

6 Ethics Statement

The procedures applied throughout of this dissertation complied with national and EU legislation and institutional guidelines. According to the current Hungarian law (1998. évi XXVIII. Törvény — the Animal Protection Act, 3rd paragraph, 9th point), non-invasive data collection on dog demographics and behaviour are not considered as animal experiments and are therefore allowed to be conducted without any special permission from the University Institutional Animal Care and Use Committee (UIACUC). The application number of the ethical commission for studies performed by the Senior Family Dog Project is PE/EA/2019-5/2017. The filling out of the questionnaires was voluntary and anonymous so the studies did not violate respondents' privacy. In case of behavioural tests, owners provided written consent to their participation. Our Consent Form was based on the Ethical Codex of Hungarian Psychologists (2004). We took special care to ensure that the consent process was understood completely by the dog owners. In the Consent Form, participants were informed about the identity of the researchers, the aim, procedure, location, expected time commitment of the experiment, the handling of personal and research data, and data reuse. The owners were not informed about the exact aim of the test. The information included the participant’s right to withdraw their consent at any time. Participants could at any point decline to participate and could request for their data not to be used and/or deleted after they were collected during the experiments. The study was performed in strict accordance with the recommendations in the International Society for Applied Ethology guidelines for the use of animals in research.

7 The prevalence of age-related cognitive decline in companion dogs across the entire adult lifespan[3]

7.1 Abstract

Currently several scales are used in parallel for assessing cognitive decline, showing huge variation in the estimation of the proportion of affected dogs (14-68%). To describe the extent of age-related cognitive decline in dogs, information regarding the baseline occurrence of associated behaviours in the general population is necessary. In addition, it would be important to describe the extent of behavioural signs associated with cognitive decline across the whole adult lifespan. Whether cognitive decline associated behaviours are detectable before 8 years of age has not been investigated before. Moreover, previous studies did not specifically address the influence of sensory decline, although the prevalence of sensory impairments seems to increase with age. With a seven-item, data driven Age-Related Changes scale, we evaluated the relationship between sensory functions, training, sex, and the occurrence of behavioural signs associated with cognitive decline across the whole adult lifespan. The twofold difference in lifespan between small and large dogs presents challenges for aging studies, with no widely accepted method to control for body size as it relates to chronological age and longevity, when comparing behavioural signs of cognitive decline. To address this issue, we utilized relative age, calculated using the estimated expected lifespan of the individuals in our questionnaire study. Signs of cognitive decline were already detectable in ’Mature’ dogs (at 50-75% of the expected lifespan). Visual, auditory and olfactory impairments all resulted in significantly higher scores on the Age-Related Changes scale. Participating in dog training activities was revealed to be protective against behavioural signs of cognitive decline in aged dogs as perceived by the owners. These results revealed possible beneficial effects of training on cognitive aging and emphasize the importance of routinely screening the sensory capacities of aging dogs. Data are expected to aid differentiating between dogs showing signs of normal aging or early signs of cognitive dysfunction.

7.2 Introduction

Most scales utilize separate subscales (domains), such as spatial orientation or house soiling as a basis of their scoring system, but the lack of item stability within categories across questionnaires is problematic. For instance, the item “Decreased recognition of/Does not recognise familiar people” has been classified as a sign of impairment in three different domains depending on the study: “Disorientation” (Osella et al., 2007), “Social interactions” (Azkona et al., 2009), and “Learning and memory: work, tasks, commands” (Golini et al., 2009). To date no report of the internal consistency of these domain specific scales has been published. In case of the Canine Dementia Scale (CADES), the four domain specific scores (spatial orientation, social interactions, sleep–wake cycles and house soiling) were highly correlating with the sum score (Madari et al., 2015). The missing information regarding the internal consistency of the scales is problematic, as behavioural and questionnaire studies often use the domain specific subscales as a basis for categorization of the various stages of cognitive

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[3] Based on: Szabó, D., Miklósi, Á., & Kubinyi, E. (2018). Owner reported sensory impairments affect behavioural signs associated with cognitive decline in dogs. *Behavioural Processes, 157,* 354-360.

dysfunction syndrome. For example, a domain (e.g. social interaction) is considered impaired if the dog shows more than one domain specific behavioural sign and the dog is categorized to suffer from severe CDS if at least three domains are impaired (Azkona et al., 2009; Neilson et al., 2001; Rosado et al., 2012).

Another problematic issue with previous reports based on owners' assessments is that they did not specifically address the influence of sensory decline on the reported behaviours in the questionnaires. This can be a confounding factor in these type of studies, as the prevalence of sensory impairments seems to increase with age. Urfer et al. (2011) reported that in dogs older than 5 years the incidence of cataract was 8.7%, while among old dogs (7-10 years) 14.1% of the population was affected, and this rate is probably even higher in senior dogs. A study by Ter Haar et al. (2010) showed auditory impairment in middle aged or older dogs compared to young ones.

Nevertheless, little effort has been taken to account for dogs displaying perceptual impairments in case of online questionnaire studies implying to evaluate cognitive dysfunction. For example, in Salvin et al. (2010), the data of the 957 dogs used to develop (Salvin et al., 2010, 2011) and test (Salvin et al., 2011) the CCDR scale, included 290 dogs reported to suffer from deafness and 226 dogs from blindness. The authors concluded that dogs categorized as suffering from CCD have an almost threefold increase (Odds ratio=2.93 after correction for age) in the likelihood of being also blind (Salvin et al., 2010).

The considerably shorter lifespan of larger dogs (Galis et al., 2006) has been rarely taken into account. Demographic data suggests that large dogs do not simply suffer from a higher mortality in general (small breeds are expected to live about 10–14 years, some large breeds in contrast only live about 6–8 years), partly because they age faster (Kraus et al., 2013). This results in a decrease in the proportion of large and giant dogs in the oldest age groups in these studies, another possible confounding factor (Szabó et al., 2016). As the size of the dog is connected to differences in both the dog and owner characteristics (e.g. smaller dogs were reported to be more anxious in general and owners' of smaller dogs engaged less in training activities and play with their dogs) (Arhant, Bubna-Littitz, Bartels, Futschik, & Troxler, 2010), not controlling for this effect may influence the behaviours associated with cognitive decline.

In the current study we aimed to control for lifespan differences to ensure the presence of large dogs in our oldest age groups, with a formula provided by Greer et al. (2007).

This formula estimates the expected lifespan in years based on the height and weight of the subjects (see 0 and 0 for further details). We decided to calculate the expected lifespan for purebred dogs instead of using breed specific lifespan data available in the literature, because the reported values were suggested to underestimate population lifespan due to right censored data (Urfer, 2008) and we wanted to include mixed breed dogs, too. We divided the age of the subject with its expected lifespan to control for the fact that e.g. at 7 years a large dog is nearly at the end of its expected lifespan (100%), while a small dog of the same age is only at half of its expected lifespan (50%). This step was necessary to create a more balanced sample across the age groups of different sized dogs. Because large dogs die younger, smaller dogs are overrepresented among old dogs. However, we should note, that using the relative age might decrease the prevalence of cognitive decline in the geriatric cohort, as currently there is no evidence about cognitive decline in giant dogs (i.e. it is possible that they generally die before the onset of cognitive decline).

We decided to collect data across the entire adult lifespan of dogs, as we were interested in the baseline prevalence of the associated behaviours in a young population, and because laboratory beagle studies reported changes in cognitive performance as early as 6-9 years (Studzinski et al., 2006).

Our goal was (1) to evaluate the internal consistency of the most widely used domain specific scales (Golini et al., 2009) on a large sample of dogs, and (2) to investigate the impact of various factors (sensory deficits, training) on the occurrence of behavioural signs associated with cognitive decline, taking into account the differences in expected lifespan of small and large dogs.

7.3 Methods

Subjects

This study used 1343 online questionnaires from dog owners who volunteered to fill in the survey about dogs older than one year. For the descriptive data of the sample see Table *1*.

Item	Answers
Age	8.45 ± 4.5 years (mean ± SD) Range: 1-25 years
Age group	1-4 years: 342 (25.5%) 5-7 years: 265 (19.7%) 8-10 years: 264 (19.7%)) 11-13 years: 265 (19.7%) 14+ years: 206 (15.4%)
Relative age	0.69 ± 0.36 (mean ± SD) range: 0.07-2.77
Relative age group (percentage of lifespan)	Junior (≤25 %): 157 (13.0 %) Adult (25 % - ≤50 %): 268 (22.2%) Mature (50 % - ≤75 %): 252 (20.9%) Senior (75 % - ≤100 %): 245 (20.3%) Geriatric (100 %≤): 284 (23.5%) 137 missing data
Breed	Purebreds: 927(1-53 individuals from 122 breeds) Mixed breeds: 416
Sex	Intact male: 452 (33.7%) Intact female: 284 (21.1%) Neutered male: 192 (14.3%) Neutered female: 415 (30.9%)
Weight	Small (below 12 kg): 429 (34.8 %) Medium (between 12 and 30 kg): 478 (38.8 %) Large (over 30 kg): 324 (26.3 %) Missing data: 112
Height	Small (below 35 cm): 386 (32 %) Medium (between 35 and 43 cm): 218 (18.1 %)

	Large 602 (over 43 cm): (49.9 %) Missing data: 137
Hearing impairment	No: 800 (60.0%) Probably no: 204 (15.3%) Probably yes: 143 (10.7%) Yes: 186 (14.0%) Missing data: 10
Visual impairment	No: 768 (57.8%) Probably no: 218 (16.4%) Probably yes: 161 (12.1%) Yes: 182 (13.7%) Missing data: 14
Olfactory impairment	No: 968 (72.8%) Probably no: 263 (19.8%) Probably yes: 84 (6.3%) Yes: 15 (1.1%) Missing data: 13
Did the dog receive any kind of training certification, or entered any kind of competition?	No: 746 (74.4%) Yes: 257 (25.6%) Missing data: 340
Where does the dog spend most of the time?	House/Flat: 724 (72.2%) Garden: 259 (25.8%) Kennel: 20 (2.0%) Missing data: 340

Table 1. Descriptive statistics of the sample. Size data was calculated from AKC breed standards in the case of purebred dogs and from individual data in mixed breed dogs.

Calculating relative lifespan

To control for the shorter lifespan of larger dogs, we utilized relative age in our analysis (chronological age in years divided by the expected lifespan in years). Based on the equation provided by Greer et al. (2007), we calculated the individual's expected lifespan from weight and height data (see 4.2 for the formula). Regarding individual's weight and height data, in the case of purebred dogs, we relied on the American Kennel Club (AKC) breed standards (the mean values calculated from the limits of the breed standards). In the case of mixed breed dogs, due to the huge variance in their size, we decided to calculate lifespan from the individuals' height and weight data provided by the owners in the questionnaire. Mixed breed dogs without weight and height data were excluded from further analysis.

Based on the relative age of the dog, we decided to allocate dogs to age groups as previous research has shown that multiple behavioural traits display a quadratic relationship with age in dogs (Lisa J Wallis et al., 2014). We grouped the dogs into five age groups according to the American Veterinary Medical Association (AAHA) Canine Life Stage Guidelines (Bartges et al., 2012):

1) junior (relative age up to 25 % of the expected lifespan),
2) adult (relative age between 25-50%),
3) mature (relative age between 50-75%),
4) senior (relative age between 75-100%)
5) geriatric (individuals which have already outlived their expected lifespan).

Based on the relative age calculation and grouping, an 8-year-old Bichon Bolognese would be categorized as mature, while a Great Dane of the same age would be categorized as senior. With this approximation, our aim was to compensate for the difference seen between large and small dogs in their expected lifespans and create categories that correspond better to the life stages than chronological age itself, therefore make comparisons and generalisations among dogs of different sizes possible.

Online survey

The online Hungarian survey contained questions about the dog's demographic factors and about the dog's current behaviour based on a questionnaire by Golini et al. (2009), which contained 31 behaviour related questions (see
Table 2). We decided to select the questionnaire that covered the widest range of domains and questions available, and which has been validated by independent behavioural or neural measures (neurologic evaluation by a veterinarian). Regarding sensory impairments, owners indicated on a four-level scale whether they were aware of a suspected sensory decline in the given domain (no, probably no, probably yes, yes). Owners were offered the option to select: 'I don't know/ I don't want to answer this question' to avoid forced answers. The latter answer resulted in exclusion from further analysis.

Statistical analysis

Statistical analysis was carried out via SPSS v 22. The internal consistency within the domain scales was investigated by Cronbach's alpha. We used Spearman' rho for examining correlations between relative age and the 31 questionnaire item scores. After evaluating the internal consistencies of the domains, we decided to use a short data driven general scale instead of these domain specific scales. To retain questions from as many domains as possible, we selected within each domain the item that correlated positively with relative age with the highest rho, as age is the greatest known risk factor for cognitive decline. We calculated the mean score of these items and labelled the scale as Age-Related Changes (ARC). A univariate General Linear Model (GLM) with a backward elimination process was used for testing the effect of the explanatory variables on the Age-Related Changes scale. The main effects for all variables included: relative age group, sex & neuter status, height (small, medium, large), weight (small, medium, large), hearing impairment, visual impairment, olfactory impairment, training history (yes/no), keeping condition (house, garden, and kennel) and 2-way interactions with the relative age groups. Significant effects were tested with Student-Newman-Keuls (SNK) post hoc test to examine differences in group means.

7.4 Results

Regarding the behavioural signs of cognitive decline, first we calculated the internal consistency of the eight domains from Golini et al. (2009, Table 2). Only 'Spatial orientation', 'Housetraining', and 'Learning and memory' domains had appropriate internal consistency based on our Hungarian population's responses (CA=0.828-0.877), the Ca of other domains were below 0.7 (0.518-0.668). Because of the discouraging results we decided to develop a short age-related behavioural decline scale (Age-Related Changes, ARC) with a more satisfying CA. We have chosen 1 item from each original scale (Table 2), the ones with the highest correlation with the relative age of the dogs. In case of the original “Anxiety” domain, only two items correlated with age (‘Is your dog recently showing increased irritability?’ and ‘Is your dog restless/agitated?’), but the absolute rho values were small (Spearman’s rho=0.078 and -0.084 respectively), therefore we decided to exclude the “Anxiety” domain from the shortened scale assessing age-related cognitive decline. The CA for the final seven items (A4, B2, C2, D1, F2, G1.5, G2.1 see Table 2) was 0.849, and the ARC scale scores ranged from 0 to 4 (mean ± SD=0.836 ± 0.830).According to the GLM, after the backward elimination process, the following explanatory variables had a significant effect on the ARC scale: relative age group*training history (F(4,785) =3.41, p 0.009), relative age group (F(4,785) =10.72, $p < 0.001$), hearing impairment (F(3,785) =23.74, $p < 0.001$), visual impairment (F(3,785) =10.79, $p < 0.001$), and olfactory impairment (F(3,785) =7.00, $p < 0.001$), partial eta squared ranged between 0.083 and 0.017. Regarding visual and hearing impairment, all severity categories differed from each other in regard the ARC scores (Figure 2). Dogs with intact sensory function scored lower on the Age-Related Changes scale, with a steady increase in score toward replies indicating more severe impairments (owners who were certain their dog is suffering from loss of sensory function). In the case of olfactory impairment, sensory intact individuals had lower Age-Related Changes scores than dogs suffering from sensory impairment and showed a gradual decline in cognition toward certain impairment, with no difference between the probably and certainly impaired groups. The largest effect size (Partial Eta 0.083) was related to acoustic impairments, exceeding the effect size of relative age (0.052).

Domains, items	**Spearman's rho**
A: CONFUSION, AWARENESS, SPATIAL ORIENTATION	
A1: Does your dog get lost in familiar locations? *	0.301
A2: Does dog goes to the wrong side of door (e.g., hinge side)? *	0.308
A3: Does your dog get stuck, cannot navigate around or over obstacles? *	0.249
A4: Is your dog barely reacting/unresponsive toward stimuli? *	**0.426**
B: RELATIONSHIPS, SOCIAL BEHAVIOUR	
B1: Is your dog recently showing decreased interest in petting/contact? #	0.223
B2: Is your dog recently showing decreased greeting behaviour when you arrive home? #	**0.438**
B3: Is your dog experiencing alterations/problems with social hierarchy? #	0.144
B4: Is your dog in need of constant contact, over dependent, clingy? #	0.201
C: ACTIVITY: INCREASED/REPETITIVE	
C1: Does your dog stare/fixate/snap at objects? *	0.183
C2: Does your dog pace/wander aimlessly? *	**0.303**
C3: Does your dog excessively lick you or household objects? *	-0.169
C4: Is your dog vocalizing a lot/excessively? *	-0.061^
C5: Has your dog's appetite increased recently? (eating too fast, would like to eat more) #	0.228
D: ACTIVITY: DECREASED/APATHY	
D1: Is your dog showing decreased exploration/activity/apathy? *	**0.464**
D3: Is your dog showing decreased self-care? *	0.362
D4: Has your dog's appetite decreased recently? #	0.244
E: ANXIETY: INCREASED IRRITABILITY	
E1: Is your dog restless/agitated? *	-0.084^
E2: Is your dog anxious when it cannot be with you? *	-0.05 NS
E3: Is your dog recently showing increased irritability? *	0.078
F: SLEEP–WAKE CYCLES: REVERSED DAY/NIGHT SCHEDULE	
F1: Is your dog recently experiencing restless sleep/waking at nights? *	0.223
F2: Is your dog recently sleeping more than usual during daytime? *	**0.587**
G1: LEARNING AND MEMORY: HOUSETRAINING	
G1.1: Does your dog eliminate indoors at random sites or in view of owners? *	0.255
G1.2: Does it happen that your dog does not or barely signals that it needs to go out? *	0.230
G1.3: Does your dog go outdoors, then returns indoors and eliminates? *	0.203
G1.4: Does your dog eliminate in its crate or sleeping area? *	0.260
G1.5: Is your dog suffering from incontinence? *	**0.290**
G2: LEARNING AND MEMORY: WORK, TASKS, COMMANDS	
G2.1: Is your dog showing impaired working ability/performs worse than it used to? *	**0.417**
G2.2: Does your dog have difficulties with or is not able to recognise familiar people/pets? *	0.270
G2.3 Is your dog less responsive to known commands and tricks? *	0.323
G2.4: Is your dog having difficulties with/is unable to carry out tasks/commands in general? *	0.348
G2.5: Is your dog slow/unable to learn new tasks? *	0.360

Table 2. List of the behaviour related items from Golini et al (2009). Two types of closed answers were utilized, when a "" is indicated at the end of the question: Never, rarely, sometimes, often, very often; and a "#": 1(strongly disagree) 2 3 4 5 (strongly agree). Owners were offered the option to choose 'Do not know/I do not want to answer this question' for every question. D2 was the repetition of A4, so we left it out. Items retained for the final Age-Relates Changes scale are highlighted*

with bold. Spearman' rho is presented to examine correlations between relative age and questionnaire item scores. The p values for Spearman's Rho were <0.001, unless indicated otherwise: NS p>0.05; ^p>0.01.

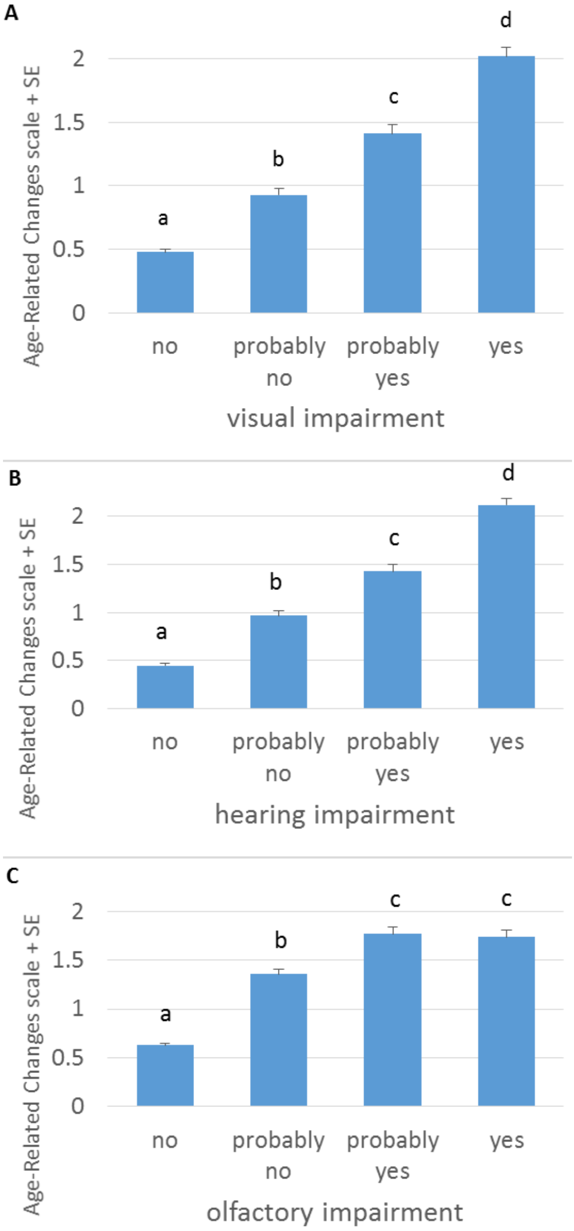

Figure 2. Mean and SE of scores of the Age-Related Changes scale (ARC) by sensory impairment categories as reported by the owner. A, visual impairment B, hearing imnpairment C, olfactory impairment. Different letters mark significant differences (p<0.05) between the categories based on the SNK post hoc test.

Mature dogs (50-75% of expected lifespan) were already reported by their owners to experience significantly more signs of cognitive decline than young dogs (≤25% of expected lifespan) and older relative age groups showed progressively more signs (Figure 3).

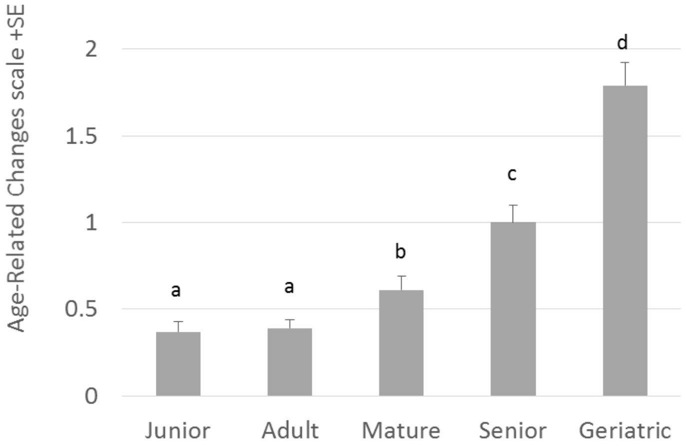

Figure 3. Mean and SE of scores of the Age-Related Changes scale (ARC) for the relative age groups. Different letters mark significant group differences (p<0.05) between the groups based on SNK post hoc test.

In 'Senior' and 'Geriatric' age groups (over 75 % of expected lifespan), lower scores were reported in the case of trained dogs, while no such difference was present among younger dogs of differing training status (Figure 4). Sex, neuter status and the size of the dog had no significant effect on the scores of the scale assessing age-related cognitive decline.

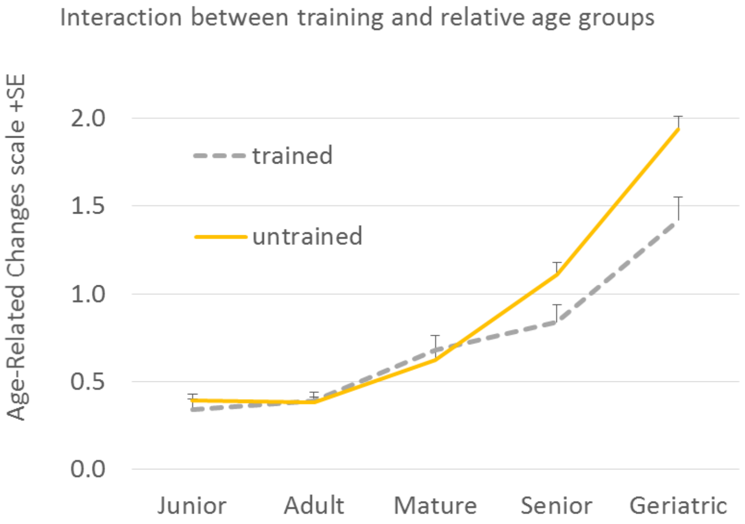

Figure 4. Mean and SE of scores of the Age-Related Changes scale for the relative age group-training interaction.

7.5 Discussion

In this study, our goal was to emphasize the relationship between the behavioural signs of cognitive decline and relative age, sensory impairments and certain demographic factors (sex, breed, training) across the whole adult lifespan in dogs. Several questionnaires exist for assessing cognitive decline, but their internal consistency have not been reported before. We tested the internal consistency of the domains of the most widely used questionnaire for grading dogs according to the severity of cognitive dysfunction (Golini et al., 2009). We found that five out its eight scales have not shown reliable internal consistency on a Hungarian sample. This warrants caution when the criteria of grouping are based on the number of signs shown within a domain.

The result prompted us to create a short, seven-items scale with high internal consistency assessing cognitive decline based on the items' positive correlation with relative age based on the items of Golini et al. (2009). Because we have not found correlation between anxiety and relative age, our resulting scale has not consisted of items assessing anxiety. The lack of correlation between anxiety and (relative) age supports the findings of Madari et al. (2015), who also found that anxiety shows a low level of predictive value for cognitive dysfunction.

As expected, we found sensory problems to be associated with a higher number of problematic behaviours regardless of relative age groups. Wayne and Johnsrude (2015) proposed a direct link between sensory systems and cognition, but the nature of this relationship (whether one precedes the other and whether there is a common underlying cause responsible for both sensory and cognitive decline) requires further experimental studies. An alternative explanation for this relationship could be that the behavioural signs of sensory impairment overlap with the signs of cognitive decline (e.g. a blind dog obviously shows signs of spatial disorientation). However, this is unlikely, given the fact that every sensory domain showed this effect, even olfaction, and that slight uncertainty regarding the complete health of the sensory organs was already associated with higher scores on the Age-Related Changes scale. Another possibility is that the dogs reported to suffer from sensory impairments were simply unreactive toward stimuli. While we cannot exclude this without a veterinary exam, the fact that owners who were simply uncertain whether their dogs suffer from sensory impairment already reported more signs of cognitive decline, supports that even the narrowing of perceptual skills, and not only the total loss of sensory function can affect the welfare and behaviour of geriatric dogs. This highlights the need for screening and detection of the early stages of sensory impairment in veterinary praxis with adequate and feasible tests.

Results have shown that sensory impairments and training history were associated with age-related behavioural problems reflecting cognitive decline in a convenience sample of family dogs. We cannot exclude (as we have conducted an online survey, similarly to e.g. Salvin et al., 2010), that the reported behaviours are related to medical conditions other than cognitive dysfunction syndrome, but we did not aim at identifying causative factors. Wayne and Johnsrude (2015) proposed a direct link between sensory systems and cognition, but whether there is a common underlying cause responsible for both sensory and cognitive decline is not known yet. It is also possible that the behavioural signs of sensory impairment overlap with the signs of cognitive decline (e.g. a blind dog certainly shows spatial disorientation). However, dogs with poor olfaction also showed prevalent age-related behavioural problems. Another possibility is that impaired dogs are unreactive toward stimuli and this is the reason why owners experience signs of cognitive decline. While we cannot exclude this without a veterinary exam, the fact that even uncertainty in the perceptual skills can affect the welfare and behaviour of geriatric dogs highlights the need for screening for the early stages of sensory impairment in veterinary praxis.

Interestingly, after controlling for differences in expected lifespan, Age-Related Changes scores were independent of the size of the dog, i.e. despite their higher chronological age, relatively old small dogs did not receive higher scores than relatively old large dogs. This suggests that cognitive decline may start earlier in larger dogs, but this issue requires further investigations on a larger sample.

The behavioural signs of cognitive decline reported by the owners started to become more prevalent already at 50-75% of the expected lifespan (which corresponds roughly to a 4-6 years old Great Dane or 7-10 years old beagle) and was followed by an accelerating decline in the older age groups. Studzinski et al. (2006) also detected signs of cognitive decline in beagles at the age of 6-8 years using a complex learning task.

Importantly, participating in formal dog training or competition in dog sports seems to delay cognitive decline among the old dogs according to our results. Among the ‘Senior’ and ‘Geriatric’ groups (above 75% of lifespan), trained dogs received significantly lower Age-Related Changes scores. Training history was also found to be a protective factor in a sustained attention task among aged dogs (Chapagain et al., 2017). Education level seems to have a similar positive effect on the retention of cognitive capacities in humans (Caamaño-Isorna, Corral, Montes-Martínez, & Takkouche, 2006). Since formal dog training has been reported to increase problem solving ability in young dogs (Marshall-Pescini, Valsecchi, Petak, Accorsi, & Previde, 2008), an alternative explanation could be that training, independently from age is associated with better performance. However, this is unlikely to be the case here, because in our sample there was no difference between trained and untrained young dogs, the difference only emerged with advancing age. Whether this is due to a cognitive reserve (Whalley, Deary, Appleton, & Starr, 2004), i.e. trained dogs have better cognitive capacities, therefore signs of cognitive decline become noticeable later in life, or if engagement in training modifies the aging trajectory remains to be uncovered. In future studies, it would be important also to investigate the role of other possible demographic reasons in this effect (e.g. owners who pay attention to the training of their dogs provide better overall care. Additionally, whether this positive effect is observable when training/enrichment is started in old age (e.g. Milgram et al. (2005) but see Davis et al. (2017)), or it is rather related to cognitive reserve acquired at a young age as suggested in humans (Lenehan, Summers, Saunders, Summers, & Vickers, 2015), requires further, more detailed studies (Kyathanahally et al., 2015).

In sum, putative signs of cognitive decline were already detectable in mature dogs (at 50-75% of the expected lifespan), which suggests that the processes resulting in cognitive dysfunction syndrome in later years start to operate before the onset of old age. This information needs to be considered when deciding on the most effective timepoint to intervene with possible therapies. Dogs suspected by their owners to suffer from sensory impairment scored higher on the Age-Related Changes scale, whereas participation in formal dog training activities was associated with delayed cognitive decline in old age. We successfully applied relative age to account for lifespan differences between small and large dogs, relying on expected lifespan calculated from the weight and size of the dogs. Based on our results, it is crucial to collect information about the sensory functions of aged dogs when evaluating cognitive decline with online questionnaires or behavioural tests.

8 Demographics of companion dogs across age groups and identifying the key variables associated with health status[4]

8.1 Abstract

Although dogs' life expectancies are six to twelve times shorter than that of humans, the demographics (e.g. living conditions) of dogs can still change considerably with aging, similarly to humans. Despite the fact that the dog is a particularly good model for human healthspan, as they generally have shorter healthspans than lifespans, there has been few previous attempts to describe demographic changes statistically. We utilized an on-line questionnaire to examine the link between the age and health of the dog, and owner and dog demographics in a cross-sectional Hungarian sample. Results from univariate analyses revealed that 20 of the 27 demographic variables measured differed significantly between six dog age groups. Our results revealed that pure breed dogs suffered from health problems at a younger age, and may die at an earlier age than mixed breeds. The oldest dog group (>12 years) consisted of fewer pure breeds than mixed breeds and the mixed breeds sample was on average older than the pure breed sample. Old dogs were classified more frequently as unhealthy, less often had a "normal" body condition score, and more often received medication and supplements. They were also more often male, neutered, suffered health problems (such as sensory, joint and/or tooth problems), received less activity/interaction/training with the owner, and were more likely to have experienced one or more traumatic events. Surprisingly, the youngest age group contained more pure breeds, were more often fed raw meat, and had owners aged under 29 years, reflecting new trends among younger owners. The high prevalence of dogs that had experienced one or more traumatic events in their lifetime (over 40% of the sample), indicates that welfare and health could be improved by informing owners of the greatest risk factors of trauma, and providing interventions to reduce their impact. Experiencing multiple life events such as spending time in a shelter, changing owners, traumatic injury/prolonged disease/surgery, getting lost, and changes in family structure increased the likelihood that owners reported that their dogs currently show behavioral signs that they attribute to the previous trauma.

8.2 Introduction

Dogs go through similar stages of development as humans, and their living conditions and nutritional requirements can change considerably as they age. However, many owners do not consider their dog's life stage when selecting a diet, feed inappropriate foods, and are unable to recognize if their pets are overweight or obese (M. Davies, 2012; Holmes, Morris, Abdulla, Hackett, & Rawlings, 2007). Therefore, e.g. in the UK, up to 60% of dogs are now classified as overweight or obese (Courcier, Thomson, Mellor, & Yam, 2010; Holmes et al., 2007; YouGov, 2017), mirroring the rise in obesity in humans (Wang, McPherson, Marsh,

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[4] Based on: Wallis, L. J., Szabó, D., Erdélyi-Belle, B., & Kubinyi, E. (2018). Demographic change across the lifespan of pet dogs and their impact on health status. *Frontiers in Veterinary Science, 5,* 200.

Gortmaker, & Brown, 2011). Obesity leads to a reduction in quality of life, shortened longevity and an increase in health issues (German, 2016). This suggests that dog owners still need to be better informed about the various aspects of dog keeping, which change as the dog ages and further studies are needed for examining what factors influence health status in pet dogs. In addition, the dog is a particularly good model species to examine healthspan, as like in humans, dogs generally have shorter healthspans than lifespans (Kaeberlein et al., 2016; Kennedy et al., 2014).

As discussed in Part I., several factors influence aging. Therefore, results from behavioural aging, longevity and health status surveys could be confounded by differences in dog and owner demographics. For example, our research group found that twelve out of 20 demographic and dog keeping factors differed between purebred and mixed-breed dogs (Turcsán, Miklósi, & Kubinyi, 2017). When we controlled for these differences, some of the previously found associations between the measured demographic and environmental factors and behavioural traits have changed, emphasizing the importance of taking into account dog and owner characteristics when examining behavioural traits in dogs (Turcsán et al., 2017). Consequently, examination into the differences in demographic and environmental factors between different dog age groups would be highly desirable, and could help to emphasize which factors are particularly relevant for aging research, related to changes in behaviour, cognition, longevity and healthspan.

The influence of environmental factors on aging and healthspan remains poorly understood, apart from the obvious culprits, smoking and obesity. Recent research has demonstrated that dogs living in smoking homes are more likely to suffer from DNA damage and show signs of premature aging than those living in non-smoking homes (Hutchinson, 2017). Previous studies have estimated that between 20 and 40% of the pet dog population are classified as obese, and these dogs have elevated levels of inflammatory markers (TNF-alpha and C-reactive protein) (German et al., 2009). Obesity can have detrimental effects on health and longevity; dogs which are overweight are at risk of developing other diseases such as diabetes mellitus, osteoarthritis and urinary incontinence, as well as altered respiratory function (German, 2016), and as such obesity is now considered the biggest health and welfare issue affecting pet dogs today. Lifestyle and dietary factors, breed predispositions, underlying diseases, neutering, and aging all may contribute to the development of obesity in dogs (German, 2016; Robertson, 2003).

There is evidence that chronic stress can have negative effects on health and lifespan in the domestic dog (Dreschel, 2010). Previous studies utilizing owner questionnaires have found that the environment, in which the dog is kept, as well as the management choices of the owner (such as how much time they spend with the dog), can vary significantly with the age of the dog, and can also influence healthspan and wellbeing. For example, Bennett & Rohlf, (2007), established that the owner’s perception of their dog’s behavior is related to the degree to which the dog is included in its owner’s activities, and suggested that the dog – owner relationship may be mediated by participation in shared activities such as hugging, taking the dog in the car, grooming, buying/giving treats, and playing games. As dog age increased, a decrease in shared activities was found, which resulted in reductions in the quality of the dog – owner relationship. Utilizing a different questionnaire, Marinelli, Adamelli, Normando, & Bono, (2007) found dog age and length of the dog-owner relationship negatively influenced quality of life, physical condition and care of the dog. Older dogs received less medical assistance, which may indicate a failure in the dog – owner relationship, and/or that owners are not well informed about geriatric dog care.

The aim of this study was to investigate the relationship of dog age on dog and owner demographics in a cross-sectional sample, and additionally, to identify other key variables associated with health status in dogs. Since this study was exploratory, we included a total of 27 dog and owner demographic factors in our analysis.

8.3 Methods

Subjects

Hungarian dog owners were invited to fill out an online questionnaire, which was advertised on the Eötvös Loránd University Department of Ethology's homepage (http://kutyaetologia.elte.hu), and on the Facebook group "Családi Kutya Program". The questionnaire was available from the middle of May to the beginning of July 2016. Dogs aged under 1 year were excluded as previous research has suggested that their behavior does not remain stable over time (Riemer, Müller, Virányi, Huber, & Range, 2014). Duplicate entries and entries with missing information were deleted, which resulted in data from a total of 1207 individual dogs. The full sample consisted of 66% pure breeds, 54% females, of which 17% were intact, and 37% were neutered (26% intact males and 20% neutered males). The most popular breeds (N=59-24, 7.5-3.0% of pure breed sample) included the Labrador retriever, Hungarian vizsla, Golden retriever, Yorkshire terrier, Dachshund, German shepherd, Bichon Havanese, Border collie, Beagle, and West highland white terrier. (As a reference, according to the HVG (Heti Világgazdaság) a Hungarian weekly economic and political magazine, in 2017 the top 10 dog breeds in Hungary included the German shepherd, French bulldog, English bulldog, Yorkshire terrier, vizsla, Dachshund, American Staffordshire terrier, Chihuahua, Boxer, and Golden retriever (HVG, 2018), indicating that owners of brachycephalic (short-headed) dogs were less inclined to participate in the study. The descriptive statistics of the sample are presented in Table 3 and Table 4.

Breed	**TOTAL count (%)**	**Sex N (%)**		**Age in months (mean ± SD)**	**Weight in kg (mean ± SD)**	**Height in cm (mean ± SD)**
		Male	**Female**			
Mixed breeds	417 (34.5)	192 (15.9)	225 (18.6)	97.50 ± 51.05	20.10 ± 11.02	43.41 ± 13.15
Pure breeds	790 (65.5)	365 (30.2)	425 (35.2)	89.8 ± 48.36	21.13 ± 13.88	43.56 ± 15.33
Chi Square test/unpaired *t* test		Chi-squared=0.003, p=0.958		*t*=2.58, df=1205, **p=0.010**	*t*=1.31, df=1205, p=0.190	*t*=0.18, df=1205, p=0.854
Grand Total	1207	557 (46.1)	650 (53.9)	92.46 ± 49.42	20.77 ± 12.97	43.51 ± 14.61

Table 3. Descriptive statistics of the subjects, including sex, age in months, weight, and height information displayed by breed group (pure breed and mixed/cross breed). A Chi-squared test was run to examine whether the proportion of males and females differed between the two breed groups, and unpaired t tests were conducted to look for mean group differences in age, weight, and height. Significant difference is highlighted in bold.

Title and description	**Categorical Variable labels:**	**N**	**%**
Age group	Group One: 1 - 3 years	185	15.3

	Group Two: >3 - 6 years	251	20.8
	Group Three: >6 - 8 years	191	15.8
	Group Four: >8 - 10 years	202	16.7
	Group Five: >10 - 12 years	170	14.1
	Group Six: >12 years	208	17.2
Neuter status	Intact	529	43.8
	Neutered	678	56.2
Sensory problems	None	980	81.2
	Vision and/or hearing	227	18.8
Off-leash activity: How long does your dog walk/run around outdoors without a leash on a typical day?	Less than 30 minutes	164	13.6
	30 minutes -1 hour	269	22.3
	>1-3 hours	367	30.4
	>3-7 hours	165	13.7
	More than 7 hours	242	20.0
Body condition score (BCS): What body shape does your dog have?	Thin (BCS 1-2)	203	16.8
	Normal (BCS 3)	784	65.0
	Over-weight (BCS 4-5)	220	18.2
Food: What food are you currently feeding your dog for its main meal?	Dry food only	267	22.1
	Tinned &/or dry food	147	12.2
	Cooked food	306	25.4
	Mixed	294	24.4
	Raw meat	193	16.0
Vitamins: Do you give your dog vitamins or supplements?	Almost never	328	27.2
	Rarely	391	32.4
	Often	244	20.2
	Regular (daily)	244	20.2
Trauma: Has the dog experienced a traumatic event, which could still have an effect on it?	No	694	57.5
	Yes	513	42.5
Health Problems: What kind of health problems does your dog have?	None	479	39.7
	Tooth problems only	182	15.1
	Joint problems + tooth problems	126	10.4
	Joint problems only	246	20.4
	Other disorders	174	14.4
Medication: Is your dog currently taking any medication?	No	1021	84.6
	Yes	186	15.4
Owner age	<29 years	385	31.9
	30-39 years	343	28.4
	40-49 years	253	21.0
	>50 years	226	18.7
Owner experience: How would you evaluate your experience with dogs?	Dogs are my hobby/profession and/or I am a dog trainer/breeder	307	25.4
	I have had a dog before	639	52.9
	I had never had a dog before	261	21.6
Other dogs in household: How many other dogs do you have living in your household? (Not including this one).	None	433	35.9
	One	474	39.3
	Two or more	300	24.9
People in household: How many people are living in the household?	One person (myself)	141	11.7
	Two people	503	41.7
	Three people	271	22.4
	Four or more people	292	24.2
	No	919	76.1

Child: Do you have a child/children living in your household?	Yes	288	23.9
Age of the dog when arrived: The age of the dog when it arrived in the Owner's household	Less than 7 weeks	265	22.0
	7-12 weeks	530	43.9
	3-12 month	198	16.4
	More than 1 year	214	17.7
Get dog: How did you get your dog?	I found it/got it from a shelter	340	28.2
	It was born at my place/ bought it	544	45.1
	I got it as a present	323	26.8
Where dog is kept: Where do you keep your dog?	Outside/inside house with garden	149	12.3
	In a fenced garden	384	31.8
	Urban/Suburban apartment	674	55.8
Dog obedience tasks: Which tasks can your dog reliably perform? (E.g., sit, lie down, come, fetch, stay, walk at heel, leave/drop it, watch me etc.). Open question.	Maximum a task	151	12.5
	2 kinds of tasks	169	14.0
	3 kinds of tasks	228	18.9
	4 or more kinds of tasks	659	54.6
Play: On an average day, how much time do you or other people spend together with your dog in different activities? (Play, walking, training)	Less than 30 minutes	122	10.1
	30 minutes -1 hours	378	31.3
	>1 -3 hours	551	45.7
	More than 3 hours	156	12.9
Commands: How many commands can your dog execute reliably?	< 10 commands	540	44.7
	11 – 30 commands	535	44.3
	> 30 commands	132	10.9
Dog training activities: How many activities are you currently doing with your dog?	One activity	385	31.9
	2 - 3 activities	527	43.7
	4 or more activities	295	24.4
Time spent alone: How much time does your dog spend alone on an average working day?	None	169	14.0
	1- 2 hours	276	22.9
	3- 8 hours	594	49.2
	More than 8 hours	168	13.9
Dog behaviour changed: Has your dog's behaviour changed over the last 3 months?	No	910	75.4
	Yes	297	24.6

Table 4. Description of categorical questions concerning the dogs and their owners (N=1207), and percentage breakdown of the groups.

Procedure

The full questionnaire can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2018.00200/full#supplementary-material.
In this study we focus on the demographic attributes of the dog and the owner. Three continuous variables were collected: the current weight (in kg) of the dog, height at the shoulder (in cm), and age (in months) (Table 3). The rest of the variables were categorical, and the main descriptive statistics of the subset of 1207 dogs and their owners are presented in Table 4. Dogs were divided into two breed groups; Mixed (including cross breeds) and Pure breeds. In addition to reporting the age in months of the dogs, we also allocated the dogs to six age groups, which would allow us to examine non-linear relationships with age. Each separate category of each variable contains at least 10% of the sample. In cases where fewer dogs were allocated, categories were collapsed. Unfortunately, owner gender was not possible to analyze, due to the

fact that only 109 male owners filled in the questionnaire, which made up only 9% of the sample. In addition, we were not able to examine individual breeds of dog, as none of the breeds in the sample exceeded 10% of the overall sample, or indeed, 10% of the pure breed sample.

Statistical analysis

In order to determine whether pure breed and mixed/cross bred) differed in sex, age, weight, and height a Chi squared test and Unpaired t tests were conducted.

To determine whether certain owner and dog demographics differ according to the age category of the dog, we ran univariate analyses (Kruskal Wallis tests (continuous variables) and Chi-squared tests (categorical variables)) on the demographic variables by dog age group.

In order to take into account multiple comparisons, we used the Benjamini–Hochberg procedure, which controls the false discovery rate (FDR, the expected proportion of false discoveries among all discoveries) and adjusts the p-values accordingly (Benjamini & Hochberg, 1995).

In order to examine the health status of the dogs, a new variable was produced. Dogs that were free from sensory problems and health problems such as allergies, teeth and joint problems, dysplasia, epilepsy, reproductive issues, heart failure, diabetes, thyroid problems, cancer and infections were defined as healthy (Waters, 2015) and were given the value “1”, and the rest received “0”. 39.4% of the sample were “healthy dogs”. This binary “health status” variable was used as the response variable in a Generalized linear model (with logit link function) that was performed in SPSS v. 22, to identify the key variables associated with health status. Weight and height were included as covariates, and the demographic variables as fixed factors. Due to the large number of predictors used in the model (26 demographic factors), we only tested for the 2-way interactions with age group: of breed (because we expected that mixed breeds would be even more healthier with age than pure breeds), weight, and body condition score (three factors that have been found to influence health), and the interaction between sex and neuter status, otherwise only the main effects were analyzed. Due to the large number of factors retained in the model, the Benjamini–Hochberg procedure was again utilized to control for the false discovery rate (FDR, Benjamini & Hochberg, 1995). The reference category used for age group was the oldest category (dogs aged>12 years), and for body condition score a normal body condition score (3) was used.

8.4 Results

Differences in owner and dog demographics in the dog age groups

We found numerous differences between the six age groups of the dogs in their demographic and dog keeping characteristics: 20 from the 27 comparisons were significant after correcting for multiple comparisons (Table 5). We list the specific characteristics of the oldest age group (> 12 years) below:

1) fewer pure breeds
2) fewer females
3) higher number of dogs with sensory problems
4) higher number of dogs that received less than 30 minutes of off-leash activity per day
5) fewer number of dogs with a normal body condition score

6) higher numbers of dogs on cooked food
7) higher number of dogs that received daily vitamins
8) higher number of dogs with previous trauma
9) higher number of dogs that had joint problems and/or tooth problems
10) higher number of dogs that were on medication
11) higher number of dogs with 2 or more other dogs in the household
12) higher number of dogs that lived outside or inside a house with a garden
13) higher number of dogs that can perform maximum one dog obedience task
14) higher number of dogs that received less than 30 minutes of play/activity with owner
15) higher number of dogs that knew fewer than 10 commands
16) higher number of dogs that participate in only one or no dog training activities currently
17) higher number of dogs whose behaviour had changed in the past three months
18) than would be expected by chance.

Deviations from chance and statistical results are displayed in Table 5.

Factor	**Category**	**Age group (years)**						
		1 – 3 N=185	**>3 - 6 N=251**	**>6 - 8 N=191**	**>8 - 10 N=202**	**>10 - 12 N=170**	**> 12 N=208**	**Statistics**
Height in cm (median±SD)		45 (15)	43 (15)	45 (14)	43 (15)	43 (14)	41 (14)	Kruskal-Wallis=11.370 P=0.055
Weight in kg (median±SD)		20 (13)	17 (13)	22 (14)	20 (13)	20 (12)	18 (11)	Kruskal-Wallis=4.666 P=0.515
Breed	Mixed	**28(35)a**	37(35)a	28(35)a	35(35)a	35(35)a	**42(35)a**	χ(5)=12.997 *P=0.031*
	Pure	**72(65)a**	63(65)a	72(65)a	65(65)a	65(65)a	**58(65)a**	
Sex	Female	58(54)ab	50(54)ab	59(54)b	56(54)ab	58(54)ab	**44(54)a**	χ(5)=14.612 *P=0.019*
	Male	42(46)ab	50(46)ab	41(46)b	44(46)ab	42(46)ab	**56(46)a**	
Neuter status	Intact	**56(44)a**	37(44)b	46(44)ab	44(44)ab	39(44)b	43(44)ab	χ(5)=18.550 *P=0.004*
	Neutered	**44(56)a**	63(56)b	54(56)ab	56(56)ab	61(56)b	57(56)ab	
Sensory problems	None	98(81)a	94(81)ab	96(81)a	86 (81)bc	78(81)c	**36(81)d**	χ(5)=375.994 *P <0.001*
	Hearing/vision	2(19)a	6(19)ab	4(19)a	14(19)bc	22(19)c	**64(19)d**	
Off-leash activity	less than 30 minutes	11(14)ab	14(14)ab	8(14)b	12(14)ab	15(14)ab	**21(14)a**	χ(20)=38.333 *P=0.014*
	30 minutes -1 hour	25(22)a	21(22)a	20(22)a	25(22)a	22(22)a	22(22)a	
	>1-3 hours	**22(30)a**	32(30)ab	32(30)ab	36(30)ab	32(30)ab	27(30)ab	
	>3-7 hours	18(14)a	14(14)a	13(14)a	12(14)a	12(14)a	13(14)a	
	more than 7 hours	25(20)ab	19(20)ab	**27(20)b**	14(20)ab	19(20)ab	17(20)ab	
Body condition score	1-2 (thin)	**24(17)a**	17(17)ab	15(17)ab	13(17)ab	11(17)b	21(17)ab	χ(10)=42.492 *P <0.001*
	3 (normal)	69(65)a	68(65)a	63(65)a	67(65)a	64(65)a	**58(65)a**	
	4-5 (overweight)	**5(18)a**	15(18)b	23(18)b	20(18)b	25(18)b	21(18)b	
Food	Dry food only	19(22)a	20(22)a	19(22)a	26(22)a	**31(22)a**	19(22)a	χ(20)=38.530 *P=0.014*
	Tinned & dry food	12(12)a	12(12)a	**8(12)a**	14(12)a	13(12)a	13(12)a	
	Cooked food	**21(25)a**	24(25)a	27(25)a	28(25)a	23(25)a	**29(25)a**	

	Mixed	25(24)a	24(24)a	**28(24)a**	**20(24)a**	25(24)a	25(24)a	
	Raw meat	**23(16)a**	21(16)ab	17(16)abc	11(16)bc	**9(16)c**	13(16)abc	
Vitamins	Almost never	26(27)ab	30(27)b	27(27)ab	32(27)b	29(27)ab	**18(27)a**	χ(15)=29.529 *P=0.021*
	Rarely	34(32)a	37(32)a	31(32)a	30(32)a	**26(32)a**	35(32)a	
	Often	20(20)a	20(20)a	22(20)a	16(20)a	**25(20)a**	19(20)a	
	Regularly (daily)	20(20)ab	13(20)b	20(20)ab	22(20)ab	20(20)ab	**28(20)a**	
Trauma	No	**72(57)a**	58(57)b	51(57)b	50(57)b	61(57)ab	54(57)b	χ(5)=24.260 *P <0.001*
	Yes	**28(43)a**	42(43)b	49(43)b	50(43)b	39(43)ab	46(43)b	
Health Problems	None	**74(40)a**	56(40)b	48(40)b	27(40)c	21(40)cd	11(40)d	χ(20)=372.200 *P <0.001*
	Tooth problems only	**2(15)a**	9(15)ab	13(15)bc	22(15)cd	26(15)d	20(15)cd	
	Joint & tooth problems	1(10)a	3(10)ab	5(10)ab	8(10)bc	17(10)cd	**29(10)d**	
	Joint problems only	16(20)a	17(20)a	17(20)a	24(20)a	22(20)a	**27(20)a**	
	other disorders	**8(14)a**	16(14)ab	17(14)ab	19(14)b	14(14)ab	13(14)ab	
Medication	No	95(85)a	90(85)ab	87(85)ab	84(85)b	84(85)b	**69(85)c**	χ(5)=60.620 *P <0.001*
	Yes	5(15)a	10(15)ab	13(15)ab	16(15)b	16(15)b	**31(15)c**	
Owner age	≤ 29 years	**49(32)a**	34(32)b	31(32)b	25(32)b	26(32)b	26(32)b	χ(15)=55.070 *P <0.001*
	30 – 39 years	**23(28)a**	30(28)a	28(28)a	33(28)a	25(28)a	30(28)a	
	40 – 49 years	**15(21)a**	24(21)a	24(21)a	21(21)a	22(21)a	20(21)a	
	> 50 years	13(19)ab	12(19)b	17(19)abc	21(19)abc	**27(19)c**	25(19)ac	
Owner experience	Hobby/profession	24(25)a	22(25)a	22(25)a	**30(25)a**	26(25)a	29(25)a	χ(10)=7.992 P=0.643
	Had a dog before	51(53)a	55(53)a	56(53)a	49(53)a	54(53)a	52(53)a	
	Never had a dog	25(22)a	23(22)a	22(22)a	21(22)a	20(22)a	19(22)a	
How many other dogs in household	0	**42(36)a**	40(36)a	37(36)a	30(36)a	35(36)a	32(36)a	χ(10)=16.675 P=0.101
	1	38(39)a	40(39)a	42(39)a	41(39)a	39(39)a	37(39)a	
	2 or more	21(25)a	20(25)a	21(25)a	29(25)a	26(25)a	**32(25)a**	
How many people in household	1	10(12)a	9(12)a	12(12)a	13(12)a	15(12)a	11(12)a	χ(15)=13.469 P=0.609
	2	43(42)a	47(42)a	42(42)a	35(42)a	38(42)a	43(42)a	
	3	21(22)a	21(22)a	24(22)a	27(22)a	20(22)a	22(22)a	
	4 or more people	26(24)a	23(24)a	21(24)a	25(24)a	26(24)a	24(24)a	
Child	No	75(76)a	80(76)a	76(76)a	73(76)a	75(76)a	76(76)a	χ(5)=3.373 P=0.643
	Yes	25(24)a	20(24)a	24(24)a	27(24)a	25(24)a	24(24)a	
Dog age when arrived	less than 7 weeks	21(22)a	21(22)a	21(22)a	23(22)a	24(22)a	23(22)a	χ(15)=37.476 *P=0.002*
	7-12 weeks	**56(44)a**	44(44)ab	48(44)ab	39(44)b	39(44)b	38(44)b	
	3-12 month	18(16)a	18(16)a	14(16)a	15(16)a	16(16)a	17(16)a	
	more than 1 year	**5(18)a**	16(18)b	17(18)b	23(18)b	21(18)b	23(18)ab	
Get dog	I found it/from shelter	22(28)a	31(28)a	21(28)a	33(28)a	29(28)a	32(28)a	χ(10)=23.066 *P=0.019*
	It was born at my place/ I bought it	**55(45)a**	46(45)ab	50(45)ab	40(45)b	39(45)b	40(45)ab	
	I got it as a present	23(27)a	23(27)a	29(27)a	28(27)a	32(27)a	27(27)a	

Where dog is kept	Outside/inside house with garden	8(12)a	10(12)ab	12(12)ab	15(12)ab	10(12)ab	**19(12)b**	χ(10)=24.942 *P=0.030*
	In a fenced garden	35(32)a	**25(32)a**	36(32)a	29(32)a	34(32)a	34(32)a	
	Suburban/urban apartment	58(56)ab	64(56)b	52(56)ab	56(56)ab	56(56)ab	**47(56)a**	
Dog obedience tasks	Maximum a task	10(13)a	7(13)a	12(13)a	10(13)a	12(13)a	**25(13)b**	χ(15)=59.777 *P <0.001*
	2 kind of tasks	13(14)ab	13(14)ab	**8(14)b**	14(14)ab	17(14)ab	19(14)a	
	3 kind of tasks	17(19)a	23(19)a	16(19)a	20(19)a	16(19)a	19(19)a	
	4 or more kinds of tasks	60(55)a	57(55)a	63(55)a	56(55)a	54(55)a	**38(55)b**	
Play	less than 30 minutes	2(10)a	5(10)ab	9(10)bc	12(10)bcd	15(10)cd	**19(10)d**	χ(15)=61.282 *P <0.001*
	30 minutes -1 hour	30(31)a	28(31)a	28(31)a	34(31)a	34(31)a	**35(31)a**	
	>1 -3 hours	52(46)ab	53(46)b	48(46)abc	45(46)abc	39(46)ac	**34(46)c**	
	more than 3 hours	16(13)a	13(13)a	15(13)a	**9(13)a**	13(13)a	12(13)a	
Commands	< 10 commands	47(45)ab	37(45)b	36(45)b	41(45)b	49(45)ab	**60(45)a**	χ(10)=38.757 *P <0.001*
	11 – 30 Commands	44(44)abc	53(44)c	52(44)bc	46(44)bc	38(44)ab	**31(44)a**	
	> 30 Commands	9(11)a	10(11)a	12(11)a	13(11)a	13(11)a	**9(11)a**	
Dog training activities	One activity	63(64)a	61(64)a	66(64)ab	60(64)bc	61(64)c	**75(64)d**	χ(10)=162.384 *P <0.001*
	2 - 3 activities	25(19)ab	20(19)b	15(19)b	22(19)ab	19(19)ab	**11(19)a**	
	4 or more activities	12(17)a	20(17)ab	19(17)bc	18(17)c	20(17)cd	**14(17)d**	
Time spent alone	None	16(14)a	14(14)a	14(14)a	15(14)a	14(14)a	12(14)a	χ(15)=20.849 P=0.167
	1- 2 hours	22(23)a	19(23)a	25(23)a	26(23)a	23(23)a	23(23)a	
	3- 8 hours	52(49)a	53(49)a	48(49)a	47(49)a	41(49)a	52(49)a	
	More than 8 hours	10(14)a	14(14)ab	14(14)ab	12(14)ab	**22(14)b**	13(14)ab	
Dog behavior changed	No	79(75)abc	88(75)c	83(75)bc	74(75)ab	69(75)ad	**56(75)d**	χ(5)=72.977 *P <0.001*
	Yes	21(25)abc	12(25)c	17(25)bc	26(25)ab	31(25)ad	**44(25)d**	

Table 5. The proportion of the dogs present in each category of the categorical variables (with the percentage expected by chance in brackets), presented separately for each dog age group. The height and weight variables alone display mean and standard deviation. Where significant group differences were found (indicated by Kruskal Wallis/Chi-squared tests), the category with the larger or smaller proportion than was expected by chance is marked in bold. P values were corrected with the Benjamini–Hochberg FDR procedure (significant corrected P values are marked in italics). Z tests were performed to compare column proportions (P values were adjusted for multiple comparison using the Bonferroni method according to the crosstabs procedure in SPSS). Each subscript letter denotes a subset of age categories whose column proportions do not differ significantly from each other at the .05 level.

Differences in owner and dog demographics in healthy and unhealthy dogs

In humans, age is the strongest predictor of healthspan, and our results confirm that this is true also in dogs (Wald Chi-squared=86.289, p<0.001; FDR p<0.001). As dogs aged, the odds of being healthy decreased. Young dogs (1-3 years old) were 22.8 times more likely to be allocated to the healthy group than dogs older than 12 years. Even the group of 10-12-year-old dogs was significantly healthier than older individuals. According to the estimated marginal means (after adjustment for the other variables in the model and differences in sample size), 49 percent of dogs aged 1 – 3 years old (N=185) were classified as healthy, but only 5% of dogs aged over 12 years (N=208) received this status.

Breed also effected health status. Mixed breed dogs that were classified as “healthy” were on average older than “healthy” pure breed dogs (unpaired t-test: t=2.404, df=474, P=0.017). Age distribution histograms for healthy dogs separated by breed group are shown on Figure 5.

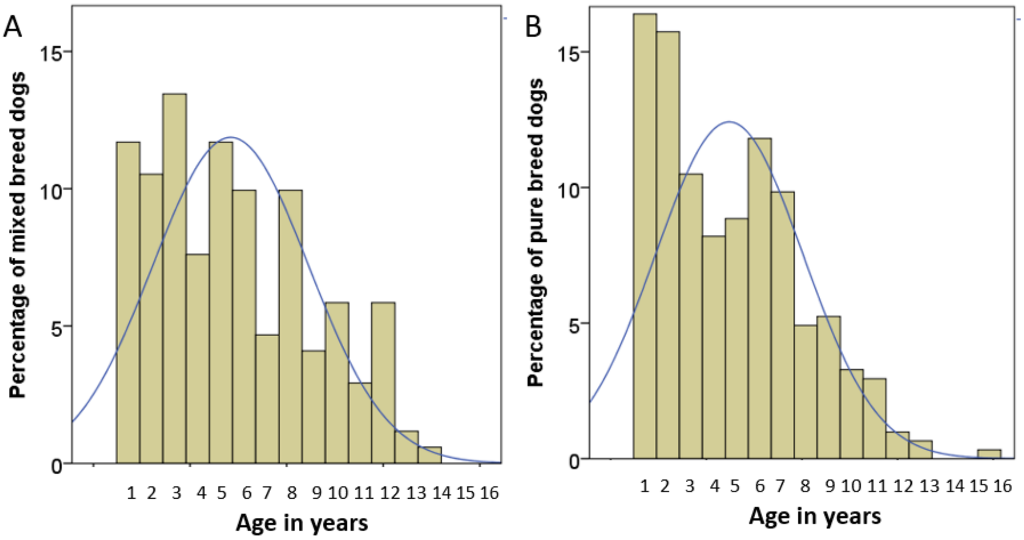

Figure 5. Distribution patterns for healthy dogs, showing the percentage that remained healthy within one-year age bands. A), mixed breed dogs B), purebred dogs.

Dogs that have experienced trauma during their lifetime were significantly more likely to be allocated to the unhealthy group (odds ratio=1.82, 12.078, p<0.001). The following factors have contributed to dogs’ trauma status significantly (odds ratio 3.87-1.38): spent time at a shelter, changed owner, traumatic injury/prolonged disease/surgery, lost for a short time, or change in the family structure (such as a death or a birth) (
Table 6). Three or more of these life events resulted in a significant increase in the likelihood that dogs were allocated to a “trauma” status by the current owner when compared to chance ($\chi(3)=162.672$, $p<0.001$).

Predictors		**df**	***Wald Chi-Square***	***P value***	**Odds ratio**	**Confidence interval**	**Estimated marginal means**
Spent time at a shelter:	Yes	1	85.844	**0.000**	3.87	2.90, 5.15	0.86
	No		-	**-**	-	-	0.61
Changed owner:	Yes	1	31.625	**0.000**	3.15	2.11, 4.69	0.85
	No		-	**-**	-	-	0.64
Trauma injury/disease /surgery:	Yes	1	22.464	**0.000**	2.42	1.68, 3.49	0.83
	No		-	**-**	-	-	0.67
Lost for a time:	Yes	1	5.196	**0.023**	2.19	1.12, 4.31	0.82
	No		-	**-**	-	-	0.68
Change family structure:	Yes	1	5.858	**0.016**	1.38	1.06, 1.80	0.79
	No		-	**-**	-	-	0.73

Table 6. Results of the binary generalized linear model with logit link function showing the direction and magnitude of effects (odds ratio and confidence interval), and the significance level of the terms in the life events associated with trauma status. iSignificant P values (in bold) indicate which group differs from the reference value in the respective analysis. (The reference value is set to the last category in the group, and is denoted by "-"). Spent more or less time alone, moved house, number of dogs changed, and pregnancy/mating were not significant at P <0.05 and were removed from the model.

8.5 Discussion

After controlling for dog and owner demographic variables, dogs that had sensory or health problems were found to be older, required medication and supplements, were more likely to have previously experienced a traumatic event, and be classified as "overweight" in body condition score at certain ages. Our results partially contradict our prediction that dogs that are heavier in weight are more likely to suffer from health problems. However, they also implicate a new factor, which appears to have an impact on health regardless of breed and age, that of experiencing one or more traumatic events at some point during the lifespan.

Many of the reported differences are not new to the literature, such as that the lifespan of pure breed dogs is lower compared to mixed-breed dogs across all weight categories (Leroy, Phocas, Hedan, Verrier, & Rognon, 2015; G. J. Patronek, Waters, & Glickman, 1997; Proschowsky, Rugbjerg, & Ersbøll, 2003). Sex differences in aging have also been examined. Some studies have reported a longer mean life span in females (David J Waters et al., 2009), and others in males, in harmony with our findings (Leroy et al., 2015; Moore, Burkman, Carter, & Peterson, 2001). Note however, that on larger sample size there is no differences in longevity between the sexes (Hoffman et al., 2018), thus our finding could be just a coincidence.

The well-known negative correlation between body size and life expectancy was shown only in a tendency for more short dogs to be represented in the aged dog population in comparison to young dogs (<12 years mean height at shoulders=41 cm, and aged 1 – 3=45 cm).

As dogs’ age, their chances of becoming obese or underweight increases, due to a decrease in metabolism and sarcopenia (the decline in skeletal muscle strength and mass) (Armstrong & Lund, 1996; Donoghue, Khoo, Glickman, & Kronfeld, 1991; Larsen & Farcas, 2014). We also found that that fewer dogs over 12 years of age had a normal body condition score.

Previous studies have shown that not only is the metabolism of aged dogs reduced, but their activity levels and mobility also decline even in the absence of any disease, and are thus a normal part of aging (Bellows et al., 2015). Therefore, the reductions in off-leash exercise, interactive play/activity with the owner, and dog training activities in aged dogs found in the current study, can be explained by a large reduction in the dogs’ activity levels, along with a higher occurrence of degenerative joint problems. Loss of muscle strength and function not only decreases mobility and quality of life, but also is related to numerous unfavorable health outcomes. Nutritional interventions combined with physical therapy to increase muscle mass and strength have been found to halt or even reverse sarcopenia in humans (Fielding, 1995; Harber et al., 2009), and therefore can increase longevity. However, to date we have found no studies that have examined pharmaceutical, nutritional, or exercise related interventions in dogs to prevent/holt the deleterious effects of sarcopenia (Freeman, 2012).

The fact that dog obedience tasks and the number of commands known by the dog were at their lowest levels in the oldest age group, indicates either that this age cohort received less training from their owners throughout their lives, or they gradually forgot the commands/tasks due to reduced/absent training in later life, or due to failing memory/advancing dementia. The prevalence of pathologic neurodegenerative cerebral changes and associated impairment of cognitive functions, increases in range from 14 – 35% in dogs more than 8 years of age (Schütt, Toft, & Berendt, 2015). Targeted programs promoting mental exercise and nutritional supplements may be used to delay progression once clinical signs have been presented (Araujo, Studzinski, Head, Cotman, & Milgram, 2005; Cory, 2013; Pop et al., 2010). Therefore, aged dogs could benefit greatly from increased training in old age, especially if they have reduced mobility, as these dogs have limited exposure to environmental enrichment (Wallis et al., 2017).

The results from the health status analysis generally confirmed our findings from the demographic age analysis that older dogs are less healthy and subsequently require medication and dietary supplementation. Our results collaborate previous research that indicates that age is the strongest predictor of health status regardless of breed, height and weight (Jin, Hoffman, Creevy, O’Neill, & Promislow, 2016), as it is in humans (Kennedy et al., 2014).

Results imply that pure breeds are more likely to suffer from health problems at a younger age. Our results corroborate previous studies that concluded that mixed breeds tend to have a longer healthspan than pure breeds (Bellumori, Famula, Bannasch, Belanger, & Oberbauer, 2013; Nicholas, Arnott, & McGreevy, 2016; O’Neill et al., 2013; N. Rooney & Sargan, 2009).

To the best of our knowledge, only one study has examined the impact of traumatic events on subsequent behavioral responses in dogs. Serpell & Duffy, (2016) reported that particularly frightening or traumatic events during the guide dog puppy-raising period (up to 6 months of age), were associated with specific behavioral outcomes at 12 months of age. Indicating that there are long-term negative consequences of traumatic experiences. Fear or anxiety disorder has also negative effects on health and lifespan. Specifically, dogs that were afraid of strangers had shortened lifespan and anxiety was associated with skin disorders (Dreschel, 2010) and premature greying in young dogs (King, Smith, Grandin, & Borchelt, 2016).

In humans, adverse childhood experiences (ACEs), result in increased mortality and decreased healthspan, through increased susceptibility to the development of diseases such as cardiovascular disease, cancer, chronic respiratory disease and diabetes, and increased risk of subsequent unintentional injury and violence (Bellis et al., 2015). Similarly to humans who suffered ACEs, preclinical studies on the effects of early stress in animals show altered neurological development, including reduced anticipatory reward response and impulse control (Dichter, Damiano, & Allen, 2012; Teicher & Samson, 2016; Teicher, Tomoda, & Andersen, 2006). Future cross-sectional and longitudinal studies should attempt to quantify and define the differing types of traumatic events experienced by dogs, and clarify whether experiencing trauma results in long-lasting personality changes, reductions in health, learning and memory, reduced anticipatory reward response and impulse control.

This cross-sectional questionnaire study had several limitations. Firstly, the sample size was relatively small for a large-scale demographic analysis, which limited our ability to detect significant associations after correction for multiple comparisons, and to examine interactions between the demographic variables. This was a hypothesis-generating study and therefore a larger confirmatory study is needed in the future. To better understand demographic changes with age group and their influence on health, future studies should examine individual breeds of different ages. Secondly, all of the health problems examined were treated equally, and the presence of any single health problem resulted in dogs receiving the status of “unhealthy”. Futures studies should determine the effect of demographics on specific health problems. Additionally, some morbidities are known to have more severe effects on physiology and health (such as diabetes). Therefore, studies that apply particular weights to health problems, examine chronic and temporary conditions and also multimorbidities, may uncover more associations with demographic variables. Thirdly, owners may under or over report the occurrence of health problems in their dogs (Wiseman-Orr, Scott, Reid, & Nolan, 2006), or incorrectly state their dog’s height/weight or classify their dogs body condition score (Holmes et al., 2007), which could potentially have confounded our results. Previous studies have indicated that owners’ consistently underestimate their dogs’ body condition score (Eastland-Jones, German, Holden, Biourge, & Pickavance, 2014; Yam, Naughton, Butowski, & Root, 2017), which could explain why we were only able to detect health associations with body condition score in some age groups. Note however, that owners in general seem to be reliable informants of demographic information (Sallander, Hedhammar, Rundgren, & Lindberg, 2001) and of dog behavioral questionnaires (Kubinyi, Gosling, & Miklósi, 2015). Fourthly, dog keeping practices, owners’ attitude and perceptions of their dogs, and their dogs behavior may vary around the world, therefore, further studies are necessary to determine whether cross-cultural differences exist (Wan et al., 2009). Traditionally, Hungarians viewed the dog as a working animal; however, more recently their opinion of dogs has changed, particularly in large cities and urban environments. The Hungarian owners who were motivated enough to fill in our extensive online questionnaire represent these changing attitudes, as in response to the question, “Why did you get your dog”, over 95% of the owners replied that they got their dog as a social partner or for companionship. Fifthly, due to time restraints we were not able to include questions regarding the owners’ education level, occupation and economic circumstances, all factors that are known to influence health in humans (Winkleby, Jatulis, Frank, & Fortmann, 1992). Future studies should focus more on owner demographic factors when examining health status in dogs. Finally, due to the correlational nature of the study design, it was not possible to determine the cause and effect of the various association found. Longitudinal studies are necessary to gather information to determine any protective and/or risk factors for successful aging to provide interventions to increase healthspan and longevity in pet dogs.

Despite the limitations, our results have shown that age and certain demographic factors are associated with health. Further research into these specific demographic variables could lead to the advancement of canine health management and keeping practices. The high prevalence of dogs that were affected by one or more traumatic life events indicate that welfare and health could be improved by informing owners of the greatest risk factors of trauma and promoting responsible dog ownership. Additionally, there is a need for greater transparency and clear guidelines for owners of dogs of different life stages.

9 Developing a behaviour test for assessing discrimination and reversal learning[5]

9.1 Abstract

Dogs are good models to study age-related cognitive decline, due to relevant neuro-biological and environmental similarities with humans. However, several tests involve laboratory dogs, large apparatus and prolonged training. We developed two discrimination learning and reversal learning tasks which could be performed in a single one hour session. We also compared dogs' performance when trained to discriminate stimuli based on their location or their physical characteristics. We tested 107 medium and large sized adult dogs, grouped according to age: "young dogs" (N=41, aged 2.5 – 6.5 years) and "old dogs" (N=66, aged 8 – 14.5 years). Our results indicated that, in the discrimination learning and in the reversal learning, young dogs learned significantly faster than the old dogs. In the reversal learning, the dogs trained based on the location of stimuli learned faster than the dogs trained based on stimulus characteristics. In the stimulus characteristics condition we observed a ceiling effect, as most old dogs did not learn the task in 50 trials. Therefore, we suggest that training based on objects' location is more informative for reversal learning tasks. Overall, these results suggest that our discrimination learning and reversal learning tasks could successfully be used to investigate differences in spatial function between young and old dogs.

9.2 Introduction

During discrimination learning dogs learn to respond differently to different stimuli. Reversal learning tasks rely on the dog's ability to differentiate and mentally adjust previously learned behaviours (Cotman & Head, 2008). Dogs are initially trained to associate one stimulus with a reward and another stimulus with no reward. Following a delay period, the dogs are presented with the same stimuli, though the discrimination is reversed. Therefore, the subject must inhibit the previously learned response to the rewarded object and learn a new association. Older dogs usually need longer time to learn the associations (Chan et al., 2002; Head, Callahan, Muggenburg, Cotman, & Milgram, 1998; Mongillo, Araujo, et al., 2013; Salvin, McGreevy, Sachdev, & Valenzuela, 2011; Studzinski et al., 2006). However, in some simple tasks old dogs performed as well as younger ones (Milgram et al., 1994), which may reflect differential susceptibility of various cognitive functions to aging (Head, 2013). Reversal learning is probably more sensitive than discrimination learning (Milgram et al., 1994). On the other hand, the disregard of sex or environmental factors may be an underlying cause of inconsistency in the aging research findings (Szabó et al., 2016). Testing large and diverse populations could answer these questions, but the training methods of the existing research protocols require large apparatus and several weeks, days or hours. For example, Mongillo et al. (2013) re-tested dogs 2 weeks after a maze-based learning task in order to detect an effect of age. This makes the procedure less feasible in veterinary clinical settings.

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[5] Based on: Piotti, P., Szabó, D., Bognár, Z., Egerer, A., Hulsbosch, P., Carson, R. S., & Kubinyi, E. (2018). Effect of age on discrimination learning, reversal learning, and cognitive bias in family dogs. *Learning & Behavior*, *46*, 537-553.

For studying the process of aging, re-testing is important. However, the reversal learning task re-testing is strongly influenced by the subjects' previous experience. Therefore, it is suggested to test the dogs with different stimuli (e.g. either the location or the physical characteristics of the stimuli varied), in order to explore whether these would affect the dogs' performance. Previous findings reported similar effects of age on location and size discrimination, i.e. young dogs performed better than old dogs in both tasks (Dwight Tapp, Siwak, Estrada, Head, et al., 2003; Dwight Tapp, Siwak, Estrada, Holowachuk, et al., 2003).

The aim of the present study was to design a simple and reproducible version of the reversal learning task, which did not require large apparatuses or several weeks of training. The performance of the old dogs was expected to be poorer (in terms of time required to learn and number of trained dogs) during the reversal learning task. We were also interested in developing tests that could be potentially repeated over time, e.g. to monitor the progress of the condition. Therefore, we tested the dogs in this study with different stimuli, in order to explore whether these would affect the dogs' performance. The two types of stimuli varied in their physical characteristics and relied on slightly different cognitive tasks and domains, as described by Cotman and Head (2008). For one group of dogs, the stimuli varied based on their location (Mendl et al., 2010); therefore, the tasks relied on egocentric spatial coding (i.e. the animal can rely on the representation of the objects in space relative to its own body axes, such as left-right and front-back), specifically relying on learning (spatial function) and reversal learning (executive function). For the other group of dogs, the stimuli varied based on their physical characteristics, such as colour and shape; therefore, the tasks relied on visual learning and reversal learning (executive function). Both tasks also relied on visual discrimination learning and reward and object approach learning (learning domain). Previous findings suggested similar effects of age on location and size discrimination (Tapp et al., 2003); therefore, we expected young dogs to perform better than old dogs in both tasks.

In this study we decided to investigate the normal aging process, i.e. the cognitive changes that occur in all individuals as they age, rather than more severe signs occurring in a smaller part of the population, therefore the subjects were screened based on symptoms of health issues associated with age. In fact, knowing the "normal" aging process is a prerequisite for the subsequent identification of pathological phenotypes (Szabó et al., 2016).

9.3 Methods

Subjects

Power analysis was performed using G*power in order to estimate the required sample size for the study, aiming for a medium effect size (*d*=.65) and a power of .08. The analysis yielded a recommended sample of N=35 per group (old and young). A group of old dogs (N_{old}=76) had already been recruited and tested, following the same procedure as the current work, for a different study (unpublished). Therefore, a matching sample of young dogs had been recruited following the same inclusion and exclusion criteria, although the sample size had been kept closer to that calculated *a priori* (N_{young}=43) in order to avoid inflating the risk of type I errors. For both groups, the inclusion criteria were for the dogs to be free from overt signs of cognitive decline (as reported by the owner) and/or medical problems, which might have affected the performance in the tasks (e.g. untreated neurological conditions, motor-sensory deficits). All dogs were assessed for their motor-sensory skills by a qualified veterinary surgeon or a physiotherapist, following on a previously defined procedure (Bognár et al., in review). For the young dogs group, dogs between 2 and 6.5 years were recruited; for the old dogs group, dogs had to be above 8 years of age. The age limits had been set based on previous findings about the age when the first signs of cognitive decline were detected (Studzinski et al., 2006; Szabó et al., 2016 for a review). The sample included 54 mix breeds and 69 pure breeds from 17 different types of breed. In order to reduce the confounding effect of dogs' size on aging speed (see Szabó et al., 2016), only dogs of medium to large size were included in the sample. Twelve dogs were tested but excluded from the analysis due to procedural mistakes during the data collection (N=11) or due to technical issues with the electronic spread sheet (N=1). The demographic characteristics of the final sample (N=107) are described in (Table 7).

Age cohorts	**N**	**Mdn Age [years] (min – max)**	**M : F (Neutered N)**	**Mdn Weight [Kg] (min – max)**	**Mdn Height [cm] (min – max)**
Young	41	4.5 (2.5-6.5)	1 : 1 (27)	20.2 (9.5 – 44.2)	50.00 (27.00 - 84.00)
Old	66	10.5 (8-14.5)	1 : 1 (53)	18.65 (5.9 – 78)	50.00 (35.00 - 70.00)

Table 7. Demographic information of the dogs included in the analysis.

Procedure

Overview: Dogs were trained to associate either the location or the physical characteristics of an object with the presence or absence of food. Different sets of stimuli were used to train the dogs to discriminate the stimuli based on their location ("location" group) or based on their physical characteristics, i.e. size, colour, and shape ("physical characteristics" group). For a successful learning dogs had to pass a threshold (the longest latency for going to the correct location was shorter than any latency for going to the wrong location within the five vs. five most recent trials). The learning tasks consisted of a maximum of 50 trials. The discrimination learning and the reversal learning lasted between 15 and 35 minutes each, depending on the dog.

Groups:

1) Location group: Dogs had to associate the presence (P) or absence (N) of food with the specific location where the stimulus was placed. The stimulus was a blue plate (plastic, round shape, 20 cm of diameter) and it was placed on the floor either on the left or the right hand side of the experimenter. For about half of the dogs in the location group, the positive location was on the left and the negative was on the right, while for the other half the opposite was true (for the exact numbers see Piotti et al. 2018).

2) Physical characteristics group: The stimuli were always placed on the floor right in front of the experimenter and the dog had to learn the association between presence (P) or absence of food (N) based on the shape and colour of the stimuli. The two plates used as P and N were a large, black, rectangular plate (plastic, 23 x 15.5 cm) and a small, white, round plate (plastic, 12 cm).

Pre-test: All dogs had been pre-tested to ensure that they could reliably find a small object placed on the floor – this was done to ensure that their visual abilities were sufficient for them to perform successfully in the current test. Additionally, in all cases, from their starting position the dog could not see the food inside of the plate as we were interested for the dogs to decide whether to approach a stimulus or not based on the outcome associated with it. Finally, previous findings indicate that dogs do not rely on smell in such settings (Szetei, Miklósi, Topál, & Csányi, 2003); nevertheless, all plates were smeared with food prior to testing in order to control for odour cues. A thin slice of sausage or a piece of cheese of equal size was used as food reward in all conditions (one dog was on a medical diet, so was tested using pieces of food from their own diet); the dog owners were asked about the dog's preference and dietary requirements.

Set-up: The study was performed at the Department of Ethology, Eötvös Loránd University (Budapest, Hungary), in a small room (2.8 x 5 m). Only the dog's owner, the dog, and the experimenter were in the room. The room was empty beside a chair placed at one end of the room (for the owner). The owner held the dog's collar in order to keep the dog in front of the chair (on a spot marked with tape on the floor). The experimenter was standing approximately 3 meters in front of the dog-owner dyad. Two cameras were mounted on the ceiling, one behind the experimenter and the other behind the dog-owner dyad (Figure 6).

For the "location" group, the plates were placed towards the experiment's left or right-hand side and at arm's length distance from her. For the "physical characteristics" group, the plates were always placed in front of the experimenter.

Detailed acquisition procedure: The aim of the learning phases was to teach the dogs that one stimulus was associated with food (P=positive) and the opposite stimulus was associated with no food (N=negative). This was achieved by presenting the dogs with the two stimuli in consecutive trials; the stimuli were presented in a pre-determined pseudo-random order, with no more than two trials of the same type being presented consecutively.

At the beginning of each trial, the owner was asked to put the dog on the leash while the experimenter baited (or did not bait, depending on the trial valence, N or P) one of the plates. The experimenter then put the plate on the floor (based on the group type and the trial valence, and according to the pseudo-random order), and in front of her: for the location group this was placed either towards the left-hand side or the right-hand side of the dog; for the location the plate corresponding to that trial valence was placed right in front of the experimenter.

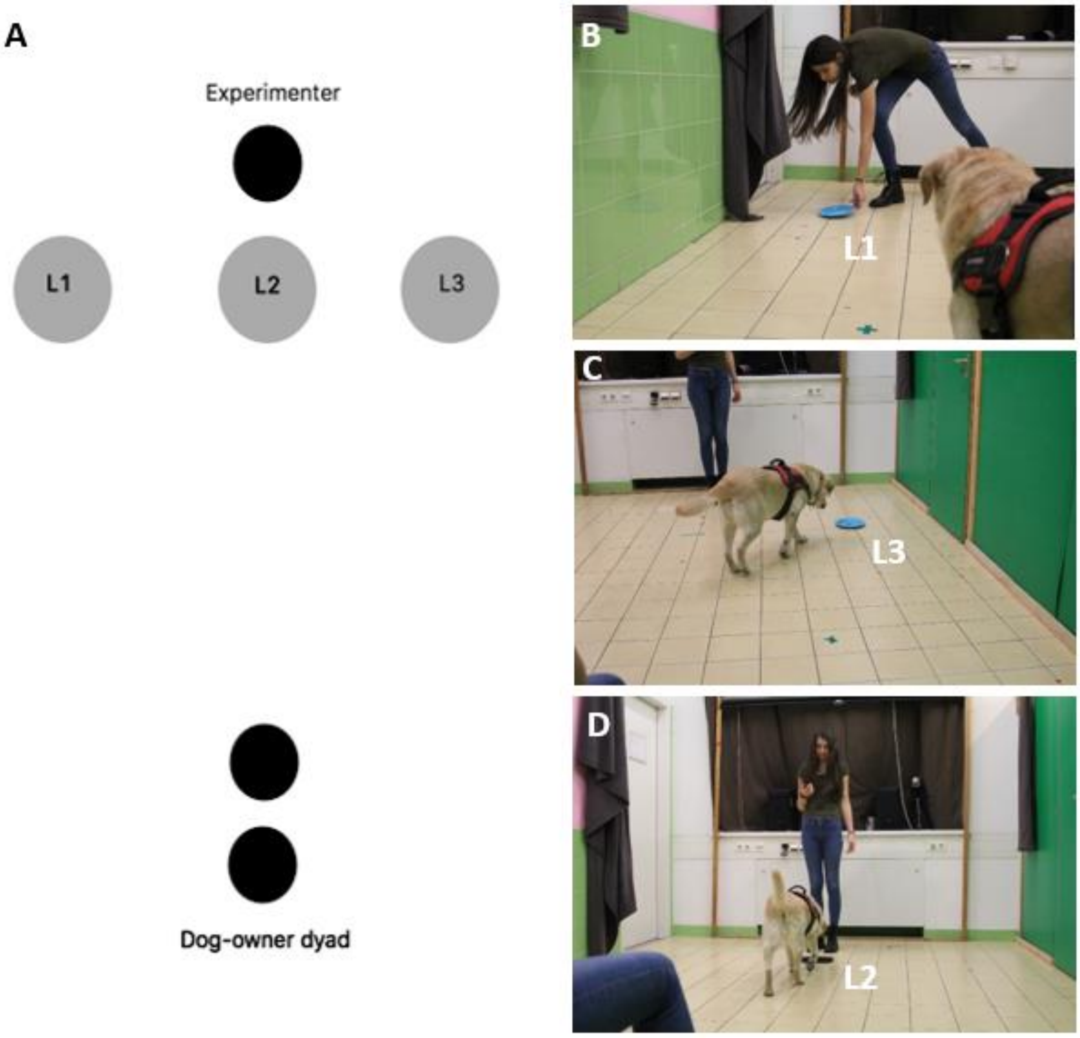

Figure 6. Room set up (A). The owner and the dog were at one side of the room and the experimenter was standing opposite to them. A line below the dog marked the starting position; lines in front of the experimenter marked the positions where the plates could be placed based on the type of the stimuli (L1 and L3=positions for the P or N stimuli in the "location" group; L2=position for the P and N stimuli in the "physical characteristics" group). Experimenter is placing P or N plate in the "location" group setting (B), the dog is approaching P/N plate in the "location" group setting (C), dog is approaching P/N plate in the "physical characteristics" group setting (D).

As soon as the plate was on the floor, the owner unleashed the dog. Owners had been instructed to unleash the dogs exactly as the plate touched the floor, so to avoid biasing the dog's behaviour. Dogs had up to 15 seconds to reach the plate and eat the food, while the experimenter looked straight ahead without making eye contact with the dog; once the dog ate the food, or after the 15 seconds had elapsed, the experimenter took the plate and the trial was over. If the dog did not immediately approach the food plates when released, the owner was allowed to encourage it with short sentences, such as "Go", or gently touching the dog. For each trial, the latency to reach the plate was recorded (defined as the amount of time from when the food plate touched the ground to when the dog crossed the line on the floor that marked the plates' position); if the dog did not approach the plate, the experimenter recorded the no-choice and the trial was given a latency of 15 seconds.

Dogs were deemed to have learned the association between the stimulus and the food when the longest latency to reach the P plate in the previous 5 trials was shorter than the latency to reach any of the previous N trials ("learning threshold"). Dogs underwent a maximum of 50 training trials; if they did not reach the learning criterion by the 50th trial, they were excluded from further testing. If the dog refused to participate in the test (i.e. did not leave the location of the chair), the training was interrupted. For each dog, the experimenter recorded the latency to reach the food in each trial. These were imported in a spreadsheet at the time of testing; after the first 10 trials, the spreadsheet compared the last 5 latencies to P and the last 5 latencies to N for each trial in order to assess whether the dog had reached the learning threshold.

The reversal learning phase was identical to the discrimination learning phase, except only the dogs that had passed the learning threshold were included (*N*=89) and the P and N were reversed. For example, for a dog in the location group, if the positive stimulus (P) was on the left, now it was on the right; for a dog in the colour group, if the P was the white plate, now it was the black plate. As in the learning phase the dogs underwent a maximum of 50 trials; the learning threshold was the same as in the discrimination learning phase

The following variables were recorded and calculated for both the discrimination and reversal learning:

- *number of learning trials*: count variable indicating the number of training trials (this could be either the number of trials required to learn the task, the number of trials before the task was interrupted because the dog refused to participate, or the maximum number of trials (50) if the dog did not learn);
- *learning success*: binary variable indicating whether the dog reached the learning criterion (0=did not achieve learning, 1=achieved learning).
- *latency*: time to reach the plate in each trial (continuous variable), if the dog did not make a choice, the maximum latency (15 seconds) was assigned to that trial;
- *learning mean latency P; learning mean latency N*: continuous variable indicating the mean latency to the last 5 P and 5 N.

Statistical analysis

Data were analysed using R statistical language (R Core Team, 2017). Our variable regarding the number of trials in the discrimination learning task was right censored (i.e. some dogs did not reach the learning outcome within the 50 trials or became uncooperative, leading to interruption of the phase). Therefore, a survival analysis was performed on the discrimination learning using a Cox proportional hazard regression model (Cox regression) implemented in the R package "survival." A Cox regression model for survival-time (time-to-event) was fit to calculate the effect of the age group upon the time (i.e. number of trials) on the occurrence of learning; types of stimuli were included in the model as covariate to evaluate their effect on the learning. The assumption of proportional hazards was tested using the function "cox.zph" and by plotting the estimates over the time to event. The analysis provided the Hazard Ratios (HR) (HRs) for each parameter, i.e. the ratio of the risk (probability) for the event to occur (i.e. for the dog to learn) for different levels of the explanatory variables. R squared indicates the proportion of the variance in the dependent variable that is attributable to the variables in the model; the log-rank test is a non-parametric test which tests the null-hypothesis that the distribution in the groups under consideration is not different.

In case of reversal learning, the effect of the age group on the time (i.e. number of trials) necessary for the occurrence of learning was analysed using Cox regression models, with the same modalities followed for the discrimination learning. Two additional Cox regression models were calculated for the location group and the physical characteristics separately.

9.4 Results

According to the log-rank tests the distribution in the groups were not different. The Cox proportional model indicated that young dogs learnt the discrimination learning task in fewer trials than old dogs (HR=1.89, test statistics=2.94, p=0.003, R^2=0.09). The type of stimuli did not significantly affect the number of trials required to learn. The median number of trials needed for learning the task was 27 vs 36, the 95% CIs were 21-34 and 31-39, respectively. 93 of young and 77% of old subjects have learnt the task within 50 trials (Figure 7A).

Importantly, dogs were included in the reversal learning task only if they have passed the discrimination learning task (N=89). According to the Cox proportional model, the number of trials that the dogs required to reach the learning criterion was influenced by both the age group and the type of stimuli (HR=2.73, test statistics=4.0, p<0.001 and HR=0.26, test statistics=-5.23, p<0.001, respectively, R^2=0.34). The location group required less trials to learn, compared to the dogs in the physical characteristics group. Both in the location and the physical characteristic group young dogs required fewer trials to reach the learning criterion than old dogs (HR=2.49, test statistics=2.658, p=0.008, HR=2.72, test statistics=2.786, p=0.005). In the location group CI was 38-45 for the old dogs (N=24) and 0-32 for the young dogs (N=18, Figure 7B). In the physical characteristics group CI was 50-NA for the old dogs (N=27) and 32-50 for the young dogs (N=20, Figure 7C).

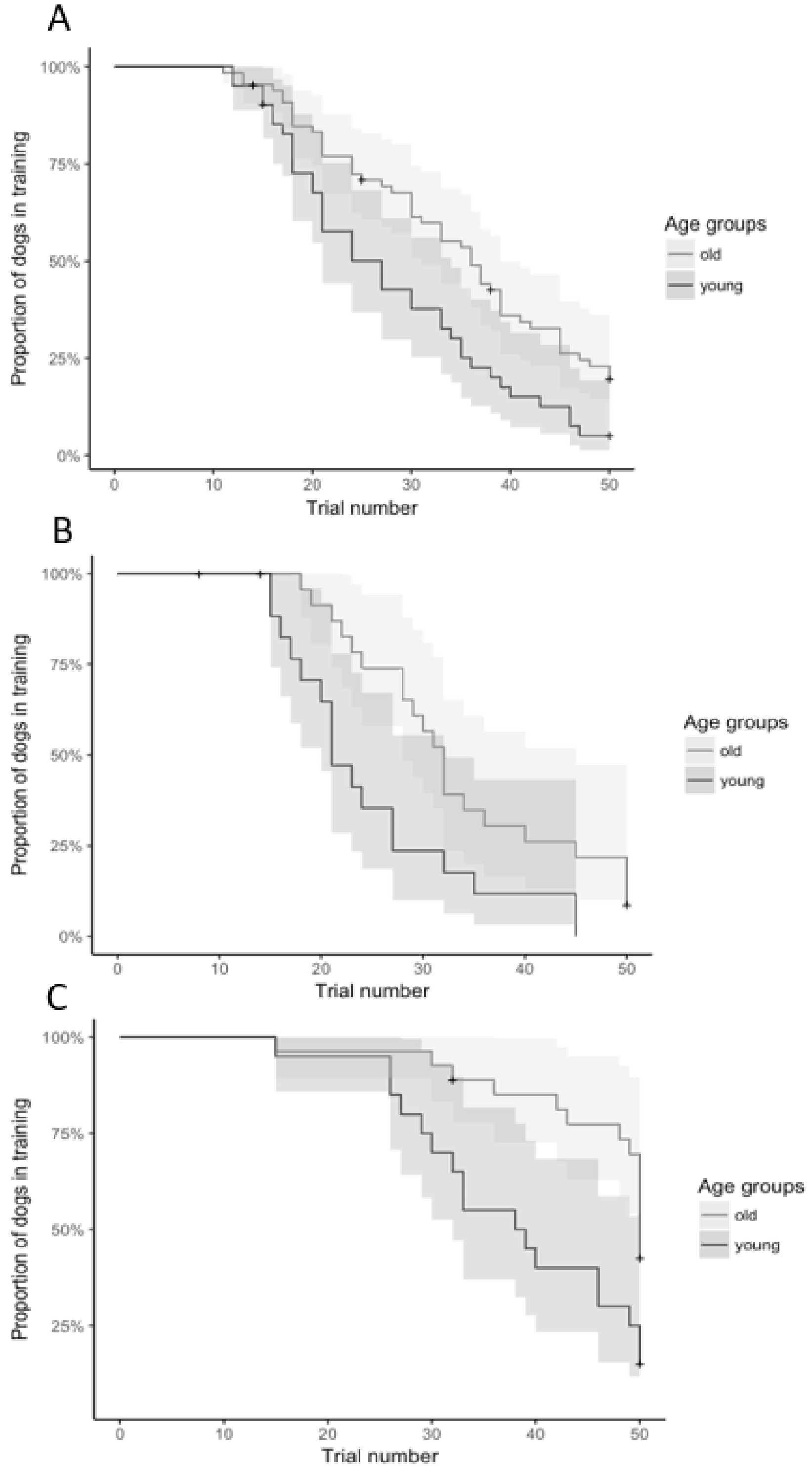

Figure 7. Survival curves for the number of trials to reach the learning criterion in the discrimination learning (A), reversal learning of the location group (B), and physical characteristics group (C) according to the age group. The darker line represents the young dogs, the lighter line represents the old dogs. The shadowed areas reflect the confidence intervals and the crosses (+) indicate censored data (i.e. dogs for which the task was interrupted but they had not learned).

9.5 Discussion

The aim of this study was to develop a reversal learning task which could detect age-related changes in the learning abilities of family dogs without overt medical problems in a short time-frame (about 1 hour). We have met this goal, as both the reversal learning and the preceding discrimination learning tasks detected age differences. Younger dogs (2-6.5 years) have learnt to discriminate two stimuli sooner than older dogs (7-14.5 years) and they were also more successful in the reversal learning task. Previous discrimination and reversal learning tests designed for the same purpose required significantly longer time-frame (e.g. Mongillo, Araujo, et al. 2013) and found that age effect is more stable when not only discrimination but also reversal learning tasks are employed, i.e. the evidence is inconsistent regarding age effects on discrimination learning (not detected effect: Christie et al. 2005; Milgram et al. 1994; Mongillo, Araujo, et al. 2013; detected effect: Tapp, Siwak, Estrada, Head, et al. 2003; Tapp, Siwak, Estrada, Holowachuk, et al. 2003; Studzinski et al. 2006; Wallis et al. 2016). Thus, our tests seem to be faster and more effective than previous tests. The focus on the discrimination learning task brings an additional level of details in the assessment of dogs' cognitive function, especially overcoming the issue of the ceiling effect observed in the reversal learning, which might be relevant in the assessment of dogs with higher deficit. Thus, tasks which are difficult for successfully aging dogs and lead to a ceiling effect, cannot be used for testing dogs with cognitive dysfunction.

Another novelty of our study is that the types of stimuli affected the learning performance during the reversal learning. Learning required more trials for the dogs trained based on the physical characteristics (size, shape, and colour) of the stimuli. One might argue that older dogs had impaired visual abilities and could not see the stimuli (plates). Although this cannot be excluded, as there is no test available to inform us about dogs' ability to see shapes or contrast, all dogs included in the current study had been preselected to ensure that they were able to reliably find small objects placed on floor.

One possible reason for the inconsistent evidence regarding age effects on learning, may be the fact that not all brain regions are affected by aging at the same pace and not all subjects are equally affected at a given age; moreover, different cognitive functions rely on different brain areas. For example, Tapp et al. (2004) observed increasing atrophy in the frontal lobe of dogs as their age increased (starting from 8 – 11 years) as well as an inverse correlation between frontal lobe volume and a discrimination learning and reversal learning task. Moreover, Studzinski et al. (2006) observed deficits in the acquisition of a discrimination task in dogs aged 10+ years, compared to dogs below 8 years of age. However, they also reported that not all dogs in their sample showed spatial impairment, with 25% of the dogs above the age of 10 showing no deficit in their performance. Similarly, Head et al. (2000) reported signs of beta-amyloid accumulation in some brain areas (enthorinal cortex, prefrontal cortex) from the age of 9 years, but only in subsets of dogs (42% vs 75% respectively), which was not consistently affected until the age of 14 years. Finally, (Siwak-Tapp, Head, Muggenburg, Milgram, & Cotman, 2008) found that the hilus of the hippocampus showed a significant loss of neurons (~30%) in the brain of 13-15 years old compared to 3-5 years old dogs. Additionally, hippocampal neurogenesis was negatively correlated with dogs' performance in a number of cognitive tasks, including object discrimination, size discrimination, and black/white discrimination (Siwak-Tapp, Head, Muggenburg, Milgram, & Cotman, 2007). The dogs in our sample were screened for neurological conditions, sensory-motor decline, and general signs of pathological aging. Therefore, our results could mirror cognitive changes of successfully aging dogs.

One advantage of the use of family dogs as models for the study of aging is the large and diverse population available, as long as non-invasive methods are employed. The learning tests described in our study could be used in combination with imaging techniques (e.g. MRI, fMRI, EEG) to further investigate the role of structural and functional changes in the brain on cognitive function. Future research should also investigate ways to overcome the issue with the non-learning dogs. This is a limitation of the current study (as well as any study requiring training), as the dogs that do not learn the discrimination cannot be assessed in such cognitive tasks. One way to attempt overcoming this issue could be to increase the number of training trials during the discrimination learning; however, this strategy has a limitation in that it prolongs the overall duration of the task.

Overall, our findings indicate that a discrimination task based on the location of two objects, accompanied by a reversal learning task, may be an efficient way to measure age related decline in learning performance in family dogs. Further studies are necessary to better understand to which degree variation in the performance of this test might relate to functional changes in the brain or whether the test is predictive to cognitive decline. The task may be associated to colour and shape discrimination, although care should be taken to avoid ceiling effects, e.g. by increasing the maximum number of trials in the test. Future research should investigate the stability over time of the learned associations. This is particularly important when other tests (for example the cognitive bias test) rely on this association. However, the effectiveness of a cognitive bias test on a population of aged dogs is yet to be demonstrated.

10 Positivity effect in dogs: do old dogs experience less negative emotions?[6]

10.1 Abstract

Age-related changes in the brain can alter how emotions are processed. In humans, attention and memory shift from negative to positive stimuli with increasing age, i.e. older people experience fewer negative emotions. Little is yet known about this “positivity effect” in non-human animals. We tested young (n=21, 1-5 years) and old (n=19, >10 years) family dogs with positive (laugh), negative (cry), and neutral (hiccup, cough) human vocalisations and investigated age-related differences in their behavioural reactions. Only dogs with intact hearing were analysed and the selected sound samples were balanced regarding mean and fundamental frequencies between valence categories. Compared to young dogs, old individuals reacted slower only to the negative sounds and there was no significant difference in latency to recover between groups. Therefore, we could reject the hypothesis that, due to cognitive decline, older dogs were slower to respond to sounds in general. Results suggest that an age-related positivity effect is present in dogs. Similarities in emotional processing between humans and dogs may imply analogous changes in subcortical emotional processing in the canine brain during aging.

10.2 Introduction

Changes in the brain during aging influence how adults process emotions (Cacioppo, 2011). Whereas the processing of positive emotions remains more or less stable, older adults experience fewer negative emotions. This phenomenon, referred to as age-related positivity effect, is present both in attention and memory (Reed & Carstensen, 2012). For example, Mather & Carstensen[3] found that older adults spent less time looking at negative features of portraits than on positive features, and that the time spent looking at negative features was shorter than in younger adults. This form of selective attention has an effect on memory, because higher attention to positive features can lead to stronger memories(Logan, 2002).

The positivity effect was also observed in the auditory domain, in regard to processing emotional prosody and valence. Prosody is defined as the properties of larger units of vocalisations that can indicate the emotional state of a speaker (Dimos, Dick, & Dellwo, n.d.), and valence describes the attractiveness/averseness or emotional association of a stimulus (Ford, Wright, & Haythornthwaite, 1985). Older adults have more trouble identifying emotional valence in sounds and pitch perception of emotional prosody can be impaired as well (Mitchell, 2007; Mitchell & Kingston, 2014). A cross-sectional study on the perception of both sounds and faces with different emotional valences showed that aged adults have a decreased ability to recognise both sadness and anger, whereas the recognition of other emotions remained intact (Mill, Allik, Realo, & Valk, 2009).

As in humans, the functions of the canine brain are known to decline with age (Borras, Ferrer, & Pumarola, 1999). Both the cognitive functions of the medial temporal lobe and the prefrontal

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[6] Based on : Smit, I., Szabó, D., Kubinyi, E. (2019) Age-related positivity effect on behavioural responses of dogs to human vocalisations. Submitted.

cortex are impaired in old dogs, as demonstrated in an indirect behavioural study by Head et al. (1995). In this study, older dogs performed worse on several cognitive behaviour tests that were used to assess age-dependent cognitive decline. This was later supported by several other behavioural studies (Norton W. Milgram, Head, Weiner, & Thomas, 1994; Mongillo, Araujo, et al., 2013; Rosen et al., 2002; Lisa J. Wallis et al., 2016). An MRI study by Tapp et al. (2004) confirmed that in 8-15 year old beagles the frontal lobe and the hippocampal volumes declined with age. However, the presence of an age-related decline in the processing of different emotionally loaded stimuli in dogs has not been studied yet.

Family dogs are able to differentiate between human emotions and owner reports suggest that dogs older than 8.9 years are less reactive toward their owner’s emotion (Szánthó et al 2017). Dogs were able to discriminate between happy and blank faces on photographs (Nagasawa, Murai, Mogi, & Kikusui, 2011) and they were most reactive to commands when their owners displayed happiness instead of neutrality or disgust with body language and sound (Borbála Turcsán, Szánthó, Miklósi, & Kubinyi, 2015). A cross-modal, preferential looking paradigm study showed that dogs spontaneously paired positive and negative emotions of both human and conspecific faces on pictures with pre-recorded emotional sounds (Albuquerque et al., 2016). Emotion processing was also tested in the head turning paradigm, where dogs reacted differently towards positive and negative sounds. This supports that dogs spontaneously discriminate between positive and negative human emotions. In addition to discriminating between the emotions, research on emotional contagion has shown that dogs are affected by both visual and auditory emotional expressions. Dogs licked their mouth more frequently when they faced pictures of human and dog faces with negative emotional expression, regardless the valence of the sound presented at the same time (Albuquerque, Guo, Wilkinson, Resende, & Mills, 2018). Dogs showed significantly more arousal and stress related behaviours following negative emotional sounds compared to positive and non-emotional sounds they were exposed to, suggesting they matched the emotional valence in the negative sounds (Huber, Barber, Faragó, Müller, & Huber, 2017). Using a non-invasive functional magnetic resonance (fMRI) procedure with awake dogs and humans, Andics et al. (2014) showed the existence of specific voice areas and the presence of emotional valence sensitivity in dogs’ brains.

We hypothesised that dogs show age-related positivity effect similar to humans, as both the general age-related changes in the brain, and the brain regions involved in auditory processing are similar in humans and dogs. Specifically, the present study focused on whether there are age-related differences in the spontaneous behavioural reaction of dogs to human vocalisations with positive, negative, and neutral emotional valences during a sound playback test. We compared latency to react and the latency of recovery of aged and young dogs with intact hearing. Via this setup we investigated whether aged dogs were (1) less responsive to all sounds in general due to e.g. general cognitive decline or (2) selectively less responsive toward negative stimuli than young dogs corresponding to a positivity effect.

10.3 Methods

Subjects

A total of 46 family dogs were tested, of which six had to be excluded from the analysis. Reasons for exclusion included excessive stress reactions to the sounds (n=1), hearing impairments (n=1), owner interference during the sound playback (n=1) and technical problems (n=3). Finally, n=21 young dogs (1-5 years) and n=19 old (>10 years) dogs were analysed in the study. Only dogs between 4 and 30 kg took part in the study, with a mean weight of 17 for old dogs and 16 for young dogs. This restriction was implemented because dogs with different sizes could exhibit a difference in aging rate. The female to male ratio was 2:5 (6:15 female to male for the young age group and 6:13 for the old age group). The dogs were required to be experimentally naive for sound playback studies. The hearing of the subjects was assessed by calling the dogs' name and via using a rattler behind them, by both the owner and the experimenter in a test following the experiment. Dogs which showed any signs of hearing impairments were excluded from the analysis (n=1). Owners were recruited via online promotion and from the Department of Ethology's database.

Test room

The tests were conducted at a room of the Department of Ethology. The room measured 3.10 x 5.40 metres. The owner was positioned on a chair in the back of the room (1 metre from the front wall) facing the speaker set (2x Logitech S-02648, 230V ~50Hz 40 mA), which were placed together 2 metres in front of the chair (Figure 8). The experimenter was standing next to camera 1, positioned next to the wall 1.25 metres away from the chair. Camera 2 was positioned 80 centimetres behind the speakers. The speakers were connected to a laptop and a media pointer was used to change the sounds using PowerPoint. The dog was positioned in front of the owner, with its back to the speakers, facing either the owner or the side walls.

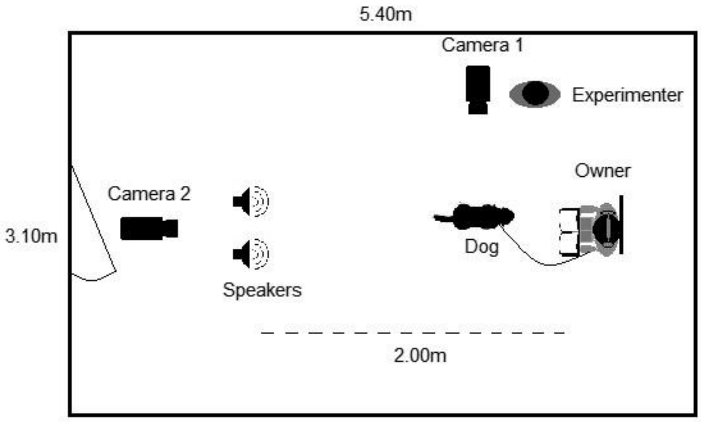

Figure 8. Test room set-up. The owner was positioned in the back of the room and the leashed dog was laying down in front of them. The experimenter was standing next to camera 1, 1.25 meters away from the owner and dog. Camera 2 was positioned behind the speakers, which were positioned 2 meters in front of the owner. The owner was reading a magazine and listening to music via headphones to minimise their effect on the dog's reaction.

Stimuli

The 6 stimuli used (2 positive, 2 negative, 2 neutral, all non-linguistic) were pre-selected from published studies. The positive and negative sounds were chosen from a study (Anikin & Persson, 2017) that validated a corpus of 260 human vocalisations using a survey on 90

participants with different nationalities who rated the sounds. They were non-acted sounds taken from real life video clips. The negative sounds featured a cry by a male and a female and the positive sounds were a male and a female laugh. As this corpus lacked neutral vocalisations, the two neutral stimuli, a hiccup and a cough, were taken from the sound database of acted vocalisations previously used in a study on intensity perception of emotional valence in humans (Farago et al., 2014). The gender of the neutral sounds was not identified in this database. All stimuli had a duration of 5 seconds, were recorded in mono and had a RMS volume of 20 dB with a limit on -3.5 dB. The sounds were edited using Audacity 2.2.2. Further details of the sounds can be found in
Table 8.

Sound ID	Emotion	Speaker gender	RMS volume	Mean frequency	Fundamental Frequency
neuSTIM1	Neutral	Unknown	20 dB	633 Hz	325 Hz
neuSTIM2	Neutral	Unknown	20 dB	500 Hz	329 Hz
posSTIM1	Positive	Female	20 dB	1040 Hz	128 Hz
posSTIM2	Positive	Male	20 dB	1828 Hz	318 Hz
negSTIM1	Negative	Male	20 dB	1481 Hz	350 Hz
negSTIM2	Negative	Female	20 dB	1465 Hz	155 Hz

Table 8. Emotional stimuli used and their sound ID, corresponding emotion, speaker gender, Root Mean Square volume and Mean and Fundamental frequency.

Procedure

The owner was sitting on the chair reading a magazine and listening to music, therefore the test sounds were inaudible for him/her. The dog was given time to acclimatise and lie down on its own. If it did not lie down within a minute, or if it lied down facing the speakers, the owner was asked to position the dog in front of him/her so it was laying down with either its back or side to the speakers. Once the dog lied down and was looking away from the speaker, the sound playback started, and the first sound (testSTIM1) was played. Once the dog recovered from the sound, but at least 10 seconds later and maximum 1 minute later, the owner was asked to reposition the dog if necessary. Once the dog was again not focused on environmental stimuli (i.e. it was passive), the second sound (testSTIM2) was played. This was repeated for the third sound (testSTIM3). After a 5-minute break outside the test room in which the owner was free to interact with the dog, the trial was repeated with the 4th-6th sounds (testSTIM4, 5 and 6). The playbacks of testSTIM1-6 were semi-randomised for each subject. Each session contained 1 positive, 1 negative and 1 neutral stimulus, therefore after the two sessions all subjects were exposed to two positive, two negative and two neutral stimuli. Video recording of the dogs' behaviour happened continuously during the trials and the behaviours were analysed based on the videos.

Data collection

- *Latency to react:* time in milliseconds from stimulus onset till the dog started its head/ear turn towards the source of the sound. We stopped the observation after 10 seconds.
- *Latency to recover:* time in milliseconds from latency to react until the dog started to turn away from the source of the sound. We stopped the observation after 60 seconds.

One observer scored the latencies and behaviours blindly, without hearing the sounds during scoring, after which the same observer coded the latencies using the audio stream for determining stimulus onset, based on the previously coded reactions. The second observer scored 120 trials using the same method.

Statistical analysis

R (version 3.5.1.) was used for the statistical analysis (R Core Team & R Foundation for Statistical Computing, 2018). Inter-observer reliability analysis of the latencies was calculated via coding 25 % of the data by a second, trained coder. Agreement was calculated using intraclass correlation coefficients (ICC) in a two-way mixed-effects model with a 95% confident interval on consistency in SPSS 22 (Corp., 2013). Normality of the latency to react and latency to recover was checked using Q-Q normality plots, which showed neither of the variables were normally distributed. Because of censoring within the reactivity and latency data (e.g. when the dog did not react within 10 seconds or when the dog did not redirect its attention within 60 seconds), Survival Analysis Methods were used.

First, to check whether there were any differences between the two samples within the categories we used Kaplan Meier estimates with the main factor of Sound ID and a post-hoc pair-wise comparison. Survival analysis was used because right censoring occurred during the tests. To compare the reaction and recover latencies of young and old dogs Kaplan Meier estimates were used, with the main factors being age group and sound category. Mixed Effects Cox Regression Models were used to analyse the effects of age group, emotion category and playback order on the latency to react and recover with subjects as random variable and the sound categories, trial order and the dogs' age as factor. The young age group, the neutral sound category and trial 1 were set as reference categories. Cox proportional hazards were used to analyse the confidence intervals. Since there was a trial effect, the Cox models were also run on a dataset including only the first trial for each dog. The dogs that did not react were classified as censored within the latency to react model and not used in the latency to recover model. Dogs that did not recover were classified as censored in the latency to recover model in that specific trial. Exploratory analysis with a dredge function in R showed no suggested effects of sex, thus it was excluded from the models. The dredge function also showed improved models without interactions.

Reactivity and recovery were also tested with a binominal Generalized linear mixed model to compare the number of censored trials of the old and young dogs in the different sound categories. It also included the factors age group, sound category and trial, using the subject as random variable.

10.4 Results

Intraclass correlation coefficients (ICC) for latency to react and latency to recover were excellent (0.924 and 0.793, respectively).

A log-rank test revealed no differences in the latencies to react and recover between the two different sounds within a category. Based on this, we analysed the reactions within a valence category (e.g. cry1 and cry2) together.

Latency to react

Binomial generalized linear mixed model

Out of a total of 240 trials, there were 23 trials (from 15 dogs) in which the dog did not react within 10 seconds from the onset of the stimuli (Table 9). A generalized linear mixed model with binomial distribution showed no significant effects of age group (β=-0.76, SE=0.69, p=0.27) or sound category (positive: (β=0.14, SE=0.63, p=0.81), negative: (β=-0.07, SE=0.60, p=0.90)) on the dogs' reactivity. However, a trial effect was found, dogs were less likely to react in Trial 6 than in Trial 1 (β=-2.23, SE=0.91, p=0.014).

		No reaction within 10 sec				No recover within 60 sec			
Age group	**Total n**	**n**	**Positive**	**Negative**	**Neutral**	**n**	**Positive**	**Negative**	**Neutral**
Young	126(100%)	9 (7%)	3(2%)	1(1%)	5(4%)	1(1%)	1(1%)	0	0
Old	114(100%)	14(12%)	4(4%)	7(6%)	3(3%)	7(6%)	0	5(4%)	2(2%)

Table 9. Number of censoring in the latency to react and recover. Showing the total censoring per age group and the number of censored trials in each sound category and their percentages of the total number of trials out of a total of 47 dogs and 240 trials.

Survival probability

A log-rank test showed that old dogs responded to the sounds with a longer latency (Chi X^2=5.2 p=0.02) (Figure 9). The difference between young and old dogs within the negative sound category was significant (Young: (95%CI: 300; 300), Old: (95%CI: 300; 800), p=0.021). Within the negative sound category, young dogs showed an increased hazard to react compared to the old dogs (Figure 10). We found no similar pattern within the neutral (Young: (95%CI: 300; 400), Old: (95%CI: 300; 400), p=0.913) or the positive sound category (Young: (95%CI: 300; 400), Old: (95%CI: 300; 400), p=0.521). This indicates that old dogs reacted significantly slower than young dogs only in the negative sound category.

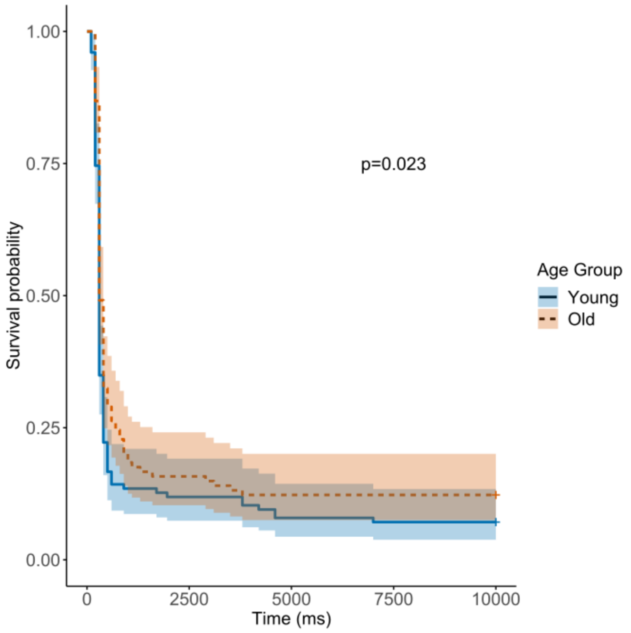

Figure 9. Kaplan Meier survival curve estimates of dogs' latencies to react comparing trials with young dogs with trials with old dogs. The blue continuous line represents the old age category and the red interrupted line represents the young age category.

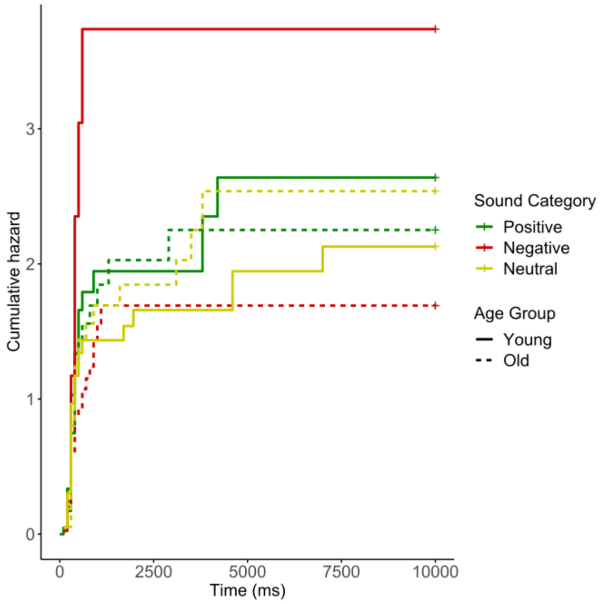

Figure 10. Cumulative hazards plot for the latency to react, grouped per age and sound category. The positive, neutral and negative sound categories are represented by the green, yellow and red coloured lines respectively. The young age group is shown with a solid line and the old age group with an interrupted line.

<u>Mixed Effects Cox regression</u>

We tested whether dogs became habituated to the sounds across trials via a Mixed Effects Cox regression model. The cumulative hazard results of the Cox regression showed a significant hazard decrease (exp(β)=<1) in trial 5 and 6, of 0.56 (exp(β)=0.57, 95%CI=(0.37;0.94), p=0.03) and 1.04 (exp(β)=0.35, 95%CI=(0.25;0.67), p<0.001) respectively (for statistical details see Table 10). An exp(β) below 1 for these factors suggests an increased latency to react, i.e. dogs habituated to the sounds in trial 5 and 6.

	Trial 1-6				Trial 1			
	p	β(SE)	exp(β)	95% CI	p	β(SE)	exp(β)	95% CI
Trial 2	0.65	0.11±0.24	1.11	0.69;1.70	-	-	-	-
Trial 3	0.61	-0.13±0.25	0.88	0.54;1.34	-	-	-	-
Trial 4	0.43	0.19±0.24	1.21	0.71;1.77	-	-	-	-
Trial 5	**0.024**	-0.56±0.25	0.57	0.37;0.94	-	-	-	-
Trial 6	**0.0001**	-1.04±0.26	0.35	0.25;0.67	-	-	-	-
Age group old	0.084	-0.43±0.25	0.64	0.52;0.89	**0.0064**	-1.11±0.41	0.33	0.16;0.74
Sound category positive	0.40	0.14±0.17	1.15	0.83;1.60	0.7600	0.13±0.42	1.14	0.52;2.46
Sound category negative	0.56	0.10± 0.17	1.11	0.78;1.52	**0.0380**	-0.99± 0.48	0.37	0.16;0.93

Table 10. Cox Mixed Effects regression results for latency to react. Showing p-values, the coefficient (β) ± the standard error, hazard ratio (exp(β)) and the 95% confidence interval for a model including trial 1-6 and a model including only 1 trial. Trial 1, age group young and sound category neutral are set as reference categories. The significant values (p<0.05) are represented in bold.

Latency to recover

The trials where the dogs did not react were excluded (due to the lack of latency to recover), resulting in a total of 217 trials being used for the analysis of the latency to recover. Out of a total of 217 trials in which the dog reacted, 5 dogs did not recover in 8 different trials (i.e. did not return to passive, unfocused state) within the time limit of 60 seconds after the onset of sound, or before another event, (e.g. due to owner interference or external distraction) (Table 8).

Survival probability

The log-rank test showed that there was no significant difference in the latency to recover between young and old dogs (Young: (95% CI: 7700msec; 9800 msec), Old: (95% CI: 6100 msec; 9100 msec), p>0.9). A Cumulative Hazard analysis paired with a log-rank test showed no age differences in recovery time from the negative (Young: (95%CI: 7500; 10700), Old: (95% CI : 7000; 13000), p=0.93), positive (Young: (95% CI: 7300; 11300), Old: (95% CI :5500; 9100), p=0.93) or neutral (Young: (95% CI: 7100; 11100), Old: (95% CI: 5600; 10800), p=0.93) sounds. This indicates that there was no significant difference in latency to recover between young and old dogs in any sound category.

Mixed Effects Cox Regression

The results of the Cox model (Cox mixed-effects model fit by maximum likelihood) showed a significant effect only in case of trial 4 (for statistical details see Table 11). The hazard of the latency to recover increased (exp(β)=>1) in trial 4 by 0.19 (exp(β)=2.25, 95% CI=(1.14;2.88), p=0.001), showing that the dogs had a significantly shorter latency to recover during the first trial after the short break.

	Trial 1-6				Trial 1			
	p	**β(SE)**	**exp(β)**	**95% CI**	**p**	**β(SE)**	**exp(β)**	**95% CI**
Trial 2	0.310	0.25±0.25	1.29	0.72;1.80	-	-	-	-
Trial 3	0.700	0.10±0.26	1.10	0.71;1.80	-	-	-	-
Trial 4	**0.002**	0.81±0.26	2.25	1.14;2.88	-	-	-	-
Trial 5	0.090	0.44±0.26	1.56	0.74;1.89	-	-	-	-
Trial 6	0.240	0.33±0.28	1.39	0.68;1.85	-	-	-	-
Age group old	0.600	0.16±0.32	1.18	0.78;1.35	0.80	0.09±0.37	1.10	0.54;2.25
Sound category positive	0.240	0.21 ±0.18	1.24	0.84;1.63	0.74	-0.14±0.42	0.87	0.38;1.98
Sound category negative	0.280	-0.20 ±0.19	0.82	0.67;1.33	0.42	0.35±0.44	1.42	0.60;3.37

Table 11. Cox Mixed Effects results for latency to recover. Showing p-values, the coefficient (β) ± the standard error, hazard ratio (exp(β)) and the 95% confidence interval for a model including trial 1-6 and a model including only 1 trial. Trial 1, age group young and sound category neutral are set as reference categories. The significant p values (p<0.05) are represented in bold.

10.5 Discussion

Cognitive decline in older dogs, which is thought to be linked to the temporal and frontal cortex (Head, 2011) can cause longer processing, namely slower reaction times and a longer latency to recover (Head et al., 1995; Siniscalchi, D'Ingeo, Fornelli, & Quaranta, 2018; P. D. Tapp et al., 2004). However, in the current study we showed that older dogs are not simply less responsive or slower to respond to the used stimuli in general. Latency to react did differ significantly between young and old dogs, but only in the negative sound category where the young dogs reacted faster than the old dogs, while latency to recover was not different between young and old dogs. The number of not recovering dogs also did not significantly differ between the old and young dogs across the different sound categories. Thus, it can be concluded that the old dogs that passed the hearing test were not less responsive to all sounds and the difference is more likely caused by changes in the processing of certain sound types than by general cognitive decline.

While attention span is generally decreased in older dogs (Wallis et al. 2014) and the likelihood to recover is smaller than in younger dogs (Siniscalchi et al., 2018), we found no differences between old dogs and young dogs in whether they recovered from the stimuli within 60 seconds. Most dogs kept their attention towards the speakers after the sound with a duration of 5 seconds had ended, suggesting that the latency to recover in the current setup may not indicate attention to the sound per se but rather the arousal induced by it. Testing the phenomenon in a different setup, for example with projected images (Racca, Guo, Meints, & Mills, 2012) could provide information whether attention span toward emotional stimuli also changes in aging dogs. Alternatively, comparing the performance of young and old dogs trained to classify portraits based on the displayed emotions (Corsin A. Müller, Schmitt, Barber, & Huber, 2015) could reveal whether the positivity effects extends into the classification performance of old dogs and whether attention toward negative stimuli is selectively decreased in old dogs during an active choice task.

It is suggested that prosody discrimination in humans is, among other factors, based on the duration and fundamental frequency of the sounds (Faragó et al., 2014; Marx et al., 2015). The effect of the prosody in dogs is seen in stress and arousal indicating behaviours (Albuquerque et al., 2018; Huber et al., 2017). The sounds used in this study were similar in their fundamental frequency (Table 8) and they all had a duration of 5 seconds. Additionally, in the selected sound set the negative samples did not deviate strongly from the other samples (the two negative samples were not the two most extreme ones neither regarding mean nor fundamental frequencies, see Table 8) meaning that the findings cannot be explained based on e.g. diminished reactivity to above/below a certain frequency range.

After multiple human studies reporting an age-related positivity effect (Brosgole & Weisman, 1995; Mill et al., 2009; Ryan, Murray, & Ruffman, 2010) , the results of our study suggests the presence of a similar valence related phenomenon in dogs. While a previous study by Siniscalchi et al. (2017), in line with our current results showed a significant difference between young and old dogs in reactivity to emotional sounds in general, our study is the first to look at the role of valence in reactions of aging dogs and the presence of the positivity effect. Further studies including more emotional categories are needed to investigate the level of similarity between positivity effect in dogs and humans, for instance whether the processing of other negative emotional vocalisation (e.g. anger) are also affected in dogs, similarly to humans (Mill et al., 2009).

Multiple theories have been proposed to explain the positivity effect in humans. The two main mechanisms differ in regard to their complexity, required level of abstract thought and the involved brain areas. The aging-brain model ascribes changes in the processes in the anterior cingulate gyrus to downregulate the response of the amygdala to negative stimuli and thereby influence the way these emotions are processed and reacted upon (Nashiro, Sakaki, & Mather, 2012; Reed & Carstensen, 2012). In contrast, the Emotional Selectivity Theory (Mather & Carstensen, 2005) proposed the role of cognitive control in the shift in attention and memory away from negative towards more positive emotions. Based on the Emotional Selectivity Theory, these changes are caused by greater emotion regulation of older adults, and a change in perception of experiences, with positive experiences being valued higher by older individuals due to awareness of time left alive (Mather & Carstensen, 2005; Nashiro et al., 2012). Since dogs are less likely to reflect upon their longevity and ultimate death and have yet to show signs of both emotion regulation and awareness of future life events, the current results cannot be explained by a positivity effect resulting from the Emotional Selectivity Theory. Thus, the presence of an age-related positivity effect in dogs supports the aging-brain model.

Degeneration in the brain of older dogs is mainly found in the frontal and temporal cortex (Head, 2011). Processing of auditory stimuli is associated with the temporal lobe, while the attention to stimuli is regulated in the frontal lobe (Baars & Gage, 2010). There are multiple similarities in the processing of emotional human sounds in both the primary and secondary auditory regions between humans and dogs (Andics et al., 2014). Processes and interactions involved in the prosody discrimination are also suggested to happen mainly in the frontal and temporal lobe (Bush, Luu, & Posner, 2000; Canli, Zhao, Desmond, Glover, & Gabrieli, 1999; Hamann, 2001; Vogt, 2005) and the anterior cingulate gyrus (in the primary auditory cortex of the temporal cortex) and amygdala (in the prefrontal cortex) are also located here. With age, the amygdala is known to decrease in volume (Allen, Bruss, Brown, & Damasio, 2005) and this is shown to affect the way emotional cues are processed in humans (De Winter et al., 2016).

There are two general pathways for auditory processing in the brain; the thalamic, or subcortical, and the cortical pathway (Lee, 2013; Pannese, Grandjean, & Frühholz, 2015). It has been suggested that the subcortical pathway subconsciously processes sounds and visual stimuli before the cortical level, to provide the amygdala with a brief preliminary characterisation before further and slower processing using the cortical pathway takes place (Pannese et al., 2015; Pessoa & Adolphs, 2010). Auditory nerve fibres located in the brainstem are involved in the preliminary processing of fundamental frequency and harmonic cues in sounds (Felix, Gourévitch, & Portfors, 2018). Harmonic cues, like timbre, are used by the auditory regions in the brainstem to encode sounds that are not easily recognisable by frequency (de Cheveigné, 1993). Auditory brainstem response (ABR) peaks are seen to be reduced in older adults, which suggests that the number and synchrony of the auditory nerves are reduced (Konrad-Martin et al., 2012).

The fact that in our study the latency to react, but not the latency to recover showed significant differences between the old and young dogs in the negative sound category, may suggest that the age-related changes take place in subcortical processing rather than in cortical processing, involving both the aging of auditory nerve fibres of the brainstem and the degeneration of the amygdala. However, further research involving brain imaging is necessary to determine the specific cause of these effects, the brain regions involved and nature of the differences.

11 Age-related effects in looking at faces of humans and conspecifics[7]

11.1 Abstract

The gaze of other dogs and humans is informative for dogs, but it has not been explored which factors predict face-directed attention. Based on findings in the human literature we expect responsiveness to the eyes and gaze to decrease with age through change in perceptual processes or to change specifically due to alterations in processing visual social stimuli. We used image presentations of unfamiliar human and dog heads, facing the observer (portrait) or facing away (profile), and measured looking time responses. We expected dog portraits to be aversive, human portraits to attract interest, and tested dogs of different age, sex, skull length and breed function, which in previous work had predicted human-directed attention. Dog portraits attracted longer looking times than human profiles. Overall, brachycephalic (short headed) dogs displayed the longest and dogs of unspecified breed function (such as mixed breeds) displayed the shortest looking times. Among the latter, females observed the images for longer than males, which is in line with human findings on sex differences in processing faces. In a subsequent experiment, we tested whether dog portraits functioned as threatening stimuli. We hypothesized that dogs will avoid food rewards or approach them more slowly in the presence of a dog portrait, but found no effect of image type. In general, older dogs took longer to approach food placed in front of the images due to changes in facial perception or due to a decline in sensorimotor functions which typically accompanies the aging process and mesocephalic dogs were faster than dogs of other skull length types. The results suggest that short-headed dogs are more attentive to faces, while sex and breed function predict looking times through complex interactions.

11.2 Introduction

In comparative research, the dog has become a favourable subject for studying gazing behaviours. Gaze-directed attention might even pre-date domestication: gaze following, both in distant space and around barriers, is present in the wolf, dogs' closest relative (Range & Virányi, 2011), and eye contact with a human experimenter can be trained, albeit with more effort than in the dog (Gácsi, Győri, et al., 2009). The morphology of the head and eyes make them relevant cues for predicting conspecific behaviour (Ueda, Kumagai, Otaki, Yamaguchi, & Kohshima, 2014), but in wolves eye contact is used mostly to signal threat (Rudolf Schenkel, 1967) suggesting that the eyes and gaze of conspecifics are aversive stimuli for canines in the wild.

On the other hand, it appears that dogs preferentially follow the gaze of humans if eye-contact was established first (Téglás, Gergely, Kupán, Miklósi, & Topál, 2012); that they use alternated gazing to 'show' a human the location of hidden toys (Miklósi, Polgárdi, Topál, & Csányi, 2000) and that they can distinguish human emotional facial expressions (Nagasawa et al., 2011; Ruffman & Morris-Trainor, 2011; Borbála Turcsán, Szánthó, Miklósi, & Kubinyi, 2014). Visual cues in general play a pivotal role in dog-human communication, like the pointing gesture (Soproni, Miklósi, Topál, & Csányi, 2002). Dogs can follow pointing gestures

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[7] Based on: Bognár, Z., Iotchev, I. B., & Kubinyi, E. (2018). Sex, skull length, breed, and age predict how dogs look at faces of humans and conspecifics. *Animal Cognition*, *21*, 447-456.

better than chimpanzees, humans' closest living relatives (Miklósi & Soproni, 2006). Miklósi et al. (2003) suggested dogs' attention towards the human gaze may explain dog-wolf differences in learning to follow human pointing (so far only one study has found wolves to perform better than dogs (Udell, Dorey, & Wynne, 2008)). Although there is some support for this capacity being an extension of the predatory (motor) sequence (Udell, Ewald, Dorey, & Wynne, 2014) i.e. orienting towards and subsequently following the prey, the human gaze may as yet play a crucial role in creating the necessary context for dogs to interpret pointing as a communicative signal and distinguish it from similar, but unintentional movements. Kaminski and Nitzschner provided an extensive review (Kaminski & Nitzschner, 2013) – one common finding is that dogs preferentially respond to pointing after eye-contact has been established with the experimenter. Another recent argument that the visibility of the human face and eyes signal communicative intention to dogs, comes from a study showing dogs' facial expressions are preferentially displayed in response to people facing them upfront (Kaminski, Hynds, Morris, & Waller, 2017). A special role for the human gaze as a signal in dog-human communication could be the result of dogs' adaptation to life with humans during their unique domestication process (Hare & Tomasello, 2005; Miklósi, 2008) (though see Udell, Dorey, & Wynne, 2010 for an alternative account).

The relationship between sex and attentiveness to the eyes and gaze is relevant to cross-species comparisons, as some studies have suggested better female performance in the social cognitive domain may persist across mammalian species (Bartal et al., 2011; de Waal, 1996). Research on canine visual abilities, however, has not explored sex differences yet.

Previous work had also shown that dog's responses to human cues (such as pointing) and their perceived trainability vary with breed characteristics like their cephalic index (skull length) (Gácsi, McGreevy, Kara, & Miklósi, 2009; Helton, 2009) and breed function (Gácsi, McGreevy, et al., 2009; Udell et al., 2014).

Skull length might have an impact due to associated differences in the position of the eyes, allowing for binocular vision and the density of retinal ganglion cells (McGreevy, Grassi, & Harman, 2004). So far it has been demonstrated, that trainability and responsiveness to pointing vary with skull length (Gácsi, McGreevy, et al., 2009; Helton, 2009). 'Cooperative worker' breeds (i.e. gundogs and herding dogs which work with continuous visual contact of their human partner) were found to be better at following human cues than 'independent worker' breeds which work without human visual contact (e.g. sled dogs, hounds, guarding dogs) (Gácsi, McGreevy, et al., 2009; McKinley & Sambrook, 2000; Wobber, Hare, Koler-matznick, Wrangham, & Tomasello, 2009). The success of cooperative dog breeds in following human pointing might be specifically related to the demands of human-dog cooperation, but alternatively they could be due to a more persistent predatory response (Udell et al., 2014) i.e. to fixate and follow moving stimuli, since pointing implies movement and directionality.

How these breed dimensions could affect gazing, previously implicated as an explanation for dogs' competence in following human pointing (Miklósi et al., 2003), has not been explored before.

Based on findings in the human literature we can also expect responsiveness to the eyes and gaze to change with age, through change in perceptual processes that specifically affect face perception (Owsley, Sekuler, & Boldt, 1981; Thomas et al., 2007) or due to a decline in the ability to process visual social signals (Chan, Pianta, & McKendrick, 2014). A comparison with dogs would be relevant as they have been argued a suitable model animal for studying human aging (Adams, Chan, Callahan, & Milgram, 2000; Cummings et al., 1996; Szabó et al., 2016). This could affect orientation to socially relevant visual cues and explain socially inappropriate behaviour in the elderly (Henry, von Hippel, & Baynes, 2009; Slessor, Laird, Phillips, Bull, & Filippou, 2010).

Previous work using two-dimensional images of dogs and humans, as well as still facial expressions, suggests the features visible in pictures resemble the real stimulus sufficiently to elicit corresponding behavioural responses in the dog (Somppi, Törnqvist, Hänninen, Krause, & Vainio, 2012; Ruffman & Morris-Trainor, 2011; Törnqvist et al., 2015). In the present study, we investigated how sex, skull length, breed function, and age could predict the responses of dogs to images of human and dog faces, shown either as facing the observer (portrait, both eyes are visible) or a side (profile) view corresponding to averted gaze. We operationalized attention as the duration of looking time dogs displayed toward still images of human and dog portraits and profiles. In accordance with the findings discussed above, we expected that younger dogs, female individuals, as well as dogs of cooperative breeds and brachycephalic dogs, will look longer at the images, specifically the portraits, which could attract attention due to either their role in dog-human communication or as threatening cues. To test more specifically if the images were perceived as threatening, considering the aversive nature of eye contact in wolves (Rudolf Schenkel, 1967), we measured approach latency to food rewards placed in front of the pictures, expecting, in particular, longer approach times for food rewards placed in front of dog images.

11.3 Methods

Subjects

The owners of 38 family dogs (20 males, 18 females, 1-15 years old, mean age=5.7 years) volunteered to participate in the study. For each breed related category (i.e. skull length, breed function) we recruited dogs from breeds of different size (see Table 1), i.e. the sample was balanced for size. Each dog participated in two conditions without delay. In a pilot study we observed that dogs looked less at the images after having received food, therefore the foraging situation condition followed the spontaneous looking condition. Only the spontaneous reaction of the animals was of interest, therefore there was no pre-training. Using the classification of Gácsi et al. (2009) the dogs were characterized along two dimensions. First, the breed function – cooperative or independent work breeds, i.e. cooperative dogs rely on visual feedback from the human partner during work, independent dogs do not, see above. Some breeds and mixed-breed dogs could not be reliably characterized, so they were listed under ‘unspecified’. Second, dogs were also classified according to their skull length, defined by the skull-index (width/length*100 (Evans & De Lahunta, 2013)) as brachycephalic (≈81), mesocephalic (≈52) and dolichocephalic (≈39). In most anatomic investigations to date (Georgevsky, Carrasco, Valenzuela, & McGreevy, 2013; M. J. Schmidt, Neumann, Amort, Failing, & Kramer, 2011; Stone, McGreevy, Starling, & Forkman, 2016) the width to length ratio is usually given as varying between 50 and 60 for mesocephalic dogs. Dogs with values above and below this range are identified as brachycephalic or dolichocephalic respectively. Each of these categories was represented by at least 4 male and 4 female dogs (Table 12).

ID	Name	Sex	Breed	Skull Length	Breed Function	Age (years)
1	Áfonya	Female	Mix	Mesocephalic	Unspecified	2
2	Bruni	Male	Mix	Mesocephalic	Unspecified	10.5
3	Connor	Male	French bulldog	Brachycephalic	Unspecified	1.5
4	Csele	Female	Mudi	Mesocephalic	Cooperative	5
5	Daisy	Female	Staffordshire terrier	Brachycephalic	Independent	8
6	Dorka	Female	Labrador retriever	Mesocephalic	Cooperative	2
7	Ebola	Female	Boxer	Brachycephalic	Unspecified	9
8	Foltos	Female	Beagle	Mesocephalic	Independent	8
9	Freddie	Male	Dachshound	Dolichocephalic	Independent	4
10	Fruzsi	Female	Dachshound	Dolichocephalic	Independent	9
11	Hummer	Male	Mix	Mesocephalic	Unspecified	4
12	Jacko	Male	Boxer	Brachycephalic	Unspecified	9
13	Joker	Male	Parson russel terrier	Mesocephalic	Independent	1
14	Kamilla	Female	Labrador retriever	Mesocephalic	Cooperative	8
15	Koda	Male	Siberian husky	Mesocephalic	Independent	2
16	Lajos	Male	French bulldog	Brachycephalic	Unspecified	1
17	Lili	Female	Mix	Mesocephalic	Unspecified	2
18	Liza	Female	Mix	Mesocephalic	Unspecified	2.5
19	Manfréd	Male	Dachshound	Dolichocephalic	Independent	9
20	Mangó	Male	Golden retriever	Mesocephalic	Cooperative	14.5
21	Mása	Female	Boxer	Brachycephalic	Unspecified	2.5
22	Matyi	Male	Mix	Mesocephalic	Unspecified	7.5
23	Maya	Female	Cairn terrier	Mesocephalic	Independent	1
24	Miró_1	Male	Golden retriever	Mesocephalic	Cooperative	5.5
25	Miró_2	Male	Beagle	Mesocephalic	Independent	4.5
26	Mixi	Female	Foxterrier	Dolichocephalic	Independent	9
27	Mogyoró	Male	Mix	Mesocephalic	Unspecified	6
28	Molly	Female	Mix	Mesocephalic	Unspecified	5
29	Odie	Male	Beagle	Mesocephalic	Independent	6
30	Pötyi	Female	Sheltie	Dolichocephalic	Cooperative	9
31	Samantha	Female	Barzoi	Dolichocephalic	Independent	8
32	Scooby	Male	Border collie	Mesocephalic	Cooperative	12.5
33	Sophie	Female	Westie	Mesocephalic	Independent	9
34	Szuszi	Female	Pug	Brachycephalic	Unspecified	4
35	Twister	Male	Boxer	Brachycephalic	Unspecified	4.5
36	Walter	Male	Golden retriever	Mesocephalic	Cooperative	3.5
37	Zozito	Male	Barzoi	Dolichocephalic	Independent	3.5
38	Zufi	Male	Barzoi	Dolichocephalic	Independent	3.5

Table 12. Name, sex, breed, skull length, breed function and age of the dogs.

Dogs were assessed for their vision loss by dropping a cotton ball in front of them (part of the standard veterinary examination for visual impairment) and presenting a food pellet on a plate 2 meters in front of the dog; dogs with poor vision were excluded from testing. Five dogs, older than 11 years, were dropped from the original sample (N=43) because of poor vision.

Procedure

A set of 20 pictures showing 20 faces was used in both conditions. The set contained four types of images. All images were taken from the web and edited to match in luminance and contrast features. Women's faces were shown due to previous findings suggesting dogs are more likely intimidated by men (Bálint, Faragó, Miklósi, & Pongrácz, 2016). The sex of the dogs was unknown. Five women portraits, five women profiles, five dog portraits, five dog profiles, all with a closed mouth, 20 in total, 90 cm tall on white background, were used as test-stimuli. The size was chosen to make the relevant cues, like eye-orientation, easy to spot. Faces with closed mouths were chosen to control against emotional expressions being an alternative explanation to eye-orientation (Darwin, 1872) (Figure 11).

The pictures were projected, at a rate of 120 Hz and with a 1024x768 resolution, on a screen on the wall for 15 seconds each; they were presented in a pre-determined pseudo-random order, chosen to avoid more than two similar pictures in succession e.g. two portraits. Every picture defined a trial, the end of which was signalled by a blank slide. The dogs were accompanied by their owner, who sat 4 meters away from the screen, seated in front of the experimenter and behind their dog. Owners were not informed about the hypotheses of the study and were instructed to remain motionless, silent and not look at the dog during the trials.

Both conditions consisted of 10 trials on which 10 of the 20 pictures were presented. In total, a session lasted from 10 to 30 minutes maximum. The image of a bouncing yellow ball and a clicking sound was used to attract the dog's attention to the screen between the trials. A camera placed under the screen, pointing in the direction of the dog, was used to capture screen-directed looking.

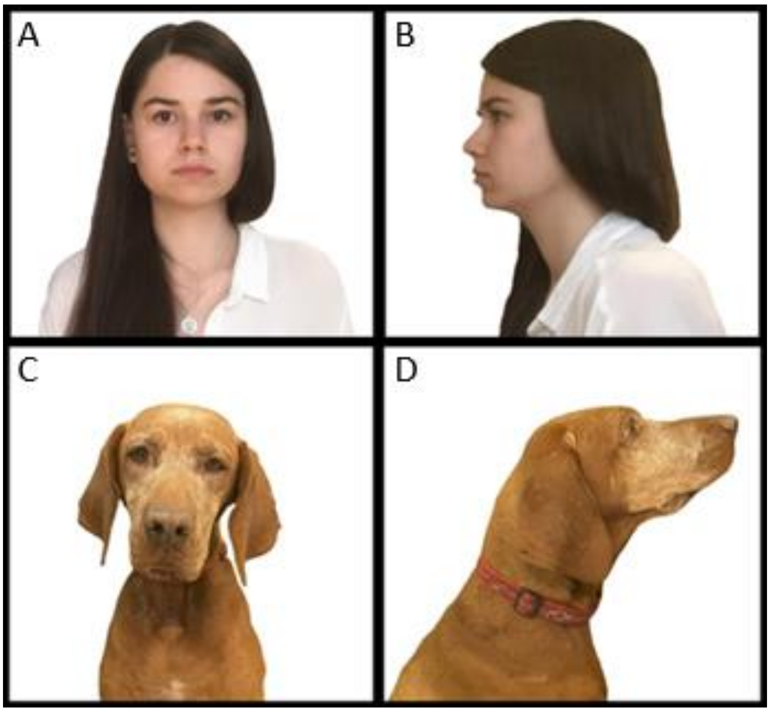

Figure 11. Four types of image were used as test-stimuli. Human portrait (A), human profile (B), dog portrait (C) and dog profile (D).

Spontaneous looking condition

The dog was kept on a tight leash by the owner in front of him/her, facing toward the canvas during the whole condition.

Duration of looking time: We measured the percentage of time the dog spent looking at the screen during the picture projection, i.e. the total looking time (in seconds) over the 15 second stimulus presentation.

Foraging situation condition

The experimenter placed a bowl and in it one piece of dry commercial dog food on the floor, 30 cm away from the centre of the canvas. The placement of food in the bowl was performed while making sure the dog is attending the procedure and looking at the experimenter. Owners were instructed to let the dog off the leash at the beginning of each stimulus presentation, and call their dog back after the trial was over.

Approach latency: We measured the approach time (in seconds), i.e. the latency to approach the bowl containing the food in front of the canvas, from the moment when the picture appeared. The maximum duration for a trial was determined by the maximum duration of the image presentation – 15 seconds. Figure 12 shows the set-up of both conditions.

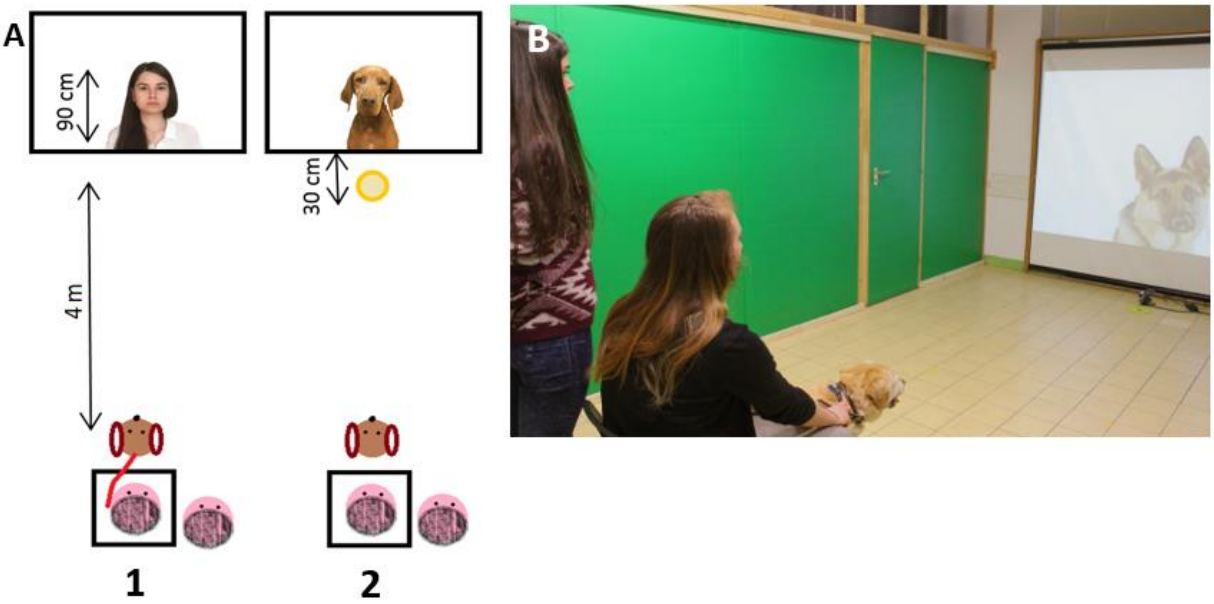

*Figure 12. In the spontaneous looking condition, dog and owner (seated on a chair, depicted on (A) as a rectangular box) were positioned 4 m away from the centre of a canvas, facing it. A bouncing ball was projected to capture the dog's attention, and once it looked in the direction of the wall a new image, 90 cm tall, was projected, starting a new trial (**1**). In the foraging situation condition, a bowl of food was placed 30 cm in front of the centre of the canvas and the dog was allowed to to approach when a new image was projected (2), defining a new trial. (B) Photo about a trial.*

Each session was video-recorded. The owners were instructed to provide no feedback during a trial, and if they talked to the dog or petted it, the trial was repeated (this happened six times for a total of five dogs) or, if noticed only later during coding – excluded (14 trials of 10 dogs). If more than 3 trials had to be excluded the dog's data was discarded. Following these criteria one dog was removed from the data in the Spontaneous Looking Condition. In the Foraging

Situation Condition two other dogs were excluded because they couldn't be motivated to approach the food reward.

A trained observer using Solomon Coder software (beta 091110, developed by András Péter (copyright 2006–2008) at the Department of Ethology, Eötvös Loránd University, Budapest, Hungary) coded the digital video footage. A second coder, naïve to the hypothesis of the study, coded a random selection of the video material (≈30% of the trials). These were 155 trials for measuring looking time, respectively 76 trials for approach latency. We analysed this sample using intra-class correlations to establish the inter-rater reliability. We found robust reliability for looking time duration (N=155; ICC=0.991, P<0.001 average measure for absolute agreement, 2-way random model) and approach time (N=76; ICC=0.999, P<0.001, average measure for absolute agreement, 2-way random model).

Statistical analysis

Statistical analysis was performed using SPSS IBM Statistic version 22. Generalized Linear Mixed Models (GLMMs) with identity function, fit by residual maximum likelihood (REML), were calculated for the dependent variables of each condition, i.e. duration of looking time in the spontaneous looking condition and approach time in the foraging situation condition. A Kolmogorov-Smirnov test indicated that looking time was not normally distributed. The variable was therefore log-transformed, in accordance with recommendations in the literature (Csibra, Hernik, Mascaro, Tatone, & Lengyel, 2016), in order to fulfil the assumptions of normality and homogeneity of variances. To account for any remaining deviation from normality, a robust estimation for the model assumptions was chosen. A Satterthwaite approximation for estimating the degrees of freedom was also applied, as the data were not perfectly balanced across conditions. In each model we included as fixed factors: age (in years), sex (male or female), picture type (human portrait or profile, dog portrait or profile), cooperativeness of breed (cooperative, independent, or unspecified; see Gácsi et al. (2009)), and skull length (brachy-, meso-, or dolichocephalic). We further tested the interactions sex with breed function (2-way interaction) and sex with skull length (2-way interaction), as well as picture type with each of the other predictors, to establish how age, sex, breed function and skull length specifically affect selectivity for the presented stimuli. The model was optimized with backwards elimination. Pairwise post-hoc comparisons for the fixed factors retained in the final model were obtained. For pairwise analysis between categorical factors, in the absence of interaction, a type III test was used to test the significance of the estimates; in the presence of an interaction, estimated marginal means were calculated instead. Main effects of variables involved in interactions were listed and interpreted in the results and discussion if their effect could not be reduced to the interference of the interacting variable.

11.4 Results

Spontaneous looking condition

Results of the GLMM are shown in Table 13.

The effect of picture type was significant (GLMM, $F_{3,240=}$2.893, p=0.036). Overall dog portraits attracted longer looking times than human profiles (estimates M ± SE, ln of duration: 2.4 ± 0.1 versus 2.1 ± 0.1, $t_{1,272}$=2.437, p=0.015).

Breed function predicted looking times (GLMM, $F_{2,239=}$15.708, p<0.001). Overall dogs classified as 'unspecified' displayed the shortest looking times (estimates M ± SE, ln of duration: 1.9 ± 0.1, $p \leq 0.001$).

Skull length predicted looking times (GLMM, $F_{2,254=}$21.946, p<0.001), which were overall longest for brachycephalic dogs (estimates M ± SE, ln of duration: 2.7 ± 0.1, p<0.001); also mesocephalic dogs looked longer at the images than dolichocephalic dogs (estimates M ± SE, ln of duration: 2.1 ± 0.1 versus 1.9 ± 0.1, $t_{1,246=}$2.844, p=0.005).

We found two interactions. Picture type interacted with skull length (GLMM, $F_{6,236=}$2.376, p=0.03,

Figure 13). In mesocephalic dogs (N=22) portraits elicited longer looking times than profiles, whether the image displayed a human or a dog face. There was no significant difference between human and dog faces of the same orientation (portrait or profile). Brachycephalic dogs (N=8) looked significantly longer at dog portraits than at human portraits. Dolichocephalic dogs (N=8) looked longer at dog profiles than at human portraits.

Sex interacted with breed function (GLMM, $F_{2,244=}$5.5, p=0.005). In breeds classified as 'unspecified' (N=15, mixed breed=8) females (N=7) looked significantly longer at the pictures than males, but for cooperative and independent work dogs there was no difference (Figure 14).

Age had no effect on the variables.

Factors	**df**	**F**	**p-value**
picture type	3,240	2.893	**0.036**
sex	1,229	2.153	0.144
breed function	2,239	15.708	**< 0.001**
skull length	2,254	21.946	**< 0.001**
picture type*skull length	6,236	2.376	**0.03**
sex*breed function	2,244	5.5	**0.005**

Table 13. P-values and related parameters for the main effects and interactions on looking time in the spontaneous looking condition.

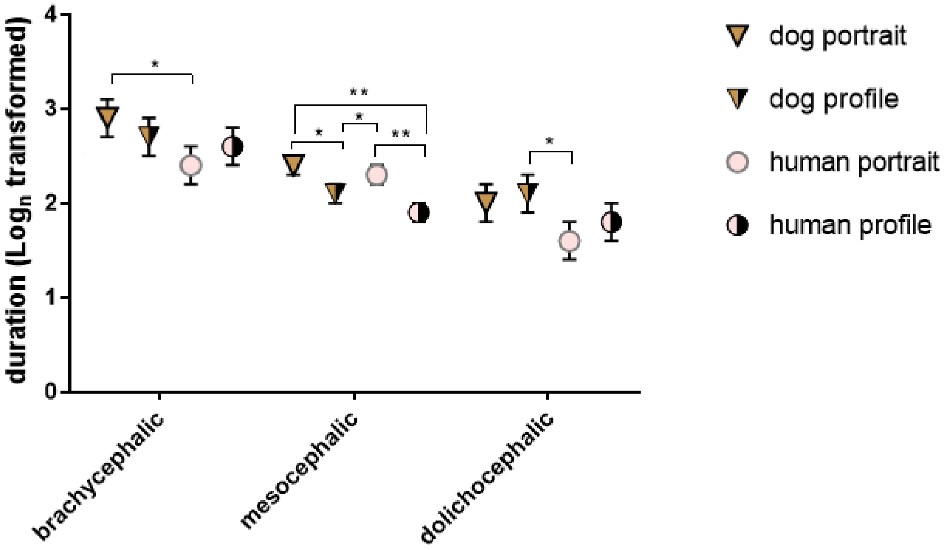

Figure 13. Means and standard errors of log-transformed looking times in the Spontaneous Looking Condition for the picture type x skull length interaction.

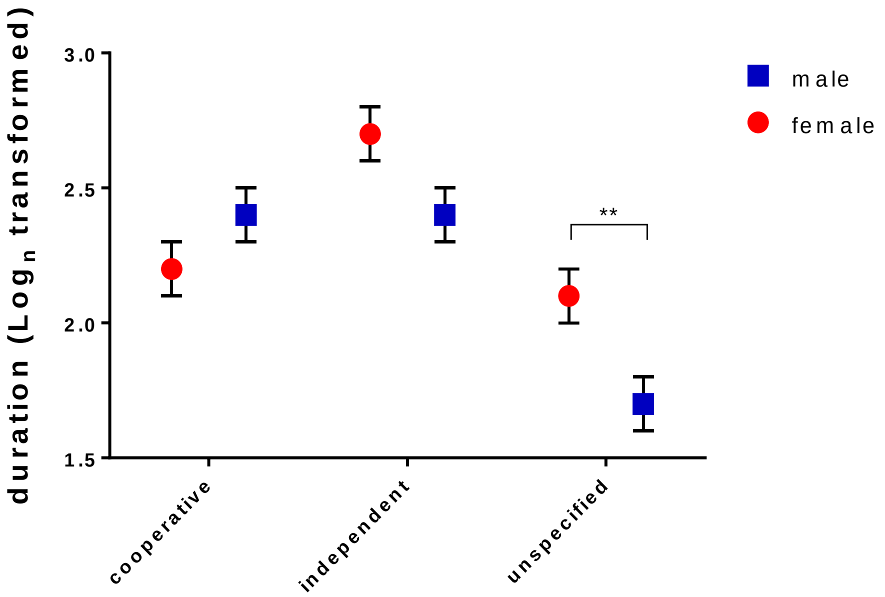

Figure 14. Means and standard errors of log-transformed looking times in the Spontaneous Looking Condition for the sex x breed function interaction.

Foraging situation condition

Results of the GLMM are shown in Table 14.

Factors	**df**	**F**	**p-value**
Age	1,295	15.835	< **0.001**
Sex	1,284	0.712	0.4
skull length	2,285	22.04	< **0.001**

sex*skull length	2,284	28.833	**< 0.001**

Table 14. P-values and related parameters for the main effects and interactions on approach latency in the foraging situation condition.

Approach latency significantly increased with age (GLMM, $F_{1,295}$=15.835, p<0.001, Figure 15).

Skull length influenced approach latency (GLMM, $F_{2,285}$=22.04, p<0.001). Overall mesocephalic dogs approached the fastest (M ± SE, seconds: 3.4 ± 0.1, p<0.001).

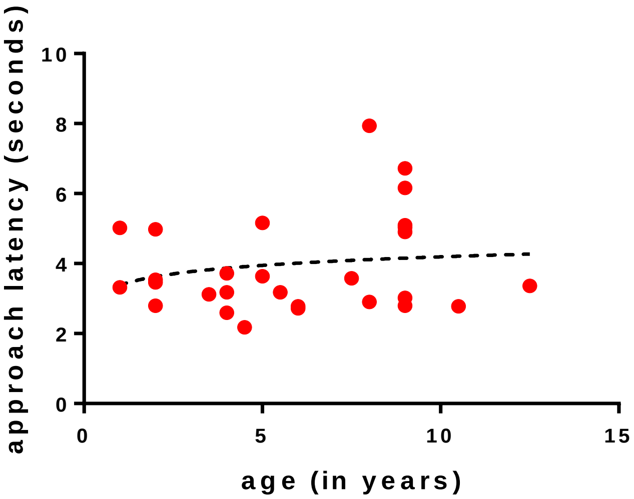

Figure 15. Scatter plot of the average approach latency (seconds) and age of the dogs (in years), in the Foraging Situation Condition.

We found one interaction between sex and skull length: males (N=4) approached the food slower than females among brachycephalic dogs (N=8). In mesocephalic (N=22) and dolichocephalic dogs (N=8) males (N=16) were faster (GLMM, $F_{2,284}$=28.833, p<0.001, Figure 16).

Picture type and breed function had no effect on approach latency.

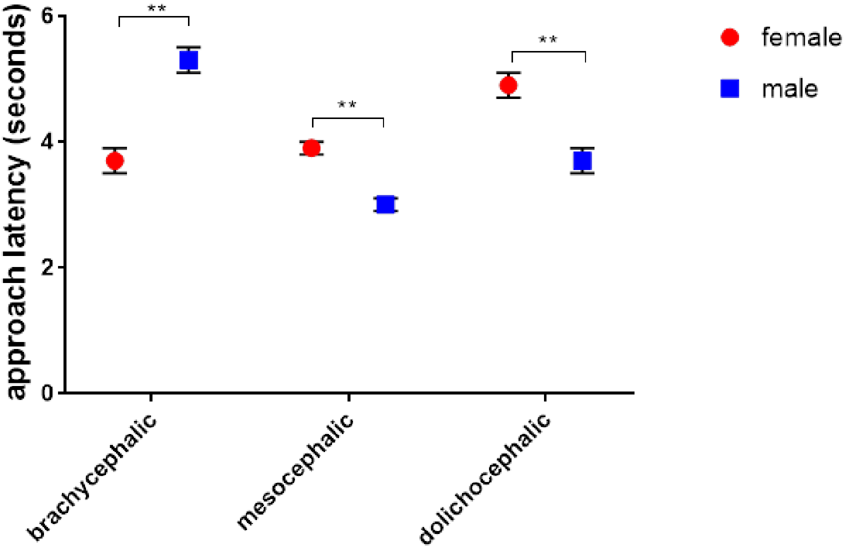

Figure 16. Means and standard errors of approach latency (seconds) in the Foraging Situation Condition for the sex x skull length interaction.

11.5 Discussion

In the present study we measured dogs' reaction to facial images of unfamiliar humans or dogs, shown as portraits or profiles, and compared their responses based on sex, skull length, breed and age.

One prediction was that looking time, measured during a Spontaneous Looking Condition, would be higher in the case of human portraits, reasoning that dogs' gazing behaviour would reflect a preference for eye contact engagement in response to the human images. Eye contact has been found crucial in human-dog bonding and communication (A. Miklósi et al., 2000; Téglás et al., 2012). In contrast, we expected that dog portraits will elicit avoidance, because the literature suggests that in dog-dog interactions eye-contact signifies threat (Öhman, 1986; Rudolf Schenkel, 1967). However, increased looking at images of conspecifics has been reported previously (Somppi et al., 2012) which suggests that aversive stimuli tend to be highly salient and might demand increased attention (Armony & Dolan, 2002). To test whether the images were observed out of interest or perceived as aversive, we additionally tested the dogs' approach behaviour in the presence of the same pictures during a Foraging Situation Condition.

The type of picture used (portrait or profile) influenced looking times differently for dogs of different cephalic index. Only mesocephalic dogs reacted with longer looking times to all portrait images, regardless if humans or dogs were shown. Brachycephalic dogs looked longer at dog portraits than at human portraits, and dolichocephalic dogs looked longer at dog profiles than at human portraits. Across dogs of different skull length, the difference in looking times directed at dog portraits versus human profiles was persistently significant, indicating that these two images were clearly distinguishable for most dogs. Our data, in contrast to our expectations, does not support the notion that dogs prefer to look at human portraits.

Among dogs with 'unspecified' breed function, females observed the images longer. Many dogs classified as 'unspecified' were mix-breed dogs (8 out of 15, 53.3%). This group showed the shortest looking times overall. Possibly sex differences in gazing disappear in specialized breeding due to a ceiling effect on the possible increase of spontaneous looking responses in dogs.

The finding that females look at faces longer than males is in line with human findings on sex differences in face-directed attention (Bayliss, di Pellegrino, & Tipper, 2005; Connellan, Baron-Cohen, Wheelwright, Batki, & Ahluwalia, 2000; Lutchmaya, Baron-Cohen, & Raggatt, 2002), which are characterized by reduced gaze following and eye contact initiation in men. Shared mammalian evolution (Decety, 2011) could underlie this difference between men and women, but reports of sex effects in the literature are scarce and inconclusive regarding non-human animals (Choleris & Kavaliers, 1999). As there was no inanimate control image, nor different effects of picture type for the sexes, the present finding could also reflect general differences in attention between female and male dogs, suggested also by previous work (C A Müller, Mayer, Dorrenberg, Huber, & Range, 2011). Future studies should investigate if these sex differences are specifically social in nature and due to similar biological substrates, as those found in humans (Lutchmaya et al., 2002).

Sex differences in approach behaviour, observed during the *foraging situation condition*, were strongly associated with skull length, which was previously found to correlate negatively with the density of retinal ganglion cells and therefore affect acuity (McGreevy et al., 2004). Mesocephalic and dolichocephalic females approached the food more slowly than males of the same skull length, but in brachycephalic dogs the relationship with sex was reversed i.e. males approached slower. Questionnaire data on dog personality suggests that females are less bold on average (Kubinyi, Turcsán, & Miklósi, 2009), which predicts that they would approach the image of an unfamiliar dog slower, but this may interact with the higher trainability of brachycephalic dogs (Helton, 2009). Alternatively, the better visual acuity of brachycephalic dogs gives them more certainty to approach novel images, which interferes with the effect of sex on boldness. It would also be interesting to explore in future work how dogs, based on their sex and breed, are expected to behave by the owner. Classical work from human psychology suggests for instance that an individual’s behaviour and performance can be guided by confrontation with stereotypes or bias about that individual’s group/category (Spencer, Steele, & Quinn, 1999; Steele & Aronson, 1995) – a phenomenon known as ‘stereotype threat’.

The study of canine perception and cognition has recently also received attention with regard to age induced changes (Chapagain et al., 2018; Head, Cotman, & Milgram, 2000). The dog has been proposed on several occasions as a model animal for studying human aging (Adams, Chan, Callahan, & Milgram, 2000; B J Cummings et al., 1996). In one of the more recent investigations, border collies of different ages were found to perform differently in sustained attention tasks with a peak at middle age (Lisa J. Wallis et al., 2014). The slowed approach in the Foraging Situation Condition could be either due to above mentioned changes in facial perception (Cynthia Owsley & Sekuler, 1981; Thomas et al., 2007) or due to a decline in sensorimotor functions, which typically accompanies the aging process (Doherty 2003; Wallis et al. 2014).

Overall, our study provided several interesting insights on how dogs process human and conspecific faces. We found that skull length, which was previously shown to indicate quality of vision (McGreevy et al., 2004), attention for visual cues (Gácsi, McGreevy, et al., 2009), and trainability (Helton, 2009), is possibly the most relevant breed characteristic to predict dogs’ facial perception as well. Skull length did not only affect how dogs of different sexes would approach food in the presence of the images in the Foraging Situation Condition, but even more importantly, how looking time changed for different picture types. Dogs within and across breed related categories showed looking times dependent on the type of image used, which was an important internal control for the behavioural significance of the images. Moreover, independent of the picture type, brachycephalic skull length predicted longer looking times, suggesting that between dogs of different cephalic index, differences in gazing behaviour might arise from differences in visual processing (McGreevy et al., 2004).

To highlight the effect that human faces might have on the gazing behaviour of dogs, different methods could prove to be more useful, for instance, comparing the ease with which dogs can be trained to approach portraits or profiles of human or dog faces to obtain rewards. Alternatively, using images of familiar faces the dogs were socialized with could reveal differences in looking behaviour more specifically associated with communicative intent.

The data can be interpreted more consistently if we do not assume that looking time measures preference in the present study. Looking times seem instead to have increased with the behavioural salience of the image. Of the dogs for which audio data was available, more than half vocalized and of the presentations which elicited barking 68% were images of dogs, which also suggests that some, and especially dog pictures had a negative physiological effect. Assuming that vocalizations were caused by the threatening features of dog and/or eye-contact signalling images also offers a plausible explanation for the interactions observed between the type of image and cephalic index of the dogs in the Spontaneous Looking Condition. As brachycephalic dogs have more developed visual capacities – binocular vision and a higher density of retinal ganglion cells (McGreevy et al., 2004), they might more easily distinguish the inherently threatening canine portrait (Schenkel, 1967) from a frontal presentation of the human face, which otherwise shares some basic features: for instance both eyes are visible. Within the same frame of interpretation, the looking duration patterns of mesocephalic dogs might reflect their relatively weaker acuity: while portraits were distinguished from profiles, the dog and human portraits did not elicit different responses in this group. Still, overall mesocephalic dogs were fastest to approach rewards placed in front of the pictures. It is possible that relative to mesocephalic dogs, who occupy an intermediate position with regard to visual acuity (McGreevy et al., 2004), brachycephalic dogs were more distracted by the pictures, as they should be able to extract more information from them, while dolichocephalic dogs were possibly less certain and confident to approach as their visual skills are the weakest. Their skull shape is associated with impaired binocular vision and fewer ganglion cells in the retina. Because picture type did not affect approach latency in the present study, future work will need to address this question more specifically.

Because dogs, at least in some contexts, rely on our faces to understand our behaviours and intentions (Miklósi et al., 2000; Miklósi et al., 2003; Müller et al., 2015; Nagasawa et al., 2011; Téglás et al., 2012) it is important to consider for the refinement of dog-human communication, training and welfare, how the animal's age, sex and breed characteristics impact facial perception. So far previous work could show that trainability and sensitivity to human pointing are impacted by skull length or breed function (Helton, 2009; Udell et al., 2014), but our work to our knowledge is the first to explore how these dimensions of breed categorization and also sex and age affect dogs' response to faces.

12 Age-related changes in human-based personality traits and associations with owner and dog demographics[8]

12.1 Abstract

The aim of this study was to analyze the relationships between four canine personality traits (calmness, trainability, dog sociability and boldness) and dog and owner demographics on a large sample size with 14,004 individuals. German speaking dog owners could characterize their dog by filling out a form. There were five demographic variables for dogs and nine for owners. Two statistical methods were used for investigating the associations between personality and demographic traits: in addition to general linear methods we also used regression trees that are ideal for analyzing non-linear relationships in the structure of the data. The results showed that age had a strong effect on all traits: older dogs were calmer (analogous to human Emotional Stability or reversed Neuroticism), less trainable (human Openness/Intellect), less social (human Agreeableness) and less bold (Human Extraversion) than younger dogs. Calmness was influenced primarily by the dog's age, the neutered status, the number of different types of professional training courses (e.g. obedience, agility) the dog had experienced and the age of acquisition. The least calm dogs were less than 2.5 years old, neutered and acquired after the first 12 weeks of age, while the calmest dogs were older than 6.9 years. Trainability was affected primarily by the training experiences, the dog's age, and the purpose of keeping the dog. The least trainable dogs had not received professional training at all and were older than three years. The most trainable dogs were those who participated in three or more types of professional training. Sociability toward conspecifics was mainly determined by the age, sex, training experience and time spent together. The least sociable dogs were older than 4.8 years and the owners spent less than three hours with the dog daily. The most sociable dogs were less than 1.5 years old. Males were less sociable toward their conspecifics than females. Boldness was affected by the sex and age of the dog and the age of acquisition. The least bold were females acquired after the age of one year or bred by the owner. The boldest dogs were males, acquired before the age of 12 weeks, and were younger than two years old. Other variables, including the owner's gender, age, education, previous experience with dogs, the number of people and dogs in the household, and purpose of keeping the dogs had minor, but detectable effects. Importantly, the characteristics of dogs in the present study were reported by the owners, and the associations with the traits do not necessarily represent a causal relationship.

12.2 Introduction

Personality is often defined as an individual's distinctive pattern of behavior (besides feeling and thinking) that is consistent across time and situations (e.g. Pervin and John 1999). Dog personality is a matter of great public concern, and besides theoretical interest, it has a wide range of practical applications, including significant influence on the dog-human bond.

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[8] Based on: Kubinyi, E., Turcsán, B., Miklósi, Á., (2009). Dog and owner demographic characteristics and dog personality trait associations. *Behavioral Processes, 81,* 392-401.

Personality research has focused on (1) developing tools for characterizing behavior (e.g. Hsu and Serpell 2003; Ley, Bennett, and Coleman 2008; Sheppard, Mills, and Kingdom 2002), (2) looking at breed (genetic) differences (e.g. Strandberg, Jacobsson, and Saetre 2005; Svartberg 2002, 2006; Wilsson and Sundgren 1997), and (3) studying the effect of development or stability of the behavior characteristics over an extended time. In the latter case, individuals are repeatedly tested in early puppyhood, at a juvenile age (time of sexual maturation) and later in adulthood with the aim of evaluating the predictability of certain early behavioral characteristics (e.g. Slabbert and Odendaal 1999; Wilsson and Sundgren 1998).

Importantly, however, as Jones and Gosling (2005) pointed out, the vast majority of dogs tested were in working contexts, and pet dogs, with a fuller representation of dog breeds, were relatively neglected. Also, they mention that few studies investigated dogs over the age of four years, so we know little about how aging affects personality traits. The situation is similar with neutering, although previous studies suggest that there are several personality differences between intact and neutered dogs (e.g. Podberscek and Serpell 1996). Some studies compensated for this imperfection and investigated the associations between dog behavior and independent variables. For example, Bennett and Rohlf (2007) studied the relationship between demographic variables (several were unusual, like the amount of experience the owner reported having with dogs, owners' age, family size, etc.) and dog behavior with a questionnaire survey in 413 adult individuals. They found that problematic behaviors were associated with numerous owner and dog characteristics, although most differences were small. For example, the number of people in the household positively correlated with aggression and disobedience. Dogs acquired from a pet shop had more problematic behaviors. However, involvement in professional training courses and other shared activities decreased the occurrence of problematic behaviors (see also Jagoe and Serpell 1996; Kobelt et al. 2003 for similar results, both based on questionnaires, with several relatively rarely observed characteristics).

However, surveying problematic behaviors exposes only a facet of dog personality. People who would voluntarily complete a questionnaire about their dogs' behavior experience relatively few problematic behaviors (Bennett and Rohlf 2007). The interaction of demographic variables in a large sample of dog owners might reveal yet uncovered associations.

In this study, we aimed to reveal associations between dog personality traits and both dog and owner demographic variables in a large sample size. Demographic variables were chosen mainly based on previously described effects in the literature, like dog's age and sex (in test batteries: see Saetre et al. 2006; Seksel, Mazurski, and Taylor 1999; Strandberg, Jacobsson, and Saetre 2005; Svartberg 2006; Wilsson and Sundgren 1997; in questionnaire-based ratings of individual dogs: Hsu and Serpell 2003; Jagoe and Serpell 1996; Ley, Bennett, and Coleman 2009; Rooney and Bradshaw 2006), but some sparsely investigated variables were also introduced, like the dog's age at acquisition, number of people and dogs in the household, owner's experience with previous dogs, purpose of keeping the dog and time investment in the dog.

As in similar studies (see Chapters 7,0,13,15), we used the Internet as the medium for reaching a large audience within a short time period. Our sample is representative of people who are relatively engaged with their dog: they are probably reading dog magazines, are interested in the popular dog literature and are keen on learning some new aspects of their dog's personality. Accordingly, the present sample is a sub-population of German speaking dog owners, but could be considered to resemble dog owners in Western Europe with similar socioeconomic status. External validity of the present questionnaire was investigated by looking at reports available in the literature on dog personality and behavior.

12.3 Methods

Subjects

In this study we collected owner’s reports on 14,004 dogs. Owners filled in an online questionnaire in German which was advertised in the “Dogs” magazine (published by Living at Home Multi Media GmbH, Hamburg, August 2007 issue) and the magazine’s website (www.dogs-magazin.de). It was accompanied by a short article and was available from the end of August 2007 to the beginning of January 2008. The first questionnaire arrived on August 28, 2007 and the last one on January 8, 2008.

Dogs younger than one-year-old (26.8%) were excluded from this sample, because literature suggests that their behavior cannot be considered as stable over time. After this correction, the sample size was 10519. The sample comprised 267 breeds which were represented by one to 527 individuals per breed (the latter was the Labrador retriever). 3920 dogs were categorized by their owners as mixed-breed. The descriptive statistics of the sample are presented in Table 15.

The mean age (± SD) of the dog in the sample was 4.2 ± 3.1 years. 56.1% of the dogs were males. 43.1% of the dogs were neutered (39% of males and 48% of females). Half of the dogs were acquired before the age of 12 weeks (53.7%), and only 1.9% were bred by the owner. Approximately one-third of the dogs (35.3%) had not participated in any kind of professional training courses. The respondents were mainly female (79.6%). The majority of them (64.9%) were between 31 and 60 years old, and only 5.3% were younger than 18 years old. Most of the respondents (40.3%) had secondary education, 26.0% had high school, 22.3% had primary school and 11.4% had a university degree. The average number of people in the households (± SD) was 2.8 ± 1.4. The majority (41.7%) of the respondents resided in a two-person household. Participants reported owning only one dog (66.9%) or two dogs (20.6%). Family member (93.3%) was marked as the most common purpose of keeping the dog. Other, non-exclusive functions were mentioned less frequently (hobby: 49.5%; protection: 10.1%; other work: 2.0%, breeding: 2.4%). 66.8% of the respondents had experience in keeping a dog previously (the mean (± SD) number of previous dogs was 1.2 ± 2.2). Approximately 70% of the respondents claimed spending more than three hours with the dog per day and playing with it every day.

DOGS		**Missing data**
age	mean ± SD=4.2 ± 3.1	0
sex	male: 56.1%; female: 43.9%	0
neutered status	intact: 56.9%; neutered: 43.1%	0
age at acquisition	bred by the owner: 1.9%; 2-12 weeks: 53.7%; 3-12 months: 22.6%;>1 year: 21.7%	0
training experience	nothing: 35.3%; 1 type: 23.3%; 2 types: 21.5%; 3 types: 11.6%; 4 or more types: 8.3%	1
OWNERS		**Missing data**
gender	man: 20.4%, woman: 79.6%	1
age	< 18 years: 5.3%; 19-30 years: 26.9%, 31-60 years: 64.9%,>60 years: 2.9%	1
education	primary school: 22.3%; secondary school: 40.3%; high school: 26.0%; college degree: 11.4%	1
number of people in the household	mean ± SD=2.8 ± 1.4	10
number of other dogs in the household	0: 66.9%; 1: 20.6%; 2: 7.7%;>2: 4.8%	1
purpose of keeping the dog	familymember exclusively: 45.1%; familymember + other: 48.2%; not familymember: 6.7%	1
number of previous dogs	mean ± SD=1.2 ± 2.2	19
hours spent with the dog per day	0-1: 3.2%; 1-3: 27.0%; >3: 69.8%	1
frequency of playing with the dog per week	1: 3.3%; 2-3: 9.5%; 4-5: 10.6%; 6-7: 76.6%	1

Table 15. Descriptive statistics of the dogs older than one year in the present study (N=10,519).

Procedure

Dog owners were asked to complete two different questionnaires.

1. The “Demography Questionnaire” inquired about demographic attributes of the dog and the owner and social attributes of their interactions (Table 15).

2. The “Personality Questionnaire” was based on a 48-item Human Personality Inventory which is available at de.outofservice.com/bigfive/ and adapted for dog behavior. Scoring: Is the statement true for your dog? 0: not true, 1: partly true, 2: true. Items: The dog (1) Is ingenious, inventive when seeks hidden food or toy (2) Is sometimes distressed, desolate, (3) Is calm, even in ambiguous situations, (4) Fights with conspecifics frequently (5) Is active, eager; (6) Is stubborn, energetic; (7) Can be stressed easily; (8) Is ready to share toys with conspecifics; (9) Is intelligent, learns quickly; (10) Is rather cool, reserved; (11) Is shy with conspecifics; (12) Is not hostile with people; (13) Is very easy to warm up to a new toy; (14) Is unassertive, aloof when unfamiliar persons enter the home; (15) Is emotionally balanced, not easy to rile; (16) Is passionless and holds him/herself apart; (17) Often does not understand

what was expected from him/her during playing; (18) Is sometimes fearful, awkward; (19) Is cool-headed even in stressful situations; (20) Is bullying with conspecifics; (21) Is not much interested except in eating and sleeping; (22) Is very self-confident; (23) Is sometimes anxious and uncertain; (24) Gets on well with conspecifics.

42 owners were asked to complete the questionnaire a second time, one day after the first completion.

Statistical analysis

SPSS 13.0 was used for the analysis. To condense the items of the Personality Questionnaire, principal component analysis was used with Varimax rotation with Eigenvalue>1 (Kline, 1994). The number of extracted factors was decided after visual inspection, using the rules of the Scree test (Cattell, 1966). Factor scores were calculated automatically by the SPSS software using the Regression method. Cronbach's alpha was calculated to assess the internal reliability of extracted factors and for testing the repeatability of the questionnaire (Xie & DeVellis, 1992). Univariate general linear model tested the main and all two-way interaction effects of independent variables on the personality traits. Dog age, number of people in the household and number of previous dogs were covariates, sex of the dog, neutered status, age at acquisition, training experience, gender of the owner, age of the owner, education of the owner, number of other dogs in the household, purpose of keeping the dog, hours spent with the dog per day, frequency of playing with the dog per week were fixed factors. We carried out four regression tree analyses, one for each personality trait, to examine the relation between the demographic variables and the traits. Regression trees are ideal for analyzing complex numeric and/or categorical data and detecting non-linear relationships in the structure of the data (Karels, Bryant, and Hik 2004; Nagy et al. 2010). We decided to use this method, because the large number of explanatory variables used in this study does not facilitate the revealing of complex interactions by the generally used univariate analyses. The process resembles a human judgment process. The tree is constructed by dividing data into mutually exclusive groups, called nodes. In one node, individuals have similar values for the dependent variable. The output is a tree diagram with a parent node at the top containing the entire data set. The parent node is split into child nodes based on the independent variable that reduces the most total variation within the dependent variable. Having considered all possible splits, the most suitable split is retained. The process is repeated on the next grouping level. The number of data divisions is determined using a cross-validation procedure by randomly drawing samples from the data set to evaluate the predictive error of the tree (De'Ath & Fabricius, 2000).

We used the CHAID statistical technique (De'Ath & Fabricius, 2000). CHAID uses an F test if the variable is continuous (e.g. the dog's age in our case) and $\chi2$ if the variable is categorical (e.g. gender of the owner). In order to facilitate interpretation, we specified the minimum number of cases as 2000 for parent nodes and 1000 for child nodes.

12.4 Results

Factor extracting

17 of the 24 items were grouped into four factors that accounted for 58% of the common variance in item scores (Table 16).

The stability of the factors was tested on a derived sample. We have randomly chosen 25 individuals from each breed with at least 25 representatives. Principal component analysis was run on this sample with the same parameter setting. Factor structure and the item-loadings were exactly the same as on the original sample that confirmed the stability of the factors (see Svartberg and Forkman 2002).

Items loading higher than 0.5 were used for the interpretation of the factors. The factors were given the following labels: Calmness (five items), Trainability (five items), Dog sociability (sociability towards dogs) (four items), and Boldness (three items). Three out of the four Cronbach's alpha values were above 0.7, but the value for the Boldness factor was lower (0.65), indicating that more related items would need to be added Table 16). However, values above 0.6 are usually considered as satisfactory (e.g. Hsu and Serpell 2003).

ITEMS	**CALMNESS**	**TRAINABILITY**	**DOG-SOCIABILITY**	**BOLDNESS**
19: Is cool-headed even in stressful situations	**0.82**	0.04	0.15	-0.03
15: Is emotionally balanced, not easy to rile	**0.79**	0.06	0.16	0.04
3: Is calm, even in ambiguous situation	**0.78**	-0.01	0.11	-0.07
23R: Is sometimes anxious and uncertain	**0.73**	0.07	0.05	0.33
7R: Can be stressed easily	**0.71**	0.05	0.18	0.22
9: Is intelligent, learns quickly	0.10	**0.72**	0.03	-0.14
17R: Often does not understand what was expected from him/her during playing	0.16	**0.71**	0.01	-0.01
13: Is very easy to warm up to a new toy	-0.04	**0.68**	0.07	0.23
1: Is ingenious, inventive when seeks hidden food or toy	0.06	**0.64**	-0.04	0.06
21R: Is not much interested except in eating and sleeping	-0.10	**0.62**	0.13	0.17
24: Gets on well with conspecifics	0.19	0.08	**0.82**	0.01
4R: Fights with conspecifics frequently	0.15	0.02	**0.81**	-0.08
20R: Is bullying with conspecifics	0.09	0.06	**0.76**	0.19
8: Is ready to share toys with conspecifics	0.10	0.02	**0.54**	-0.09
10R: Is rather cool, reserved	-0.08	0.19	0.04	**0.77**
14R: Is unassertive, aloof when unfamiliar persons enter the home	0.12	-0.05	0.00	**0.71**
18R: Is sometimes fearful, awkward	0.32	0.15	-0.09	**0.70**
Explained variance	23.81%	13.86%	11.41%	8.60%
Cronbach alpha	0.85	0.71	0.75	0.65
Eigenvalues	4.05	2.35	1.94	1.46

Table 16. Items, factor structure, loadings of items, explained variance, Cronbach's alpha and Eigenvalues of factors. Item numbers followed by R were reverse coded.

Test-retest reliability

The test-retest reliability of the factor was found to be acceptable: Cronbach's alpha for the factor scores of the first and second questionnaire-filling was 0.88 for calmness, 0.87 for trainability and sociability and 0.83 for boldness.

Interactions between the personality factors and independent variables

Calmness

Figure 17 shows the regression tree model predicting calmness. Age, age at acquisition, neutering and training experience had the most significant effect on the calmness factor. The crossvalidation method separated the whole sample into four subgroups by age ($F=89.72_{3,10515}$, $p<0.001$). Dogs older than 6.9 years were subdivided by the age at acquisition ($F=8.88_{1,2177}$, $p<0.001$). Dogs acquired before the age of 12 weeks were reported to have the highest mean calmness in the sample (mean±SD=0.35±1.0). This node consisted 10.2% of the dogs.

The 1-2.5-year old and 2.5-4.8-year-old subgroups were subdivided by neutered status ($F=81.86_{1,4318}$, $p<0.001$; $F=36.30_{1,2668}$, $p<0.001$ respectively). Unaltered dogs had higher mean calmness in both subgroups. Dogs with the lowest mean calmness were neutered and less than 2.5-year-old (mean±SD=-0.32±1.0). This node consisted of 13.6% of the sample.

The node of unaltered dogs younger than 2.5 years were further divided; the split was according to the dog’s training experience ($F=14.54_{1,2875}$, $p<0.001$). Dogs without any or with one type of training courses (e.g. guarding, agility) had lower calmness score.

In short, older dogs were calmer than their younger counterparts, and neutering was related to less calmness. Earlier acquisition of the dog was reported to enhance its calmness.

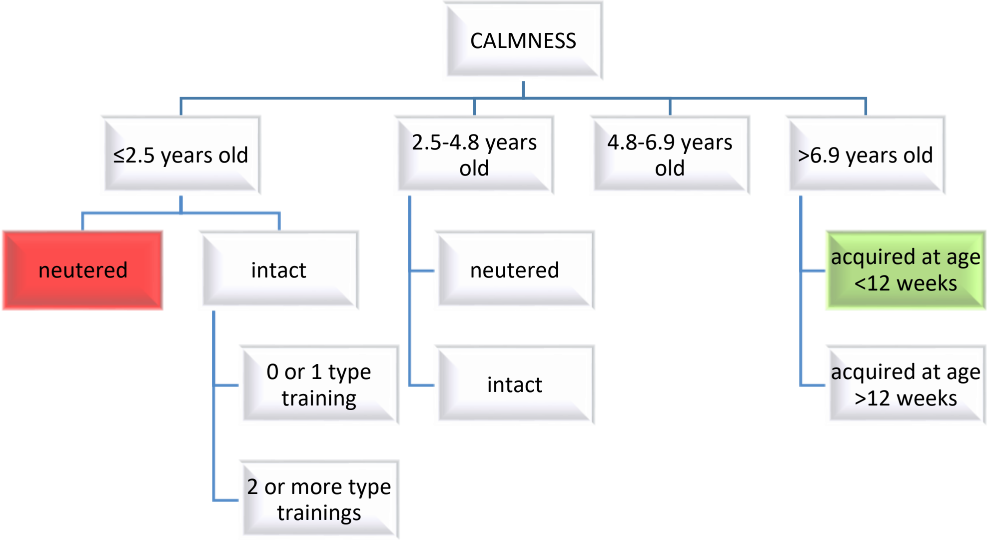

Figure 17. Regression tree model for calmness trait. Green highlights the highest mean, red the lowest mean.

According to the GLM analysis, every independent variable had an effect on the calmness trait ($F=10.63_{78}$, $p<0.001$). Below we list those effects that were not presented on the regression tree.

Main effects:

Owner gender: Men had calmer dogs than women ($F=9.59_1$, $p<0.001$).

Owner age: Owners under the age of 18 reported to have calmer dogs than others. 19-30-year-old owners had less calm dogs than others ($F=8.57_3$, $p<0.001$).

Other dogs in the household: More dogs were related to higher calmness ($F=4.24_3$, $p<0.01$).

Interactions:

Neutered status and age: Calmness positively correlated with the age of the dogs in both neutered and intact dogs. However, the correlation was stronger in neutered dogs ($F=19.03_1$, $p<0.001$).

Sex and neutered status: Intact dogs were reported to be calmer than their neutered counterparts in both sexes. However, the difference was less significant in case of females than in case of males ($F=3.94_1$, $p<0.05$).

Sex of the dog and number of people in the household: In female dogs the number of people was positively correlated with the calmness score: more people around was related to higher calmness. However, in male dogs there was no correlation ($F=11.81_1$, $p<0.001$).

Neutered status and frequency of playing with the dog per week: Daily playing was related to a significantly higher calmness in intact dogs, however, this difference disappeared in case of the neutered dogs ($F=3.96_3$, $p<0.05$).

Age at acquisition and education: Higher calmness was detected at dogs bred by the owner. In case the dog was acquired after its first birthday, secondary and high school educated owners had calmer dogs than primary school and university educated owners ($F=2.02_9$, $p<0.05$).

Age at acquisition and age: Calmness positively correlated with the age in every 'age at acquisition' groups, except in the 'bred by the owner' group, at which there was no correlation ($F=3.39_3$, $p<0.05$).

Age at acquisition and owner age: Children had the calmest dogs. Owners above the age of 60 years had generally less calm dog than others. However, in case of other owners, those, who acquired their dogs before the age of 12 weeks, reported moderately higher calmness compared to a delayed acquisition ($F=1.89_9$, $p<0.05$).

Purpose of keeping the dogs and hours spent together: Calmness increased with the longer time the owner and the dog spend together. However, among owners, who claimed that their dog has other function besides being the member of the family, the reported calmness did not differ between the dogs (since it was relatively high primarily, $F=2.83_4$, $p<0.05$).

Purpose of keeping the dog and owner age: Owners above the age of 60 years had less calm dogs in case they kept their dog as family member only ($F=2.46_6$, $p<0.05$).

Owner age and training experience: Dogs with at least two types of training experience were generally calmer than others, except in the group of owners under 18 and above 60 years ($F=2.18_{12}$, $p<0.05$).

Trainability

The regression tree model for predicting the trainability of dogs is illustrated in Figure 18. Number of professional training courses the dogs has received (e.g. puppy class, obedience, agility), the age of the dog and purpose of keeping the dog were detected to have the most significant effect on the trainability score.

The first split was predicated on training experience ($F=220.13_{3,10515}$). The subgroup of dogs who attended at least three types of professional training courses had the highest mean trainability (mean±SD=0.38±0.8). This group consisted of 19.8% of the sample.

The group of dogs without professional training experience was further split into two child nodes based on the age of the dog ($F=123.88_{1,3707}$). Untrained dogs which were around or older than three years, had the lowest mean trainability in the sample (mean±SD=-0.47±1.2). This node consisted of 17.2% of the dogs.

Dogs in the one type of training experience group generally attended basic obedience courses (puppy class and basic class). Their group was divided to two terminal nodes based on the purpose of keeping the dog ($F=9.10_{1,2463}$, $p<0.01$). Dogs described as the member of the family without any special purpose had lower mean scores on trainability than those dogs that had a more specific function in addition (e.g. work, guarding, etc.).

The two types of training experience group, similarly to the untrained dog-group, was split based the age of the dog ($F=40.19_{1,2259}$, $p<0.001$). 2.5-year-old or younger than 2.5-year-old dogs were reported to be more trainable than older dogs with similar types of training experience.

In short, according to the regression tree, the most important factor related to the trainability is the training experience of the dog. Additionally, younger dogs were generally reported to be more trainable than older dogs.

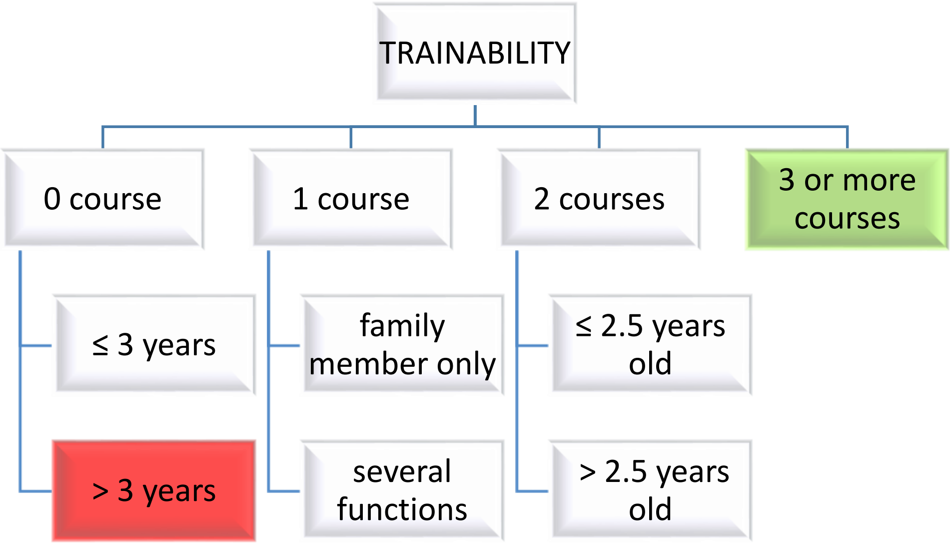

Figure 18. Regression tree model for trainability trait. Green highlights the highest mean, red the lowest mean.

The GLM analysis revealed that every independent variables had detectable effects on the trainability variable ($F=17.30_{111}$, $p<0.001$). Below we list those main effects and interactions that are not represented on the regression tree of Figure 2.

Main effects:

Number of people in the household: More people around the dogs was related to less trainability ($F=5.03_{1}$, $p<0.01$).

Purpose of keeping the dog: Dogs were reported to be more trainable in case their owner attributed specific function to the dog (work, guarding, etc.) not only being a family member ($F=13.70_{2}$, $p<0.001$).

Playing per week and hours spent together: Frequent playing was related to higher trainability. However, those owners who played only once with their dog weekly reported higher level of trainability than it could be expected compared to others ($F=14.44_{3}$, $p<0.001$).

Interactions:

Sex and neutering: Neutered females and intact males were reported to be more trainable compared to neutered males and intact females ($F=4.76_{1}$, $p<0.05$).

Neutering and the age of the dog: In the group of neutered dogs the negative correlation between the trainability and the age of the dogs was stronger than in the group of unaltered dogs ($F=9.23_{1}$, $p<0.01$).

Age at acquisition and owner gender: Women had more trainable dogs than men, except if they acquired the dog after its first birthday ($F=3.01_{3}$, $p<0.05$).

Age at acquisition and number of other dogs: Trainability decreased with the delayed acquisition of the dog. Dogs acquired after their first birthday and kept alone were reported to be the least trainable ($F=2.33_{9}$, $p<0.05$).

Neutering and owner age: Trainability of neutered dogs slightly decreased with the age of the owners. However, in case of intact dogs, 19-30- and >60-year-old owners reported lower trainability ($F=2.99_{3}$, $p<0.05$).

Training experience and age of the dog: Age negatively correlated with trainability in every 'training experience' group, but the correlation was less strong in case of more trained dogs ($F=2.84_{4}$, $p<0.01$)

Age of the dog and hours spent together: The negative correlation between the age of the dog and the trainability score was the strongest in the group of dogs which spent less than one hour with their owner daily ($F=7.59_{2}$, $p<0.001$).

Dog sociability

The regression tree of dog sociability factor can be seen in Figure 19. The first split on the sample was determined by the age of the dog ($F=195.69_{4,10514}$, $p<0.001$). Five age-groups were formed. The youngest group (dogs under or around the one and a half year) had the highest sociability toward other dogs (mean±SD=0.41±0.8). The oldest age group (dogs above 4.8 years) was divided into subgroups by the hours spent together with the owner daily ($F=24.06_{1,3527}$, $p<0.001$). The least sociable dogs were older than 4.8 years and spent less than three hours together with the owner (mean±SD=-0.40±1.0). Dogs who spent more than three hours with the owners were subdivided again, by the sex of the dog ($F=20.37_{1,2437}$, $p<0.001$). Females were found to be more sociable toward their conspecifics than males.

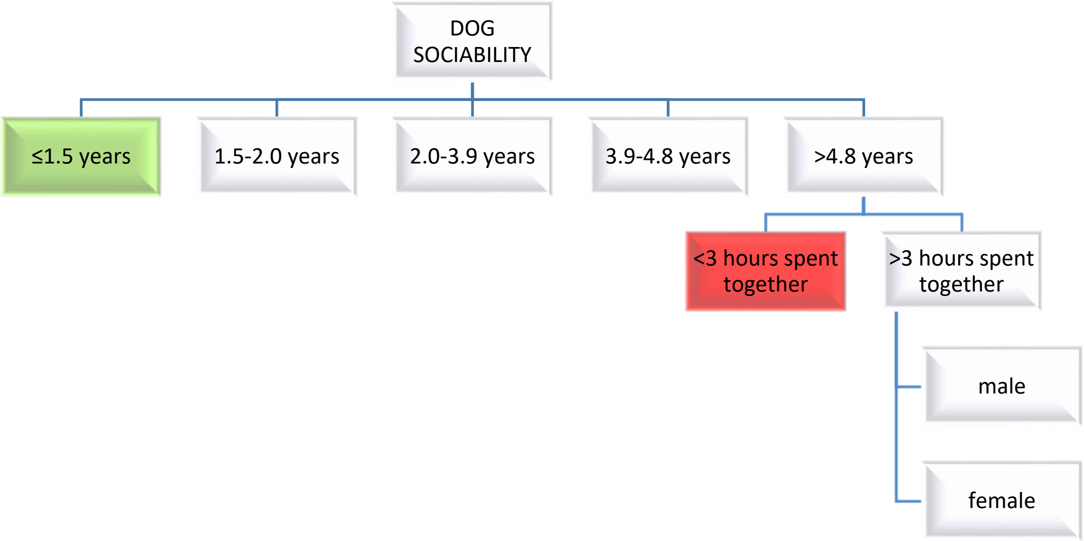

Figure 19. Regression tree model for dog sociability trait. Green highlights the highest mean, red the lowest mean.

According to the GLM, every independent variable had effect on the Dog-sociability factor. ($F=12.19_{81}$, $p<0.001$). Below we list those results that cannot be observed on the regression tree.

Main effects:

Education of owner: Owners who had basic school education only reported the lowest dog-sociality, while owners with a university degree the highest. Secondary and high school educated owners were in between, at a similar level ($F=4.38_3$, $p<0.01$).

Purpose of keeping the dog: Non-family member dogs were less sociable than familymember dogs ($F=7.24_2$, $p<0.001$).

Number of people in the household: Owners from more populated households reported having less dog-sociable dog ($F=23.68_1$, $p<0.001$).

Frequency of playing with the dog: More frequent playing was related to higher dog-sociability ($F=6.26_3$, $p<0.001$).

Interactions:

Acquisition of the dog and gender of the owner: Delayed acquisition of the dog was related to lower sociability in both genders of owners. However, dogs of men, bred by the owner or acquired after the age of first year had the lowest mean of sociability ($F=3.40_3$, $p<0.05$).

The number of professional training courses does not affect the sociability of the dog towards other dogs if they were acquired between the age of two weeks and 12 months. However, dogs that were bred by the owner and received one-two or four or more training courses were reported as less sociable. Dogs acquired after the age of one year and with three types of training experience were also found to be less sociable than the others ($F=1.96_{12}$, $p<0.05$).

Neutered dogs who live with at least with two other dogs were the least sociable compared to others. Intact dogs had a mid-level of sociability except for those who live with at least two other dogs. These dogs were the most sociable compared to the whole population ($F=4.87_3$, $p<0.01$).

Boldness

Figure 20 presents the regression tree model of boldness. The sex of the dog, age at acquisition, and age of the dog had the strongest predictivity on the boldness factor. The first split was related to the dogs' sex ($F=196.59_{1,10517}$, $p<0.001$). Males were divided into three subgroups ($F=28.97_{2,5898}$, $p<0.001$) and females into two subgroups ($F=51.23_{2,4616}$, $p<0.001$) based on their age at acquisition. Two out of these five child nodes were further subdivided by the dogs' ages (2-12 week in males node: ($F=39.51_{1,3179}$, $p<0.001$ and 2 week-12 month in females node $F=34.59_{1,3526}$). The boldest dogs were males, acquired before the age of 12 weeks, and were younger than two years old (12.3% of the sample; mean±SD=0.31±0.8). The least bold dogs were females either acquired after the age of one year or bred by the owner (10.4% of the sample, mean±SD=-0.35±1.1).

In short, younger dogs were bolder in both sexes. Females were outstandingly fearful if they were either bred by the owner or acquired after the age of their first year.

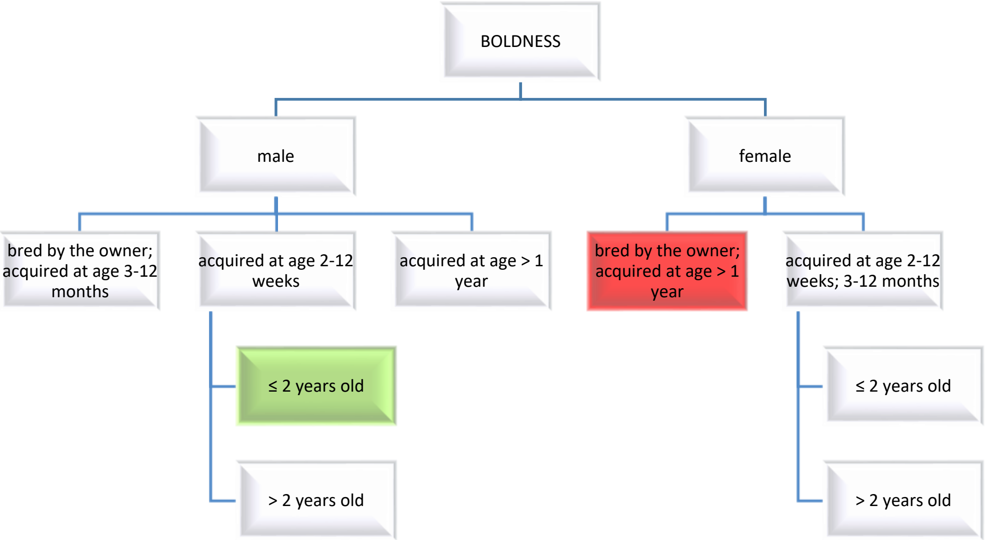

Figure 20. Regression tree model for boldness trait. Green highlights the highest mean, red the lowest mean.

According to the GLM analysis 11 independent variables had effect on the fear factor ($F=10.73_{49}$, $p<0.001$).

Main effects:

Owner gender: Women's dogs were reported to be less bold ($F=6.93_{1}$, $p<0.01$).

Number of other dogs: Single dogs were the boldest, while dogs who shared the household with two other dogs were reported to be the least bold ($F=3.16_{3}$, $p<0.05$).

Interactions:

Age at acquisition and age of the dog: Among dogs who were acquired between the age of 2-12 weeks, boldness negatively correlated with the age of the dog. In other groups there was no correlation ($F=6.61_{3}$, $p<0.001$).

Training experience and age of the dog: Among dogs who attended 0-2 types of professional training courses, age negatively correlated with boldness: older dogs were more fearful.

However, in dogs with three or more training courses there was no correlation ($F=3.74_4$, $p<0.01$).

Neutered status and age at acquisition: Among dogs acquired after the dog's first birthday intact individuals were reported to be bolder than neutered dogs. ($F=3.40_3$, $p<0.05$).

Neutered status and hours spent together: Among dogs who spend less than three hours with their owner daily, neutered dogs were reported to be bolder. However, this trend has changed in the group of dogs who spend more than three hours with their dog daily: in this case neutered dogs were less bold than their unaltered conspecifics. Boldness decreased with the longer time the dogs spend together with their owners in both groups ($F=3.37_2$, $p<0.05$).

Purpose of keeping the dog and number of previous dogs: In dogs who were kept as family members exclusively, more experienced owners had less bold dog. In the groups of dogs who were kept for other purposes as well, there were no correlations ($F=4.06_2$, $p<0.05$).

Sex of the dogs and number of previous dogs: In male dogs, more experienced owners had less bold dogs. In female dogs there was no correlation ($F=6.74_1$, $p<0.01$).

Age of owner and training experience: Dogs of 19-30-year-old owners had bolder dogs than 31-60-year-old owners, except in the case of dogs who attended four or more training courses. Here the trend was opposite ($F=2.15_{12}$, $p<0.05$).

12.5 Discussion

In the present questionnaire-based study with more than 14,000 respondents we identified four dog personality traits: calmness, trainability, dog-sociability and boldness. These traits are analogous to the human traits Emotional Stability, Openness/Intellect, Agreeableness, Extraversion.

The four traits and their converses were described previously by several authors. Jones and Gosling (2005) conducted a meta-analysis on 51 empirical studies that were published before 2004 and extrapolated seven personality traits: reactivity, fearfulness, activity, sociability, responsiveness to training, submissiveness and aggression. Our four traits fit satisfactorily into Jones and Gosling's framework. Their reactivity trait is similar to our calmness trait, and our boldness trait is the converse of their fearfulness trait. While the meta-analysis of Jones and Gosling (2005) covered all areas of dog personality, our study did not touch upon activity, submissiveness and aggression in dogs, although some items in our sociability trait were aggression-related and there is some debate whether activity and submission should be considered a separate or independent personality traits (Gosling and John, 1999; Jones and Gosling, 2005).

The review of Jones and Gosling (2005) covered all types of temperament assessment methods and merged the results of the test batteries, ratings of individual dogs, expert ratings of breeds and observational tests. As Gosling argued elsewhere (2001), owner's ratings of individual dogs are as adequate as behavioral observations in case the ratings' validity and reliability were proved to be appropriate. Our personality traits in the questionnaire showed convincing repeatability and internal consistency. Our constructs had similar associations with independent variables as has been previously reported, which supports convergent validity (see below).

Although working out a new tool (e.g. a questionnaire in this case) for collecting information about dogs' behavior in the home environment might be useful, the more important aspect of this study arises from the multiple demographic questions collected about both dogs and their owners. These variables allowed an extensive study on demographic and trait associations, some of them is new to the literature.

We used two statistical methods for investigating the associations. The more traditional univariate general linear models tested linear relationships and two-way interactions, however, due to the large number of independent variables, the numerous interactions are difficult to interpret. Regression trees are ideal for analyzing complex numeric and/or categorical data and detecting non-linear relationships in the structure (Karels, Bryant, and Hik 2004; Nagy et al. 2010). As far as we know, this method has not been used to analyze large data sets in personality research on dogs, despite the fact that the method shows some advantages over other statistical approaches.

Below we summarize which independent variable had the most significant effect on our personality constructs and then we list the variables one by one for setting them in the literature. Note that the characteristics of dogs in the present study were reported by the owners, and the association with other traits or factors do not necessarily represent a causal relationship.

The results showed that calmness is influenced primarily by the dog’s age, the neutered status, training experience and the age of acquisition. The least calm dogs were less than 2.5 years old, neutered and acquired after the first 12 weeks of age, while the calmest dogs were older than 6.9 years. Neutered dogs of either sex were less calm than their intact counterparts. More daily interaction and more experience with previous dogs on the owner’s part was related to higher calmness. These findings are in accordance with the literature (Bennett and Rohlf, 2007; Wilsson and Sundgren, 1997; Kobelt et al., 2003) which support the convergent validity of this trait. Trainability is affected primarily by the number of types of professional training courses the dog received, the dog’s age, and the purpose of keeping the dog. The least trainable dogs had not received professional training courses at all and were older than three years. The most trainable dogs were those who participated in three or more types of professional training courses. Daily interactions were also crucial for trainability (see Ley and Bennett 2008; Bennett and Rohlf, 2007; Jagoe and Serpell, 1996; Kobelt et al., 2003). Dog sociability was mainly determined by the age, sex, training experience and time spent together. The least sociable dogs were older than 4.8 years and the owners spent less than three hours with the dog daily. The most sociable dogs were less than 1.5 years old. Males were less sociable toward their conspecifics than females. Higher number of people in the household was related to less sociability too (see Bennett and Rohlf, 2007). Boldness was affected by the sex and age of the dog, the age of acquisition. The least bold were females acquired after the age of one year or bred by the owner. The boldest dogs were males, acquired before the age of 12 weeks, and were younger than two years old. These results are in harmony with previous findings (Goddard and Beilharz 1983; Wilsson and Sundgren, 1997; Ruefenacht et al. 2002; Svartberg, 2002; Strandberg et al, 2005).

Regression trees showed which demographic factors are the most predictive for the described personality traits. However, other variables, including the owner’s gender, age, education, previous experience with dogs, the number of people and dogs in the household, and purpose of keeping the dogs also had less significant, but detectable effects.

Several findings revealed in the present study resonate with earlier reports. The dog owners of this study reported that generally older dogs were calmer, less trainable, less social and less bold than younger dogs. This is in harmony with the findings of Bennett and Rohlf (2007) who showed, using questionnaires in a volunteer sample, that the age of the dog was positively associated with unfriendliness and negatively associated with anxious behaviors. In another questionnaire-based study Ley and Bennett (2008) found that extraversion negatively correlated with age, also using a questionnaire. In contrast with these results, Seksel et al. (1999) did not find any associations with age in a behavioral test battery, while Strandberg et al. (2005) observed higher boldness in older dogs.

Age of acquisition is commonly believed to affect the adult behavior of the dog, but scientific evidence is rare. Bennett and Rohlf (2007) even called this belief a misconception, as they did not find any associations between that variable and problematic behaviors. However, according to our results, this variable had a significant effect on all personality traits in this study: dogs acquired before the age of 12 weeks were described as being calmer, more trainable, more social and bolder than dogs acquired later, especially those acquired as adults. This finding could be explained from two different perspectives. On the one hand, owners who acquire a dog before the age of 12 weeks could be more caring and more likely to plan in advance by consulting relevant references on dog behavior. Since the work of Scott and Fuller (1965), the idea that a dog should be adopted before the age of 12 weeks has been widespread in the dog literature. However, as shown by these authors, dogs can be socialized much easier during the so called sensitive period between eight and 12 weeks of age, so that direct positive effects from relatively early interactions cannot be excluded. Importantly, Serpell and Jagoe (1995) reported a relationship between the age of acquisition and problem behaviors. These included increased fear of other dogs and of traffic and was interpreted as the result of the so called “kennel syndrome” in which young dogs are not exposed early enough to a variety of social and non-social stimuli.

From both theoretical and practical points of view, the effect of sex in dogs on various personality traits could be of great importance. We found that neutered females and intact males were reported to be more trainable compared to neutered males and intact females, which suggests that owners decide to neuter their male dogs if they have encountered behavioral problems or disobedience (see Hart and Miller 1985; Bradshaw et al. 1996; Ruefenacht et al., 2002, and Notari and Goodwin 2007 for similar results, and Seksel et al., 1999 and Bennett and Rohlf, 2007, who did not find major differences between the trainability of males and females).

Neutered dogs were less calm in both sexes. Bennett and Rohlf (2007) reported similar findings: desexed dogs were considered to be more nervous than sexually intact dogs. Intact males were the boldest group. Similar findings were reported with reference to “nerve stability” based on the behavioural test batteries (Goddard and Beilharz, 1983; Wilsson and Sundgren 1997; Ruefenacht 2002). Neutered females were less bold than all other sex-groups. Females were more sociable than males independently from altered status, as expected from Notari and Goodwin (2007). However, we have to emphasize again that the associations do not imply causal relationships. Neutering could well be the consequence of having experienced a behavioral problem, not the reason for showing a particular trait (see also Guy et al 2001).

One third of dogs in our sample did not receive any type of professional training courses, similarly to an Australian sample (Kobelt et al. 2003). Dogs without any professional training courses were less calm, less trainable and less sociable than trained dogs. Dogs that received more types of training courses (e.g. guarding, agility) had significantly higher scores for all three traits. Trained dogs were reported as being bolder in comparison to non-trained companions. This corresponds to results obtained by Svartberg (2002). He found that trained dogs tested in the Dog Mentality Assessment test were bolder than untrained companions. In parallel, in a questionnaire survey, both Bennett and Rohlf (2007) and Kobelt et al (2003) reported that trained dogs were more obedient.

Previously there were little data on the relationship between the gender of the owner and the dog's personality. Bennett and Rohlf (2007) found that men reported having more disobedient dogs. By comparing the opinions of 2146 men and 8372 women about the behavior of their adult dogs we found that women's dogs were more trainable, more sociable and less bold than men's dogs. However, neither of these findings show whether the difference is in the eyes of the beholder or indeed if interactions between dogs and humans might be influenced by human sex differences.

We have to note here, that women were considerably more frequent in our sample. This could be explained by assuming that women keep dogs more frequently than men, are more willing to fill in questionnaires, or use the Internet more frequently. However, the latter assumption might not be relevant because other authors who did not require their subjects to use the Internet for filling in questionnaires published very similar gender rates (e. g. 85% of respondents were women in Bennett and Rohlf, 2007).

Older participants in Bennett and Rohlf's study (2007) reported that their dogs were more likely to appear anxious. In our sample, people aged between 19-30 years reported having the least calm dogs. The most trainable and sociable dogs could be found in the 31-60 year-old owner-group. However, the boldness scores of 31-60 year-olds' dogs were lower than those of dogs with 19-30 year-old owners.

We did not find previous data in connection with dog-owners' educational history and their dogs' behavior. In our case primary-school educated owners reported having less trainable and less social dogs than others. People with university degrees judged their dogs to be more social dogs in comparison to secondary-school educated owners.

Number of people in the household is another variable that can influence the behavior of dogs but has received little attention so far. In an Australian sample, dogs from larger families were rated as more disobedient and more unfriendly/aggressive (Bennett and Rohlf, 2007). Based on our sample, we can confirm that dogs in larger families were reported being less social toward theirs conspecifics than dogs living in smaller families. In female dogs the number of people was positively correlated with the calmness score: more people around was related to higher calmness. Additionally, a higher number of people in the household was associated with significantly bolder dogs. One possible explanation could be that people in larger families, which usually have one or more children, show less care and devotion towards their dogs. This seems to be supported by the finding that families with infants and children express a low degree of attachment towards their pets (including dogs), and the opposite is true for single or divorced people (Albert & Bulcroft, 1987).

In a similar vein, people living without children are more devoted to their dogs according to Marinelli et al. (2007). Thus, the family size, and potentially, the quality and quantity of interaction between family members and dogs has an influence on the personality traits of dogs. This suggests that it would be advisable to take this variable into account in future studies.

According to Kobelt et al (2003), the number of dogs in the household did not affect personality traits. We found that a higher number of dogs in the household is associated with higher calmness and trainability, but decreased boldness. The reason for this finding is not clear; however, dogs could provide social partners for each other in the absence of the owner and be less bold on their own.

The reason for living with a dog is a key factor in the human-dog relationship, and it might affect the dog's personality. Companionship is a common reason for acquiring a dog – approximately 80% of a UK sample reported this as the main motivation (Jagoe and Serpell, 1996). In a random sample from Australia, 52% of owners reported that companionship was the reason for getting the dog, and 74% said companionship was the main benefit of having a dog (Kobelt et al., 2003). Dogs chosen for companionship showed lower rates of competitive aggression than dogs acquired for protection, breeding or exercise (Jagoe and Serpell, 1996), in contrast Kobelt et al. (2003) did not find such associations. In our questionnaire, the categories for the function of the dogs were not exclusive, so perhaps it is not surprising that 93.3% of the 14,004 respondents marked the 'family member' category as the function of their dogs. This suggests that German-speaking Western European residents' attitudes towards their dogs can be characterized as affection and sympathy, rather than as economic self-interest (Serpell, 2004). By surveying the adult dogs only, we found that people who consider their dogs as family members exclusively (45.1%) have less calm, less trainable, but bolder dogs than dogs in the "family member and other" category (48.2%). The results suggest that dogs kept without any specific functions (e.g. work, guarding) are not as well-trained as dogs with these functions.

Owners' experience with previous dogs seems to be an important factor. Dogs belonging to first-time dog owners were more likely to show behavior problems (Kobelt et al., 2003). Experienced owners had calmer dogs (Bennett and Rohlf 2007), and experience increased the trainability or working success of dogs (questionnaire surveys: Jagoe and Serpell, 1996; Kobelt et al, 2003; Bennett and Rohlf, 2007; Ley and Bennett, 2008; test battery: Svartberg 2002). Boldness was also higher in dogs living with experienced owners (questionnaire: Jagoe and Serpell, 1996; test battery: Svartberg, 2002). Similar to previous findings, we found that owners who had two or more previous dogs reported having calmer dogs than those owners who had no previous dogs or only one previous dog. Experienced owners had also more trainable dogs. However, contrary to the findings of Jagoe and Serpell (1996) and Svartberg (2002), boldness of the dogs in our sample did not increase with multiple training courses.

It is not surprising that owners who spend more time together with their dogs report to have calmer, more trainable, more sociable and less bold individuals. Since more time together generally means that the dog is kept in the house or in a flat rather than in a garden or a kennel, the result suggests that housing conditions probably affect the investigated traits.

Dog owners who engaged in training activities reported that their dogs were less disobedient, less nervous and more friendly towards people and dogs (Jagoe and Serpell, 1996; Kobelt et al., 2003, Bennett and Rohlf, 2007). We found that people who played every day with their dog perceived their pet to be calmer, more trainable and more social than those who played less. Importantly, this could be interpreted in two ways. People may prefer to play with calmer, more sociable dogs, or dogs could become calmer and more sociable as a result of frequent play.

Despite the virtues of the study (sample size and multiple personality trait-demographic variables associations), it has its own limitations. First, respondents were interested in reading DOGS magazine and completing a personality questionnaire about their dog, which biased the sample population. Presumably, this is one main reason why the majority of the respondents were women, although other authors published similar gender rates (e. g. 85% in Bennett and Rohlf, 2007), which suggests that significantly more women than men have dogs. Second, the demographic variables may certainly be related to each other in several ways that were not uncovered here.

In summary, this large sample of dog owners allowed us to uncover a few major factors that may act as environmental variables in influencing the development of dog personality. Some of these are in agreement with previous studies based on questionnaires or behavioral observations, while others have not previously been reported. We think that the value of such research is in providing hypotheses for future work, which then should be executed under more controlled conditions, including the careful selection of a representative sample and with more focus on using direct behavioral measures.

13 Interventions to increase play and training motivation may alleviate the negative effects of aging[9]

13.1 Abstract

Numerous cross-sectional studies in humans have reported mean-level changes in personality traits across the lifespan, and that significant life events and educational experiences can influence personality traits. The dog has been suggested as a possible model for personality development, however, there is no consensus neither about the number of personality traits, nor abut their definitions. We utilised a reliable and valid questionnaire developed specifically for dogs (Dog Personality Questionnaire) to confirm previous findings on the influence of age and dog/owner explanatory variables on personality. From a sample of 1207 dogs (M_{age}=7.71, SD=4.12, female=54%, purebred=65.5%) results revealed that the internal consistency of the factors was excellent, confirming that the translation of the questionnaire into Hungarian was successful. Three of the five factors showed significant age effects. Activity/Excitability decreased with age, and whilst Responsiveness to training also decreased, only dogs older than 12 years differed significantly from the other groups. Aggressiveness towards animals showed a quadratic developmental trajectory peaking in dogs aged 6 to 10 years. When the models were re-run including the other explanatory variables, age group was no longer significant for the Responsiveness to training trait. The amount of time spent interacting/playing with the owner partially mediated the relationship between age and this trait, implying that interventions to increase play and training motivation may alleviate the negative effects of aging on dogs' trainability. 15 out of the 28 explanatory variables were significantly associated with at least one of the five factors. Weight, breed (pure breed/mixed breed), sex, off-leash activity, diet, previous trauma, age of dog when arrived in the household, play, dog training activities, number of known commands and dog obedience tasks were all associated with personality traits in dogs. Similarly to humans, dogs that had previously experienced trauma scored higher in Fearfulness and Aggression towards people and animals. A strong link between dogs' level of basic obedience and personality was established; dogs with more training showing lower Fearfulness and Aggression towards people and animals, and higher Activity/Excitability and Responsiveness to training. However, it remains an open question whether personality or the owners' reduced training efforts in case of old dogs drives this association.

13.2 Introduction

Although personality is defined as "behavioural differences that are stable across time and situations", there is substantial cross-sectional evidence for mean personality trait change across the lifespan in humans (Roberts et al., 2006). People tend to show increased self-confidence, warmth, self-control, and emotional stability with age, with changes occurring during young adulthood, middle age, and old age. Previous studies have also indicated substantial individual differences in changes; individuals display unique patterns of development at all life stages, which appear to be the result of specific life experiences (Roberts & Mroczek, 2008). Work, marital, family, and educational experiences can all lead to changes

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[9] Based on: Wallis, L., Szabó, D., Kubinyi, E. (2019). Cross-sectional age-differences in canine personality traits and associations with owner and dog demographics. *Submitted.*

in personality traits (Heckman, Pinto, & Savelyev, 2013; Jeronimus, Riese, Sanderman, & Ormel, 2014; Jeronimus, Ormel, Aleman, Penninx, & Riese, 2015; Roberts, Wood, & Smith, 2005).

Cross-species comparisons have been used to examine the origins and adaptive significance of specific personality traits. For example, Gosling & John (1999) used the human Five Factor Model (FFM: openness to experience, conscientiousness, extraversion, agreeableness, and neuroticism) for comparing the personality factors dogs and 11 other nonhuman species. They found four of the five factors, and the canine analogues were labelled: Energy (analogous to human Extraversion), Affection (human Agreeableness), Emotional Reactivity (human Neuroticism), and Intelligence (human Openness/Intellect). The dog has been suggested as a possible model for human personality development, and the influence of personality on health (Cavigelli, 2005; Ley & Bennett, 2007; Mehta & Gosling, 2008). Dogs are observed by their owners on a daily basis, and biological, psychological, social, and health related events are often recorded. Their lifespan is much shorter than ours is, which means developmental studies can be performed in less time. Dogs share an evolutionary and developmental history with humans due to domestication. They are present in many households and are subject to the same environmental conditions. Thus, they can be tested using the same observations and experimental protocols (Ádám Miklósi & Kubinyi, 2016). The high genetic variability and differing environmental experiences found in pet dogs provides the foundation for individual differences and personality (Jones and Gosling, 2005).

However, we know little about how aging and experience may shape personality in pet dogs (Jones and Gosling 2005). Over the last twenty years, much research has focused on studying personality in dogs, as they are common household pets around the world, and play important roles in human society, such as guide dogs, assistance dogs, therapy dogs, military and police dogs, and search and rescue dogs. The number of publications on personality in dogs has increased from roughly one per year in the late nineties to a current average of eight publications per year (google scholar title word search). By far the most common method to assess personality in dogs is through owners' or care-takers individual ratings of individuals' personality traits on a Likert scale (with 1 being the least likely to exhibit the trait, and 5, or 7 as being the most likely (reviewed in (Gartner, 2015)). Evidence suggests that data collected through questionnaires can be accurate and consistent (Henriksson, 2016; Posluns, Anderson, & Walsh, 2017) with demonstrated reliability and validity (Gartner, 2015; Harvey, Craigon, Blythe, England, & Asher, 2017; Kubinyi et al., 2015; Borbála Turcsán et al., 2018). Owners can draw on their experience from a wide range of contexts and situations when they answer questions regarding their dogs' personality, while test batteries are strongly affected by the context in which they are performed, and do not necessarily reflect the dogs' behaviour on a day-to-day basis. The most commonly used questionnaires include the Canine Behavioral Assessment and Research Questionnaire (C-BARQ) (Hsu & Serpell, 2003), the Monash Canine Personality Questionnaire (MCPQ/MCPQ-R) (Ley, Bennett, & Coleman, 2008; Ley, Bennett, & Coleman, 2009), and the Dog Personality Questionnaire (DPQ) (Jones, 2009).

Most studies have concentrated on the early development up to two years, the predictability of certain early behavioural characteristics on adult behaviour, or on senior and geriatric populations (Chan et al., 2002; Neilson et al., 2001; Riemer, Müller, Virányi, Huber, & Range, 2016; Erik Wilsson & Sundgren, 1997). Early experience has been found to have a long-term effect on the personality of dogs (Harvey, Craigon, Blythe, England, & Asher, 2015; McMillan, Serpell, Duffy, Masaoud, & Dohoo, 2013; J P Scott, 1958). Additionally, several studies have established differences in personality between individuals belonging to dog breeds or breed groups (Duffy, Hsu, & Serpell, 2008; Hart & Hart, 1985; Turcsán, Kubinyi, & Miklósi, 2011), as well as between the typical personality of pure breed and mixed breed dogs (Borbála Turcsán et al., 2017).

Besides the effects of early experience and breed, the most commonly reported factors that have been found to influence personality in dogs are age, sex, and reproductive status (Lofgren et al., 2014). Previous studies (listed in Part I. of this theses) did not look for quadratic relationships with age, and in most cases, only a few age groups were compared. Therefore, more detailed questionnaire studies regarding the influence of aging on mean level personality traits are necessary, particularly as the majority of past studies typically examined only a few personality traits, used dogs in working contexts, or only specific breeds, and only a handful of studies investigated dogs of all life stages (particularly those over 4 years of age). One recent study by Chopik and Weaver (2019), is the first to use the validated Dog Personality Questionnaire (DPQ) (Jones, 2009) to examine the degree to which dog personality differs by age (including testing for quadratic relationships) whilst controlling for age differences in sex, breed (pure breed or mixed breed), reproductive status (intact/neutered), whether the dog has attended obedience training, and whether the owner trains their dog themselves or not. Although the sample was heavily biased towards undergraduate students (70% of the sample) and neutered dogs (87%), nevertheless a significant linear age effect was found for the factor Activity/Excitability, and quadratic effects were found for Responsiveness to training and Aggression towards animals. Older dogs were less active/excitable compared to younger dogs, and responsiveness to training and aggression toward other animals was highest among 6 to 8 year old dogs.

Sex effects have been reported in 38% of studies (reviewed in Gartner, 2015), however, reports are often conflicting. In general, results show that males have higher aggression (Chopik and Weaver, 2019; Hart & Hart, 1985; Lofgren et al., 2014; Wilsson & Sundgren, 1997) and boldness (Kubinyi et al., 2009; Starling et al., 2013) and lower sociability (Kubinyi et al., 2009) than females. Conversely, female dogs have higher fearfulness (Gosling, Kwan, & John, 2003; Temesi et al., 2014), and lower dominance over dogs than males (Henriksson, 2016). Neuter status often complicates sex effects, due to the absence or presence of hormones. Intact dogs were found to be bolder (Starling et al., 2013) than neutered dogs. In addition, neutered dogs were found to be less calm (Kubinyi et al., 2009), more aggressive, excitable and anxious (Farhoody & Zinc, 2010) than entire male and female dogs. In one study, entire male Labrador retrievers showed higher owner aggression, and entire females higher trainability (Lofgren et al., 2014). The results of sex effects on personality are inconsistent, so further investigations are necessary with larger sample sizes, to clarify the patterns found, and determine the importance of sex effects in relation to other biological and environmental influences.

So far, personality differences have also been described with regards to coat colour (Lofgren et al., 2014), body size (dog height is negatively associated with neuroticism, and positively with amicability (shorter dogs are considered more fearful and less sociable (Ley et al., 2009; McGreevy et al., 2013)), training history (the most calm, trainable and sociable dogs were found to be those that have participated in three or more types of professional training (Kubinyi et al., 2009)), and owner experience (experienced owners tend to have calmer and more trainable dogs (Bennett & Rohlf, 2007)). Several studies have even found correlations between the owner's and their dog's questionnaire-assessed personality traits (Chopik & Weaver, 2019; Konok et al., 2015; Turcsán, Range, Virányi, Miklósi, & Kubinyi, 2012).

Studies examining how behaviour changes with age and/or breed, rarely take into account lifestyle demographic factors, which have the potential to influence both test battery and questionnaire results (Mirkó et al., 2012; Szabó et al., 2016). For example, environmental factors (such as housing condition (living in a flat, house, and/or garden) can mask, or even enhance genetically potentiated breed differences in personality (Mirkó et al., 2012). Physiological changes with age in the dog may also have an effect on the dogs' perceived personality. Starling, Branson, Thomson, & McGreevy (2013) suggested that a sharp decrease in the personality trait boldness, in dogs aged over 13 years might be explained by age-related degenerative conditions, such as arthritis. Older dogs may suffer from physical pain and discomfort, which may cause them to take fewer risks and to become less inclined to interact with other dogs or people. Therefore, when examining personality in dogs over all life stages, it is important to include a wide range of dog demographic, health, and environmental factors.

The aim of this study was to investigate the effects of age on personality in a cross-sectional Hungarian sample. Additionally, we explored which other factors are associated with dog personality. We measured personality using the Dog Personality Questionnaire (DPQ) (Jones, 2008), as it has been shown to demonstrate reliability and validity, and has been used in numerous studies to measure personality in dogs via owner report (Ákos, Beck, Nagy, Vicsek, & Kubinyi, 2014; Corrieri, Adda, Miklósi, & Kubinyi, 2018; Kuroshima, Hori, Inoue-Murayama, & Fujita, 2016; Riemer et al., 2016). Additionally, it has been found to be the more reliable and trustworthy questionnaire in comparison to C-BARQ and MCPQ-R (Henriksson, 2016), and it achieved a slightly higher average mean consensus estimate of inter-rater reliability than the MCPQ-R (0.54 vs. 0.45) (Posluns et al., 2017). From previous studies, we predicted a strong influence of dog age on dog personality. Purebred dogs were predicted to be rated as less fearful and aggressive than mixed breeds, male dogs less fearful and more aggressive than females, reproductively intact dogs less fearful than neutered, and finally, shared activities and training was predicted to increase responsiveness to training and decrease fearfulness and aggression.

13.3 Methods

Subjects

1365 Hungarian dog owners filled out an online questionnaire, which was advertised on the Eötvös Loránd University Department of Ethology's homepage (http://kutyaetologia.elte.hu), on the Facebook page "Családi Kutya Program", and on the group "Kutyaetológia". The questionnaire was available from the middle of May to the beginning of July 2016. Dogs aged under 1 year were excluded from the full sample of 1365, as previous research has suggested that their behaviour does not remain stable over time (Riemer et al., 2014). Duplicate entries and entries with missing information were deleted, which resulted in data from a total of 1207 individual dogs. The final sample consisted of 66% pure breeds, 54% females, of which 17%

were intact, and 37% were neutered (26% intact males and 20% neutered males). The descriptive statistics of the sample are presented in Table 17.

Breed	TOTAL count (%)	Sex N (%)		Age in months (Mean ± SD)	Weight in kg (Mean ± SD)	Height in cm (Mean ± SD)
		Male	Female			
Mixed Breeds	417 (34.5)	192 (15.9)	225 (18.6)	97.50 ± 51.05	20.10 ± 11.02	43.41 ± 13.15
Pure Breeds	790 (65.5)	365 (30.2)	425 (35.2)	89.80 ± 48.36	21.13 ± 13.88	43.56 ± 15.33
Grand Total	1207	557 (46.1)	650 (53.9)	92.46 ± 49.42	20.77 ± 12.97	43.51 ± 14.61

Table 17. Descriptive statistics of the subjects, including sex, age, breed group, weight, and height information.

Procedure

The on-line questionnaire contained three sections – the demographic data of the dogs and their owners, questions relating to the dogs' personality, and questions concerning possible age-related changes in cognition, impulsivity and interspecific communication (results from this final questionnaire are presented in a forthcoming publication). The "Demographic Questionnaire" collected basic information regarding the demographic attributes of the dog and the owner and social attributes of their interactions. Details from the demographic questionnaire were previously reported in Wallis, Szabó, Erdélyi-Belle, & Kubinyi, (2018), where we examined the descriptive statistics of the variables, and whether the proportion of the dogs allocated to each category of the demographic variables varied among the dog age groups. Three continuous variables were collected from the owners: the dog's current weight (in kg), height at the shoulder (in cm), and age (in months) (Table 1). The rest of the variables were categorical. In addition to reporting the age in months of the dogs, we also allocated the dogs to six age groups, which would allow us to examine non-linear relationships with age.

To measure dog personality traits, we used the "Dog Personality Questionnaire" (DPQ) as it has been shown to demonstrate reliability and validity, and has been used in numerous studies to measure personality in dogs via owner report (Jones 2008, Table 19).

Factor 1 – Fearfulness

Facet 1 – Fear of People
R1) Dog is relaxed when greeting people
6) Dog is shy
27) Dog behaves fearfully towards unfamiliar people
Facet 2 – Nonsocial Fear
3) Dog is anxious
R11) Dog is confident
R22) Dog adapts easily to new situations and environments
Facet 3 – Fear of Dogs
13) Dog avoids other dogs
21) Dog behaves submissively (e, rolls over, avoids eye contact, licks lips) when greeting other dogs
42) Dog behaves fearfully towards other dogs
Facet 4 – Fear of Handling
16) Dog behaves fearfully during visits to the veterinarian
35) Dog exhibits fearful behaviors when restrained
44) Dog behaves fearfully when groomed (eg, nails trimmed, brushed, bathed, ears cleaned)

Factor 2 – Aggression towards People

Facet 1 – General Aggression
7) Dog behaves aggressively towards unfamiliar people
R18) Dog is friendly towards unfamiliar people
40) Dog shows aggression when nervous or fearful
Facet 2 – Situational Aggression
25) Dog behaves aggressively in response to perceived threats from people (eg, being cornered, having collar reached for)
30) Dog behaves aggressively during visits to the veterinarian
36) Dog aggressively guards coveted items (eg, stolen item, treats, food bowl)

Factor 3 – Activity/Excitability

Facet 1 – Excitability
15) Dog is boisterous
31) Dog seeks constant activity
R41) Dog tends to be calm
Facet 2 – Playfulness
R9) Dog gets bored in play quickly
17) Dog enjoys playing with toys
33) Dog retrieves objects (eg, balls, toys, sticks)
Facet 3 – Active Engagement
R4) Dog is lethargic
14) Dog works at tasks (eg, getting treats out of a Kong, shredding toys) until entirely finished
24) Dog is curious
Facet 4 – Companionability
20) Dog seeks companionship from people
R26) Dog is aloof
37) Dog is affectionate

Factor 4 – Responsiveness to Training

Facet 1 – Trainability
R29) Dog is slow to respond to corrections
R38) Dog ignores commands
43) Dog is able to focus on a task in a distracting situation (eg, loud or busy places, around other dogs)
Facet 2 – Controllability
5) When off leash, dog comes immediately when called
R10) Dog is quick to sneak out through open doors, gates
32) Dog leaves food or objects alone when told to do so

Factor 5 – Aggression towards Animals

Facet 1 – Aggression towards Dogs
2) Dog behaves aggressively toward dogs
R19) Dog is playful with other dogs
R34) Dog is friendly towards other dogs
Facet 2 – Prey Drive
8) Dog likes to chase squirrels, birds, or other small animals
23) Dog likes to chase bicycles, joggers, and skateboarders
39) Dog behaves aggressively towards cats
Facet 3 – Dominance over Other Dogs
12) Dog is dominant over other dogs
R28) Dog willingly shares toys with other dogs
45) Dog is assertive or pushy with other dogs (eg, if in a home with other dogs, when greeting)

Table 18. Dog Personality Questionnaire (DPQ, Jones, 2008). Owners scored the amount they agreed with each statement from 1 – I do not agree at all with the statement, to 5 – I fully agree. Items are grouped based on facets and factors but were presented to owners in the order of item numbers. An R in front an item indicates that the item is reverse coded.

Statistical analysis

Generation of factor scores and assessment of reliability

We used the short form of the DPQ, which consisted of 45 items that made up a five-factor solution. We translated the questionnaire into Hungarian, and then back translated into English, to ensure that each items content was preserved. To calculate the facet and factor scores we used the Scoring Key for the DPQ Short Form provided by the author. The scores for each relevant raw item were averaged to create the facet scores. The factor scores were produced by averaging the scores of the facets that made up each specific factor. The raw scores for each item on each facet were summed and then divided by the maximum score possible for that factor to create a percentage, in order to better visualise the results. The five factors were labelled by Jones as "Fearfulness, Aggression towards People, Aggression towards Animals, Activity/Excitability, and Responsiveness to Training". Cronbach's alpha was calculated to assess the internal reliability of the extracted factors (Xie & DeVellis, 1992). The five factors were divided into facets: "Fearfulness" was composed of "Fear of people", "Non-social fear", "Fear of dogs" and "Fear of handling". "Aggression towards people" was divided into "General aggression" and "Situational aggression". "Activity/Excitability" was divided into "Excitability", "Playfulness", "Active engagement" and "Companionability". "Responsiveness to training" was composed of "Trainability" and "Controllability". The last factor, "Aggression towards animals", contained "Aggression towards Dogs", "Prey Drive" and "Dominance over Other Dogs" (Jones 2008).

Statistical models to determine the effects of the demographic variables

Statistical analyses were run on the reduced dataset of 1207 individuals and performed in R 3.3.2 (R Core Team, 2013). The five factors from the DPQ were transformed using the boxcox power transformation (Package "MASS", (Box & Cox, 1982)) to fulfill the assumptions of normality and homogeneity of variance. Separate linear models were first calculated with age as a categorical variable to look for specific differences between age groups on the five factors of the DPQ. Then additional models were run with weight and height included as covariates, and all of the rest of the variables as fixed factors (age group, breed, sex, neuter status, sensory problems, off-leash activity, body condition score, food, vitamins, trauma, health problems, medication, owner age, owner experience, how many other dogs in household, how many people in household, child, dog age when arrived, get dog, where dog is kept, dog obedience tasks, play, commands, dog training activities, time spent alone, and dog behaviour changed). The aim of these linear model analyses was to investigate (1) associations between personality traits and the investigated variables (e.g. demographics of both dog and owner), and (2) to examine whether the behavioural differences between the dog age groups remained significant after controlling for the differences in the other explanatory variables. Due to the large number of predictors used in the models (28 explanatory variables in total), only main effects were analysed, and we did not examine interactions.

Normality and homoscedasticity were assessed via residuals' distribution charts and plots of residuals against fitted values. Due to the large number of variables retained in the models, the Benjamini–Hochberg procedure was utilised to control for the false discovery rate (FDR, Benjamini & Hochberg, 1995). Most of the categorical variables used were ordinal, which allowed group comparisons to the smallest or lowest category. However, post hoc Tukey tests were run on the nominal variables where significant group differences were found. To analyse the effect of outliers, any outliers of z scores of greater than ± 3 were removed from the analysis, and the models re-run.

A mediation model was proposed in order to better explain the mechanism or process that underlies the relationship between personality and dog age, if a previously significant age effect was no longer detectable in the second model including the other explanatory variables. Please note that mediation analysis does not imply a causal relationship. In the case where multiple significant explanatory variables were present in the model, we chose the variable that had the greatest variance explained by dog age (this was determined previously in Wallis et al. 2018). By implementing the Mediation package in R (Tingley, Yamamoto, Hirose, Keele, & Imai, 2015) we estimated the average causal mediation effect (ACME) and the average direct effect (ADE). First, we fitted the mediator model, where the measure of the relevant explanatory variable is modelled as a function of dog age group and confounding variables (weight, height, breed, sex, neuter status, etc. (see Table 3 for full list of final model variables). Next, we modelled the outcome variable, including the mediator, age group, and the same set of confounding variables as those used in the mediator model. We then used the mediate function to estimate the ACME and ADE. The default simulation type (the quasi-Bayesian Monte Carlo method based on normal approximation (Imai, Keele, & Tingley, 2010)) was used, with White's heteroskedasticity-consistent estimator for the covariance matrix from the sandwich package (vcovHC; (Zeileis, 2006)) by setting the robustSE argument to TRUE.

13.4 Results

Generation of factor scores and assessment of reliability

The internal consistency (Cronbach's alpha) of the five questionnaire factors in the current sample ranged from 0.71 to 0.78 (Fearfulness 0.770, Aggression towards people 0.774, Activity/Excitability 0.758, Responsiveness to training 0.714, and Aggression towards animals 0.729). This confirmed that the translation of the questionnaire from English to Hungarian, and the modification of the rating scale (from a 7 point to a 5 point Likert scale) did not cause marked changes in the factors' structure. The Cronbach's alpha values from the original study ranged from 0.73 to 0.84 (Fearfulness 0.838, Aggression towards People 0.742, Activity/Excitability 0.728, Responsiveness to Training 0.771, and Aggression towards Animals 0.748).

Linear models: Main effect of age

Linear models were run to examine the effect of dog age group on the five DPQ factors. Results revealed a significant effect of age group on Activity/Excitability, which explained 18% of the variance, Responsiveness to training, with 4% variance explained, and Aggressiveness to animals, at only 2% variance explained. Fearfulness and Aggressiveness towards humans had no relationship with dog age (F=1.35, P=0.443; F=0.88, P=0.493 respectively). Activity/Excitability showed a strong negative linear relationship with age, all dog age groups differed significantly from age group 1 (1-3 year olds). Responsiveness to training was highest in 3 to 6 year olds, and there was a tendency for Responsiveness to training to decrease from age 10, however, only dogs aged above 12 years (age group 6) had significantly lower scores than dogs aged 1 – 3 years. Aggressiveness towards animals showed a quadratic distribution with age. Dogs aged between 6 and 10 years (age groups 3 and 4) had significantly higher scores than dogs aged 1 – 3 years (Figure 21A-C).

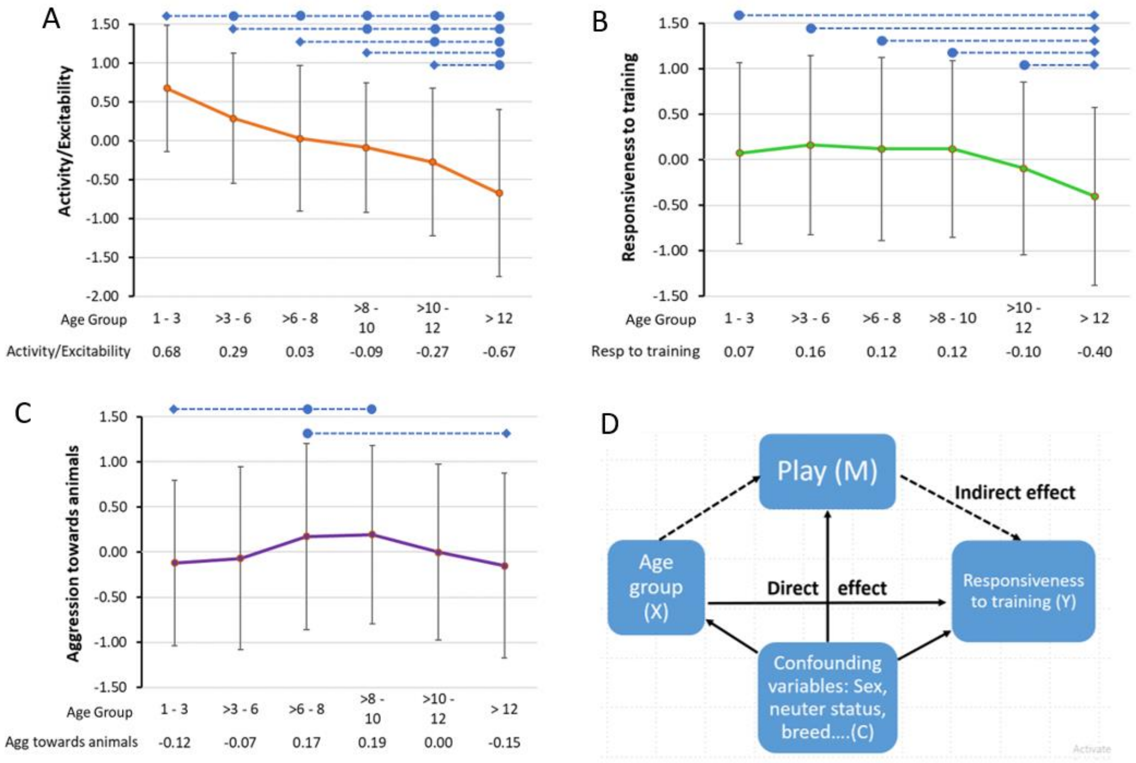

Figure 21. Mean Z score (and standard deviation) of the Dog Personality Questionnaire (Jones, 2008) factors A) Activity/Excitability, B) Responsiveness to training, and C) Aggression towards animals, in the six different dog age groups. The blue diamonds indicate the reference age group, and the blue circles and dotted lines represent significant differences between age groups p<0.05. The mean Z score for each age group is presented under each graph. D) Proposed relationship between dog age group (X), the explanatory variable play (mediator (M): "on an average day, how much time do you or other people spend together with your dog in different activities?" (Playing, walking, training)), and the Dog Personality Questionnaire factor Responsiveness to training. The dotted line represents indirect effects (i.e. similar aged dogs with different playtime differ in the trait) and the solid line direct effects (i.e. different aged dogs with similar playtime differe in the trait). Confounding variables includes all demographic and other explanatory variables retained in the final model.

Linear models: Main effects of all explanatory variables

Linear models were run to examine the effects of the explanatory variables and age group on the five DPQ factors. Since most of the demographic and explanatory variables were previously shown to differ according to the age group of the dog (Wallis et al., 2018), in order to control for these age differences, all of the variables were left in the models (i.e. the models were not reduced), except five variableswhich were not significant in the models and did not show age differences (specifically, owner experience, other dogs in the household, people in household, child and time spent alone). We found that dog age group was still significantly associated with the Activity/Excitability trait, and also for the Aggressiveness towards animals. However, the significant main effect in the linear model of Responsiveness to training disappeared after FDR correction (p=0.475, Table 19).

In addition to dog age group, we found numerous associations between the dog and owner demographics and other explanatory variables and the personality traits (Table 19).

Results for the Fearfulness factor revealed significant effects of previous trauma (3.5% variance explained), weight in kg (2.5%), breed (1.5%), number of dog obedience tasks known (1.6%), number of dog training activities currently participating in (1.2%), and sex (0.8%), which including the remaining 17 variables explained a total of 23.0% of the variance. Previously we established that dogs that have experienced one or more traumatic events (such as spent time at a shelter, changed owner, suffered traumatic injury/prolonged disease/surgery, were lost for a time, or who experienced a change in family structure), were more likely to be currently suffering from health and/or sensory problems (Wallis et al., 2018). In the current study, dogs that had previously experienced a traumatic event were scored higher in Fearfulness than dogs that had not, and dogs with a higher weight in kg were scored lower in Fearfulness than lighter dogs. Males and pure breeds scored lower in Fearfulness than females and mixed breeds. Finally, dogs that could perform three or more types of obedience tasks, and/or participated in four or more dog training activities had lower Fearfulness scores than dogs that could perform maximum one task, or one dog training activity.

The 23 explanatory variables together accounted for 13.1% of the total variance of the Aggression towards people score. Four variables had significant associations after correction for FDR: Purebreds were rated to be less aggressive than mixed breeds, and males had higher Aggression towards people than females. Dogs that had experienced trauma had higher Aggression towards people, and dogs that knew three or more dog obedience tasks had lower aggression than dogs that knew maximum one task. From the four variables, three had a higher than 1% effect size: number of dog obedience tasks known (3.1%), previous trauma (1.9%), and sex (1.3%). After excluding 16 outliers and rerunning the model, all results that were significant according to FDR Benjamini-Hochberg method remained significant.

Results for the Activity/Excitability factor revealed significant effects of age group (7.6% variance explained), age of dog when arrived (3.7%), dog obedience tasks (3.3%), body condition score (1%), where the dog was obtained from (get dog) (0.9%), and sensory problems (0.6%), which including the remaining 22 variables explained a total of 33.3% of the variance. As dogs age increased Activity/Excitability decreased, and dogs that arrived in the household aged 7 weeks or older received lower Activity/Excitability scores than dogs that were obtained from under 7 weeks. Dogs that knew two or more types of dog obedience tasks were rated as higher in Activity/Excitability than dogs that knew maximum one task. Overweight dogs and dogs with sensory problems were scored lower on Activity/Excitability levels than dogs in a normal weight range, with no sensory problems. Finally, dogs that were born in the household or bought by the owner from a breeder, had lower Activity/Excitability scores than dogs that were found as a stray, or obtained from the shelter. After excluding three outliers and rerunning the model, all results that were significant according to FDR Benjamini-Hochberg method remained significant.

The 23 explanatory variables together accounted for 45.9% of the total variance of the Responsiveness to training score. Results revealed that as a consequence of successfully uncovering one or more mediator variables, we could no longer detect age group differences in this personality trait. Six explanatory variables had significant associations after correction for FDR: Dogs that knew two or more dog obedience tasks had higher Responsiveness to training than dogs that knew maximum one task, and dogs that knew 11 or more commands were rated higher in Responsiveness to training than dogs that knew 10 or fewer. Dogs that engaged in more than one hour of off leash activity had greater Responsiveness to training scores, than dogs that received less than 30 minutes. Owners who engaged in play or other activities with their dog for more than one hour per day gave their dog higher scores in Responsiveness to training than owner who spent less than 30 minutes. Male dogs were rated to be less Responsive to training than females, and heavier dogs had higher Responsiveness to training scores than lighter dogs. From the six variables, four had a higher than 1% effect size: dog obedience tasks (18.2%), number of commands known (3.4%), off leash activity (1.4%), and time spent in play (1.1%). After excluding one outlier and rerunning the model, all results that were significant according to FDR Benjamini-Hochberg method, remained significant.

Results for the Aggressiveness towards animals factor revealed significant effects of dog obedience tasks (1.9% variance explained), age group (1.8%), diet (food) (1.2%), and previous trauma (0.8%), which including the remaining 19 variables explained a total of 11.6% of the variance. As described previously, Aggressiveness towards animals showed a quadratic distribution with age; dogs aged between six and 10 years had significantly higher scores than dogs aged 1 – 3 years. Dogs that could carry out four or more dog obedience tasks had lower Aggressiveness towards animals than dogs that could perform maximum one task. Dogs fed cooked food and/or raw meat, as well as dogs fed a mixture of foods had higher owner reported Aggressiveness towards animals than dogs fed a diet of tinned food, or tinned and dry food mixed. Finally, dogs that had previously experienced trauma scored higher in Aggressiveness towards animals.

		Fearfulness (N=1170)				Aggression towards people (N=1182)				Activity Excitability (N=1156)				Responsiveness to training (N=1183)				Aggression towards animals (N=1171)			
Source	**df**	***F***	***P***	**FDR**	**Partial eta2**	***F***	***P***	**FDR**	**Partial eta2**	***F***	***P***	**FDR**	**Partial eta2**	***F***	***P***	**FDR**	**Partial eta2**	***F***	***P***	**FDR**	**Partial eta2**
Corrected Model		**6.542**	**<0.001**		**0.230**	**3.327**	**<0.001**		**0.131**	**10.800**	**<0.001**		**0.333**	**18.830**	**<0.001**		**0.459**	**2.884**	**<0.001**		**0.116**
Age group	5	2.690	0.020	0.066	0.012	0.897	0.482	0.605	0.004	**18.153**	**0.000**	**0.000**	**0.076**	1.077	0.372	0.475	0.005	**4.113**	**0.001**	**0.012**	**0.018**
Height (in cm)	1	0.194	0.659	0.288	0.000	0.578	0.447	0.605	0.001	0.137	0.711	0.779	0.000	2.094	0.148	0.340	0.002	1.266	0.261	0.375	0.001
Weight (in kg)	1	**29.058**	**0.000**	**0.000**	**0.025**	2.569	0.109	0.358	0.002	0.938	0.333	0.418	0.001	**7.039**	**0.008**	**0.031**	**0.006**	1.512	0.219	0.336	0.001
Breed	1	**16.562**	**0.000**	**0.001**	**0.015**	**10.112**	**0.002**	**0.012**	**0.009**	**3.387**	**0.066**	**0.169**	**0.003**	0.238	0.626	0.719	0.000	5.841	0.016	0.061	0.005
Sex	1	**9.013**	**0.003**	**0.010**	**0.008**	**14.386**	**0.000**	**0.000**	**0.013**	3.806	0.051	0.147	0.003	**10.334**	**0.001**	**0.008**	**0.009**	2.739	0.098	0.188	0.002
Neuter status	1	3.173	0.075	0.173	0.003	1.097	0.295	0.590	0.001	0.000	0.985	0.985	0.000	0.993	0.319	0.459	0.001	3.065	0.080	0.178	0.003
Sensory problems	1	2.205	0.138	0.693	0.002	1.729	0.189	0.543	0.002	**6.526**	**0.011**	**0.042**	**0.006**	1.516	0.218	0.380	0.001	5.967	0.015	0.061	0.005
Off-leash activity	4	1.510	0.197	0.377	0.005	0.718	0.580	0.642	0.003	2.039	0.087	0.200	0.007	**3.872**	**0.004**	**0.023**	**0.014**	1.866	0.114	0.202	0.007
Body Condition Score	2	0.411	0.663	0.693	0.001	0.693	0.500	0.605	0.001	**5.427**	**0.005**	**0.029**	**0.010**	0.385	0.681	0.719	0.001	1.851	0.158	0.260	0.003
Food	4	0.930	0.446	0.603	0.003	1.374	0.241	0.564	0.005	1.356	0.247	0.379	0.005	2.959	0.019	0.055	0.010	**3.443**	**0.008**	**0.046**	**0.012**
Vitamins	3	0.769	0.512	0.654	0.002	0.190	0.903	0.903	0.001	1.938	0.122	0.236	0.005	1.428	0.233	0.380	0.004	0.739	0.529	0.676	0.002
Trauma	1	**40.734**	**0.000**	**0.000**	**0.035**	**22.142**	**0.000**	**0.000**	**0.019**	1.619	0.204	0.335	0.001	0.805	0.370	0.475	0.001	**8.502**	**0.004**	**0.031**	**0.008**
Health problems	4	2.719	0.029	0.082	0.010	0.356	0.840	0.878	0.001	1.225	0.298	0.403	0.004	1.635	0.163	0.341	0.006	2.051	0.085	0.178	0.007
Medication	1	0.299	0.585	0.684	0.000	0.297	0.586	0.642	0.000	1.724	0.189	0.334	0.002	0.161	0.688	0.719	0.000	0.063	0.802	0.922	0.000
Owner age	3	1.007	0.389	0.559	0.003	1.030	0.379	0.604	0.003	0.939	0.421	0.484	0.003	1.500	0.213	0.380	0.004	0.106	0.957	0.957	0.000
Age of dog when arrived	3	1.163	0.323	0.495	0.003	3.104	0.026	0.120	0.008	**14.223**	**0.000**	**0.000**	**0.037**	0.719	0.541	0.655	0.002	3.224	0.022	0.072	0.009
Get dog	2	1.163	0.313	0.495	0.002	1.180	0.308	0.590	0.002	**4.945**	**0.007**	**0.032**	**0.009**	0.029	0.972	0.972	0.000	0.069	0.933	0.957	0.000
Where dog is kept	2	1.448	0.236	0.417	0.003	2.503	0.082	0.314	0.004	1.065	0.345	0.418	0.002	1.394	0.248	0.380	0.002	2.548	0.079	0.178	0.005
Dog obedience tasks	3	**5.895**	**0.001**	**0.003**	**0.016**	**12.157**	**0.000**	**0.000**	**0.031**	**12.738**	**0.000**	**0.000**	**0.033**	**83.808**	**0.000**	**0.000**	**0.182**	**7.275**	**0.000**	**0.000**	**0.019**
Play	3	0.091	0.965	0.965	0.000	0.940	0.420	0.604	0.002	1.931	0.123	0.236	0.005	**4.207**	**0.006**	**0.028**	**0.011**	0.248	0.863	0.945	0.001
Commands	2	0.520	0.595	0.684	0.001	0.869	0.420	0.604	0.002	3.388	0.034	0.112	0.006	**19.961**	**0.000**	**0.000**	**0.034**	0.801	0.449	0.607	0.001
Dog training activities	2	**7.052**	**0.001**	**0.004**	**0.012**	0.925	0.397	0.604	0.002	1.228	0.293	0.403	0.002	4.064	0.017	0.055	0.007	0.408	0.665	0.805	0.001
Dog behaviour changed	1	4.191	0.041	0.105	0.004	1.350	0.245	0.564	0.001	0.068	0.794	0.830	0.000	5.025	0.025	0.064	0.004	2.970	0.085	0.178	0.003

Table 19. Results of the linear models on the five PCA components of the DPQ. Owner experience, Other dogs in the household, People in household, Child and Time spent alone were removed from the model, as all p values were non-significant, and none of these variables differed among the age groups. All other non-significant effects remained in the model in order to control for age effects on the explanatory variables. P values were corrected for multiple comparisons using the false discovery rate procedure (FDR). Significant predictors are highlighted in bold and darker colours (p≤0.05).

Mediation analysis: Responsiveness to training

When modelled separately, some explanatory variables and Responsiveness to training both showed an effect of age group, therefore it is possible that a mediation may take place between this factor and one or more of the variables. To follow up on this possibility we looked for potential mediator variables by examining which of the significant explanatory variables in the model had the strongest relationship with dog age (including off-leash activity, dog obedience tasks, play, and commands). Results revealed that the explanatory variable play had the greatest differences between the age groups ($\chi^{(15)}=61.282$, $P<0.001$). Please refer to Wallis et al., (2018) for results of the age analysis. Dog age may not be the real reason that Responsiveness to training decreases in the oldest age groups. We hypothesized that as dog age increases, play levels decrease in some dogs and then low play levels decreases Responsiveness to training: age group (X) → play (M) → Responsiveness to training (Y) (Figure 21D).

We estimated the average causal mediation effect of play, by first fitting the mediator model, where the categorical variable play (mediator (M)), is modelled as a function of dog age group (X) and confounding variables ((C) weight, height, breed, sex, neuter status, etc. (see

Table 19 for full list of final model variables). Next, we modelled the outcome variable Responsiveness to training, including the mediator (play), age group, and the same set of confounding variables as those used in the mediator model. We used proportional odds logistic regression for the mediator, and linear regression for the outcome model. When comparing the age group with the highest Responsiveness to training, with that of the lowest, (age group two and six (adult vs old dogs)), results from the mediation analysis indicated that there was a significant average causal mediation effect (ACME or indirect effect), but the average direct effect and the total effect were not significant. Results from the mediation analysis posits a partial mediation and the average proportion mediated was 18%. The results suggest that the mediating variable play accounts for a significant part (but not all) of the relationship between dog age and Responsiveness to training. Therefore, the difference in the play variable (mediator) in older dogs in part is responsible for the lower Responsiveness to training personality trait score. The absence of a significant total effect can be explained by the presence of several mediating paths that may cancel each other out.

13.5 Discussion

Previously we established that aging influences multiple dog and owner demographics (Wallis et al., 2018), therefore the next step was to examine whether the personality of the dog as measured by the Dog Personality Questionnaire (Jones 2008), also fluctuates with age. In this study, we demonstrated that according to their age group, dogs do indeed differ in their mean personality trait levels. Younger dogs had higher Activity/Excitability levels than older dogs, while older dogs had lower Responsiveness to training. Aggressiveness towards animals showed a quadratic trajectory with age and peaked between six to ten years.

Previous studies have observed that multiple environmental factors can mask or even enhance differences in biological factors such as age, sex, neuter status, and breed (Kubinyi, Turcsán & Miklósi, 2009; Turcsán et al., 2017). Therefore, in a second set of models we additionally controlled for demographic and other explanatory variables. Results revealed that as a consequence of successfully uncovering one or more mediator variables, we could no longer detect age group differences in the personality trait Responsiveness to training. The most important factors that influenced personality traits (that had a higher than 1% variance explained) were age, weight, breed (pure breed or mixed breed), sex, off-leash activity, diet, previous trauma, age of the dog when it first arrived in the household, number of dog obedience tasks the dog could perform, time spent in play/interacting with owner, number of known commands, and current dog training activities.

Interestingly, although we did not find an effect of age in the main Fearfulness factor of the DPQ, we did find age differences in the facets Fear of people and Non-social fear. Fear of people peaked in dogs aged three to six years and was lowest in dogs aged over ten years. Non-social fear increased with age, with dogs aged over 12 years showing the highest levels, perhaps due to a decline in environmental stimulation opportunities. Non-social fear was characterised by higher anxiety, diffidence, and difficulties to adapt to new situations and environments. Previous studies have reported increased anxiety in aged dogs, including increased neuroticism (Temesi et al., 2014), fear of handling, fear of noises (Blackwell, Bradshaw, and Casey 2013; Dale et al. 2011; Henriksson 2016) and human and object fear (Lofgren et al., 2014). Increased fear responses in older dogs is particularly relevant for the senior dog-owner bond, as fearfulness and fear-related behaviour problems result in an increase in the perceived cost of the dog owner relationship (Meyer & Forkman, 2014), which can ultimately lead to the relinquishment of the dog (New et al., 2000).

Studies have also shown increased anxiety-like behaviour in aged mice and rats, and in humans (Chemerinski, Petracca, Manes, Leiguarda, & Starkstein, 1998; Kogan, Edelstein, & McKee, 2000; Meeker, Chadman, Heaney, & Carp, 2013; Meyza, Boguszewski, Nikolaev, & Zagrodzka, 2011). Increases in anxiety is one of the symptoms of Alzheimer’s disease (Seignourel, Kunik, Snow, Wilson, & Stanley, 2008) and also Canine Cognitive Dysfunction (Schütt et al., 2015). In older humans, anxiety is often generalised, but in rats, and dogs, individual increases in social and non-social anxiety can be expressed separately (Salchner, Lubec, & Singewald, 2004; Schütt et al., 2015). In the current study, only non-social anxiety increased with age, whilst social anxiety showed a different trajectory. This finding could be explained by the fact that the same genomic region affected by structural variants in human Williams-Beuren syndrome (WBS) is associated with hyper-sociability found in most domestic dogs (vonHoldt et al., 2017) which in human often heighten non-social anxiety, too (Dodd & Porter, 2011). Another explanation for the differing cross-sectional trajectories of social and non-social fear may be due to the fact that these processes involve differing neurological regions and neurotransmitters, based on evidence from rat and human studies (Lukas & Neumann, 2012; J. N. Wood, Romero, Makale, & Grafman, 2003).

Unsurprisingly, the factor Activity/Excitability, which contains the facets Excitability, Playfulness, Active Engagement and Companionability, showed a strong decline with age in the current cross-sectional study. Several studies have reported decreases in activity levels with age in dogs within the home environment (Landsberg, Nichol, & Araujo, 2012; Siwak, Murphey, Muggenburg, & Milgram, 2002). This factor also includes questions regarding sociability (companionability, or time spent interacting with humans), and playfulness, which are also reported to decline with age in dogs (Bennett & Rohlf, 2007; Landsberg et al., 2012; Sforzini et al., 2009).

Using the same questionnaire Chopik and Weaver (2019) found a similar decline of the factor Activity/Excitability with age. Utilising a different personality questionnaire based on a Human Personality Inventory, Kubinyi et al. (2009) found that older dogs were calmer, less social and less bold than younger dogs (see also Starling et al. (2013) for decrease in boldness with age) which also points to a reduction in activity/excitability and sociability with age.

In the current study, Responsiveness to training also declined with age, after peaking in the three to six-year-old dogs. Chopik and Weaver (2019) reported a peak in Responsiveness to training in dogs aged 7 years, and no decline with age. This can be explained by the fact that their sample was skewed towards younger dogs and contained fewer senior and geriatric dogs in comparison to the current study. By measuring selective attention, sensorimotor control and trainability using a clicker training for eye contact test in a large sample of pet Border collies aged from 6 months to 14 years, Wallis et al. (2014) similarly to our results, found that the dogs' performance peaked in the three to six year olds. Kubinyi et al. (2009) and Turcsán et al. (2017) also found a reduction in Trainability in older dogs, especially those that did not take part in any training activities and whose owners spent less than one hour active with them daily.

Finally, Aggressiveness towards animals increased with age up to 10 years, but then declined. Several studies have reported increases in intraspecific aggression in dogs with age (Bennett & Rohlf, 2007; Casey, Loftus, Bolster, Richards, & Blackwell, 2013; Riemer et al., 2016). Similarly to the current study, Chopik and Weaver, (2019) documented a peak in the factor Aggressiveness towards animals at 7 – 8 years old, and a decline thereafter. However, this factor also contains the facets Prey drive and Dominance over dogs. In the current study, results indicated that the oldest age group had the lowest scores in both facets. Which explains why older dogs overall had lower Aggressiveness towards animals, despite the fact that the facet Aggressiveness towards dogs was highest in the oldest age group. This information is particularly relevant for owners of aged dogs living in multi-dog households as their management and housing could be affected. If increased aggressiveness towards dogs within the same household is observed, pain issues should be ruled out first, and preventative measures be implemented such as providing separate sleeping areas and feeding locations, in order to minimise conflicts. Prey drive and Dominance over dogs may be reduced in the oldest age group due to falling activity levels or age-related frailty and/or increased pain levels, and a corresponding decline in walks and opportunities to meet unfamiliar conspecifics and/or other animals. Indeed, an independent behaviour study from our research group supported that old dogs spent less time in proximity to an unfamiliar dog and the number of approaches also decreased with age. Previous work has reported that aging, and in particular pathological aging could affect social responsiveness in dogs (Howse, Anderson, & Walsh, 2018; Rosado et al., 2012), although it is difficult to separate these observations from a general decline in activity. In addition, age also correlates with social rank in canines (Bonanni et al., 2017; Bonanni, Cafazzo, Valsecchi, & Natoli, 2010; Pal, Ghosh, & Roy, 1998; Kubinyi et al 2019). Social rank by itself has been shown to affect some social behaviours, e.g. social learning across several species (Nicol & Pope, 1999; Pongrácz, 2014), but also active versus passive approach to social interaction (Rudolf Schenkel, 1967), with dogs of higher rank being often the recipients rather than initiators of ritualized greetings (Bonanni et al., 2010). It was also observed that younger dogs initiate more muzzle contacts (Howse et al., 2018) further strengthening the notion that higher age and/or rank are associated with being more passive in social interactions. So far only playful pursuits and attacks were observed more often in older dogs and strictly concern relative age-differences within dyads of playing animals (Bauer & Smuts, 2007). Whether or not mediated by social rank, an association between age and social responsiveness/interest in a naturalistic setting is a valuable observation, as it adds to similar observations obtained under laboratory conditions (Rosado et al., 2012).

Our next aim was to investigate if the behaviour differences between the dog age groups remained significant after controlling for any differences in the demographic and dog keeping factors, as well as to examine how the demographic and explanatory variables are associated with the behavioural traits. Results revealed that due to the fact that we successfully uncovered one or more mediator variables we could no longer detect age group differences in the personality trait Responsiveness to training. For the Activity/Excitability and Aggression towards animals behaviour traits, dog age group remained a significant predictor in the models even after controlling for the measured explanatory variables. This suggests that these behaviour differences (i.e. lower Activity/Excitability in older dogs, and higher Aggression towards animals in dogs aged six to ten), cannot be attributed solely to other demographic differences–at least not to those investigated in the current study.

When we explored the relationship between the demographic and other explanatory variables and the behaviour traits, we found that 15 out of the 28 variables were significantly associated with at least one behaviour trait, after correction for multiple comparisons. Here we will discuss only those variables that accounted for greater than 1% variance explained in the models (including weight, breed, sex, off-leash activity, food (diet), previous trauma, age of dog when arrived, dog obedience tasks, play, commands, and dog training activities).

The explanatory variables weight and breed (mixed or pure bred) were significant in the DPQ factor Fearfulness. Larger, heavier dogs scored lower in Fearfulness than smaller lighter dogs, and mixed breeds were higher in Fearfulness than pure breeds. Our results are supported by previous studies that found that smaller dogs were seen as more anxious, neurotic and fearful in comparison to larger dogs (Arhant, Bubna-Littitz, Bartels, Futschik, & Troxler, 2010; Ley et al., 2009; McGreevy et al., 2013), which might help explain why in the current study smaller dogs had lower scores in Responsiveness to training than larger dogs. Mixed breeds were also found to be more fearful than pure breeds (Bennett & Rohlf, 2007; Schneider, Delfabbro, & Burns, 2013; Temesi et al., 2014), which may heighten their tendency to show aggression towards people. The factors Aggression towards animals and Aggression towards people had a low percentage of variance explained in the models and were less influenced by environmental factors; therefore, additional variables not measured in the current study likely contribute to these personality traits. Previous studies suggest that dogs are likely to learn to show aggression only in particular contexts. Experiences that are specific to the individual and the type of training method used by the owner may also influence aggression in dogs (Casey et al. 2013; Casey et al. 2014; Hiby, Rooney, and Bradshaw 2004). Genetic factors that contribute to the DPQ factors Fearfulness, Aggression towards People and Activity/Excitability have been identified (Hejjas, Vas, Topal, et al., 2007; Liinamo et al., 2007; Van Der Waaij, Wilsson, & Strandberg, 2008; van Rooy, Arnott, Early, McGreevy, & Wade, 2014; Vermeire et al., 2009). Our results reflect the importance of genetic factors, as breed status was associated with these factors, albeit only one of them with a higher than one percent variance explained.

Similarly, although we found significant sex effects for three of the five personality traits (and a tendency in Activity/Excitability and Aggression towards animals), only one had a higher than one percent variance explained, Aggression towards people. Regardless of breed, owners rated male dogs as higher in aggression than females. Previous studies have found that male dogs score higher on owner directed aggression (Hsu & Sun, 2010), biting, growling and possessive behaviour (Guy et al., 2001), and over half the dogs reported to display aggressive behaviour towards humans are reproductively intact males (Beaver, 1983; Wright, 1996; Wright & Nesselrote, 1987). In the current study, female dogs were rated higher in Fearfulness and Responsiveness to training similarly to previous studies (Bamberger & Houpt, 2006; Fratkin, 2017; Hart & Hart, 1985; Temesi et al., 2014; Tiira & Lohi, 2015). However, another study reported no relationship between Responsiveness to training and sex, and an interaction between sex and breed was also reported (Serpell & Hsu, 2005; Wilsson & Sundgren, 1997). The current study adds to previous studies findings that there are breed and sex specific differences in behaviour, behavioural development and heritability of traits in dogs (Riemer et al., 2016; Scott, 1958; Wilsson & Sundgren, 1997; Wilsson & Sundgren, 1997).

The environmental explanatory variable, off-leash activity showed only one association with the personality traits measured. Dogs that engaged in more than one hour of off-leash activity had greater Responsiveness to training scores, than dogs that received less than 30 minutes. This finding is easily explained by the fact that dogs that have received more training and have a better recall, are likely to be allowed more off-leash time than untrained dogs. Some breeds are more commonly allowed off the lead in public by owners, which indicates that there are breed differences in off-leash activity. Additionally, dogs that are more fearful and show aggression to strangers or other dogs (and therefore might not return to the owner when called), are less likely to be allowed off-leash. A recent study found that such dogs were also more likely to be overweight, perhaps because their owners do not allow their dogs to exercise outside the house and garden, or restrict their freedom if they do (German, Blackwell, Evans, & Westgarth, 2017).

The dogs' main diet had a significant association with one behavioural trait. Interestingly, dogs fed cooked food and/or raw meat, as well as dogs fed a mixture of foods (including, cooked food, raw, as well as dry and/or canned food), had higher owner reported Aggressiveness towards animals than dogs fed a diet of only dry food, or tinned food, or tinned and dry food mixed. One explanation for this finding could be due to the fact that dogs which are prone to intraspecific aggression that are fed a higher protein diet, show heightened dominance aggression compared to when they are fed a low protein diet, or a diet supplemented with tryptophan (DeNapoli, Dodman, Shuster, Rand, & Gross, 2000). Alternatively, owners with strong opinions about their dog's diet may also be biased in their perception of their dog's behaviour.

Earlier we established that dogs that have previously experienced one or more traumatic events (such as spent time at a shelter, changed owner, suffered traumatic injury/prolonged disease/surgery, were lost for a time, or who experienced a change in family structure), were more likely to be currently suffering from health and/or sensory problems (Wallis et al., 2018). We speculated that exposure to traumatic experiences causes behavioural changes in dogs such as increased fearfulness and aggression to certain stimuli. In the current study, results showed that dogs that were exposed to previous trauma showed higher fearfulness and aggression towards people and animals, than dogs with no such negative experience. The owners of forty two percent of the dogs in our sample indicated that their dogs had experienced trauma. This figure seems particularly high, and future studies should examine whether this high percentage holds true for other dog populations. One could speculate that mixed breed dogs were more likely to experience trauma, due to the fact that many of them are obtained from shelters. However, since there were also significant breed effects in these personality traits (only a trend in Aggression towards animals), the fact that trauma was still significant, indicates that this effect was present regardless of whether the animal was a purebred or a mixed breed dog.

To date, only one study has examined the effect of previous trauma on behavioural traits in dogs. Serpell & Duffy, (2016) found that particularly frightening or traumatic events that occurred during puppyhood/adolescence were associated with differences in C-BARQ scores for some behaviours displayed at 12 months. Puppies that had been attacked by an unfamiliar dog displayed higher dog-directed fear, and stranger directed aggression. In addition, puppies that had been frightened by a person showed higher levels of stranger directed fear. However, the authors note that it was not possible to determine whether the dogs became more fearful and/or aggressive as a direct result of their experience or if they had a pre-existing disposition towards fearfulness, which resulted in a higher likelihood to become traumatised by aversive encounters. The same argument can be made of our own results. However, in the current study dogs could have suffered the trauma at any point in their life, and therefore, we can speculate that the effects of previous trauma are likely to persist much longer than the 12-month period in Serpell & Duffy's study (2016).

Studies in humans have also indicated that extremely adverse life experiences can have a profound effect on personality. Participants who reported an extremely horrifying or frightening event up to two years previously, showed increases in neuroticism, decreases in the compliance facet of agreeableness, and decreases in openness to values (Löckenhoff, Terracciano, Patriciu, Eaton, & Costa, 2009). These changes correspond to increases in fear, anger and frustration (aggression), and decreases in cooperation in interpersonal relationships. Striking similarities in dog and human neurobiological alterations in behavioural disorders, means the dog represents an interesting natural animal model for human neuropsychiatric diseases (Overall, 2000; Taylor, Audenaert, Baeken, Saunders, & Peremans, 2016).

Dogs that arrived in the household aged older than 7 weeks or that were bred by the owner received lower Activity/Excitability scores than dogs that were obtained from under 7 weeks, or that were found, or rescued from a shelter. Our results are in part agreement to those from Kubinyi et al (2009); dogs bred by the owner were described as being calmer, and bolder than dogs acquired later, especially those acquired as adults (note that a sex difference in boldness was found). In general, puppies that are removed earlier from the dam are more likely to exhibit potentially problematic behaviours (Pierantoni, Albertini, & Pirrone, 2011). However, we did not observe any other effects of the age of dog when it first arrived in the household, apart from a tendency of dogs that arrived over one year of age to have lower aggression towards people and animals, than dogs that arrived at a younger age.

The final four explanatory variables that influenced the dog behavioural traits were dog obedience tasks, amount of time in play or other activities, number of known commands, and dog training activities. Dog obedience tasks was the only demographic variable that had a higher than 1% variance explained in all five of the models. A greater number of dog obedience tasks known by the dog corresponded to lower Aggressiveness towards animals, Fearfulness and Aggression towards people, and higher Activity/Excitability and Responsiveness to training, than dogs that could perform maximum one task. These results suggest that there is a link between the number of obedience tasks known and personality as assessed by the owner in pet dogs. However, this is a correlation and although changes in the number of obedience tasks known may lead to changes in personality, it is also possible that the dogs already had the type of personality that would be amenable to training (lower aggression and fearfulness, and higher Activity/Excitability), which resulted in greater levels of obedience. Regardless of which is cause and which is effect, it is clear that obedience is an important aspect contributing to owner answered dog personality questionnaires, and the dog-owner relationship.

Formal and informal obedience training has been found to reduce aggression, and owners of obedience-trained animals reported fewer behavioural problems (Clark & Boyer, 1993; Jagoe & Serpell, 1996). However, Casey et al., (2013) found that dogs that attended obedience classes had a 1.8 times increased risk of aggression to unfamiliar dogs, perhaps because the owners were seeking assistance with their aggressive dog. A questionnaire study by Bennett & Rohlf, (2007) found that more obedient dogs (dogs that come when called, and sit and stay on command) were reported to be more friendly, and less aggressive, nervous, and anxious/destructive by their owners. Owners of obedient dogs had greater training engagement and participated in more shared activities with their dog, which could result in a stronger dog-owner bond. In the current study, dog obedience tasks, time spent in play or other activities and number of commands known explained 22% of the Responsiveness to training factor, providing construct validity for this trait. Additionally, we could show that one explanatory variable, play, partially mediated the relationship between age group and Responsiveness to training. This result is particularly important, as it implies that in older dogs, interventions to increase play and training motivation may alleviate the negative effects of aging on dogs' trainability. Finally, dogs that participated in a higher number of dog training activities had lower Fearfulness scores. Previous studies have also found a link between training and fearfulness. Owners of nervous dogs had lower training engagement (Bennett & Rohlf, 2007), and dogs that participated in more training course were rated higher in calmness (Kubinyi et al., 2009).

The fact that the dogs' level of training (or education) was found to have a stronger influence on owner perceived dog personality than breed, sex or reproductive status provides evidence that educational experiences have the power to shape dogs' personality development. It is generally accepted that children and puppies' personality is dynamic and dependent on the interaction of genetics, biology, and environmental influences. Such phenotypic plasticity allows individuals to adjust to environmental variation, and helps to explain the high heritability of personality in early childhood (Briley & Tucker-Drob, 2014). However, the idea that a dog's or indeed a person's personality is fixed at adulthood and cannot be changed has been pervasive in society in general. Whilst it is true that as individuals enter adulthood the longitudinal stability of personality increases substantially (Briley & Tucker-Drob, 2014) in humans, personality traits continue to change in response to key life stages and events (Roberts & Mroczek, 2008). A recent study has proven that interventions can change self-reported personality traits through volitional means (Hudson & Fraley, 2015). Evidence is emerging of the potential of education interventions in children and adolescents to alter personality traits in order to improve resilience (Dray et al., 2017). Here we define resilience as “any behavioural or emotional response to a cognitive or social challenge that is positive and beneficial for development” (Yeager & Dweck, 2012). Dog obedience training throughout the lifespan may help to increase resilience in dogs, and thus increase their ability to cope with potentially stressful situations, reducing fear and aggressiveness, and increasing responsiveness to training and sociability. Indeed, the success of behaviour modification by owners supervised by qualified dog trainers that use positive reinforcement as a tool to “correct” behavioural problems in dogs is a testament to the power of education in improving motivation and resilience in dogs (Blackwell, Casey, & Bradshaw, 2006; Herron, Shofer, & Reisner, 2009; Levine & Mills, 2008).

Our study is among the first that aimed to report mean-level differences in personality traits across the lifespan of pet dogs, and to describe the demographic variables that may contribute to them. However, it is important to note that a major limitation of this study is that it is based on owner reports, which are subjective, and in most cases where associations were found, it was not possible to determine the direction of the cause – effect relationships, or indeed whether a real causal relationship does in fact exist. However, the questionnaire utilised showed good reliability, and the questionnaire method has previously been proven to be reliable and valid (Jones, 2009; Kubinyi, Gosling, & Miklósi, 2015). Many of the associations found can be used to generate new hypotheses and tests that will help to validate the results. A low amount of variance explained in some of the models was likely due to the fact that we were not able to identify and measure all aspects that can influence dog personality. Other factors such as trait heritability and developmental effects like early socialisation, rearing environment and early life experience could also provide explanations for the observed behavioural differences.

In conclusion, some of the predicted relationships between demographic variables and dog personality were found (such as age, breed and sex effects), however, most were small effects, therefore their biological relevance is questionable. Instead, the amount of shared activities, specifically the number of dog obedience tasks known, and the occurrence of previous trauma proved to be more predictive of how owners viewed their dogs’ personality.

14 The relationship between age, personality, dominance and leadership in a group of dogs[10]

14.1 Abstract

Movement interactions and the underlying social structure in groups have relevance across many social-living species. Collective motion of groups could be based on an 'egalitarian' decision system, but in practice it is often influenced by underlying social network structures and by individual characteristics. We investigated whether age, dominance rank and personality traits are linked to leader and follower roles during joint motion of family dogs. We obtained high-resolution spatio-temporal GPS trajectory data (823,148 data points) from six dogs belonging to the same household and their owner during fourteen, 30-40 min unleashed walks. We identified several features of the dogs' paths (e.g., running speed or distance from the owner) which are characteristic of a given dog. A directional correlation analysis quantified interactions between pairs of dogs that run loops jointly. We found that dogs play the role of the leader about 50-85% of the time, i.e. the leader and follower roles in a given pair are dynamically interchangable. However, on a longer timescale, tendencies to lead differ consistently. The network constructed from these loose leader-follower relations is hierarchical, and the dogs' positions in the network correlates with the age, dominance rank, trainability, controllability and aggression measures derived from questionnaires. We demonstrated the possibility of determining dominance rank and personality traits of an individual based only on its logged movement data. The collective motion of dogs is influenced by underlying social network structures and by characteristics such as age and personality differences. Our findings could pave the way for automated animal personality and human social interaction measurements.

14.2 Introduction

Groups that are not able to coordinate their actions and cannot reach a consensus on important events, such as where to go, will destabilise, and individuals will lose the benefits associated with being part of a group (Larissa Conradt & Roper, 2009; Sumpter & Pratt, 2009). Decision-making usually involves some form of leadership, i.e. 'the initiation of new directions of locomotion by one or more individuals, which are then readily followed by other group members' (Krause, Hoare, Krause, Hemelrijk, & Rubenstein, 2000, p83).

Several factors may give rise to the emergence of leadership. In some species or populations, leaders are socially dominant individuals (consistent winners of agonistic interactions (Drews, 1993) and have more power to enforce their will (King & Cowlishaw, 2009). For example, in rhesus macaques (*Macaca mulatta*) the decision to move is the result of the actions of dominant and old females (Sueur & Petit, 2008). Similarly, dominant beef cows (*Bos taurus*) have the most

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[10] Based on: Ákos, Zs., Beck, R., Nagy, M., Vicsek, T., Kubinyi, E. 2014. Leadership and path characteristics during walks are linked to dominance order and individual traits in dogs. PLoS Computational Biology, 0(1): e1003446.

influence on where the herd moves. They go where they wish while subordinates either avoid or follow them (Sárová, Spinka, Panamá, & Simecek, 2010).

Leaders could appear in species or populations without any dominant individuals, or independently from social dominance. Leaders may have the highest physiological need to impose their choice of action (Conradt & Roper, 2009; Furrer, Hansjoerg, & Manser, 2012; Krause et al., 2000; Sueur, Deneubourg, Petit, & Couzin, 2010; Vicsek & Zafeiris, 2012), or they may possess special information or skill (Flack, Pettit, Freeman, Guilford, & Biro, 2012; R. Freeman, Mann, Guilford, & Biro, 2011).

Several studies have reported a positive correlation between age and dominance (Berdoy, Smith, & MacDonald, 1995). Age-related dominance might be due to greater fighting skills (e.g. Arcese and Smith, 1985)) or enhanced possibility of forming alliances with other individuals, among other factors (Datta & Beauchamp, 1991).

Finally, an individual of a personality type that is more inclined to lead or does not prefer following others may also initiate collective movements (King, Johnson, & Vugt, 2009; Krause, James, & Croft, 2010). For example, leadership is associated with boldness in sticklebacks (*Gasterosteus aculeatus*) (Harcourt, Sweetman, Johnstone, & Manica, 2009; Nakayama, Stumpe, Manica, & Johnstone, 2013). The investigation of the relationship between leadership and personality might reveal which personality types occupy particular positions in the leadership network, and conversely, network metrics could identify potential personality traits.

With this study our aim was to develop a method for revealing potential links between leadership in collective movements, motion patterns, age, social dominance, and personality traits in domestic dogs (*Canis familiaris*). It is often assumed that domestic dogs inherited complex behaviours from their wolf ancestors (*Canis lupus*). The typical wolf pack is a nuclear or extended family, where the dominant/breeding male initiates activities associated with foraging and travel (Mech, 2000). However, family dog groups may consist of several unrelated individuals with multiple potential breeders. In large wolf packs with several breeders, leadership varies among packs, and dominance status has generally no direct bearing on leadership, but breeders tend to lead more often than non-breeders (Peterson, Jacobs, Drummer, Mech, & Smith, 2002). Similarly, leadership in Italian free-ranging dogs interchanged between a small number of old and high-ranking habitual leaders. Interestingly, affiliative relationships had more influence on leadership than agonistic interactions (Bonanni, Cafazzo, Valsecchi, & Natoli, 2010).

Family dogs are often kept in groups (for instance, 33% of owners in Germany (Kubinyi et al., 2009) and 26% of owners in Australia (Kobelt, Hemsworth, Barnett J L, & J, 2003) have 2 or more dogs), however interactions within freely moving dog groups and their relationship with social dominance are still unexplored. The capacity of dogs to form robust dominance hierarchies is highly debated (Bradshaw, Blackwell, & Casey, 2009; S. Cafazzo, Valsecchi, Bonanni, & Natoli, 2010). However, the reason for the inability to detect hierarchies might be due to methodological issues in certain cases, as instead of aggression patterns, submissive behaviours appear to be better indicators of dominance relationships in dogs (Rowell, 1974).

To describe what characterises the collective movement of a group of dogs, and to investigate links between leadership, age, social dominance, personality (Jones & Gosling, 2005), and characteristics of individual motion trajectories, we collected high-resolution spatio-temporal (1-2 m, 0.2 s) GPS trajectory data from a group of dogs and their owner during everyday walks. Directional choice dynamics and potential leading activity were assessed by quantitative methods inspired by statistical physics (Nagy, Akos, Biro, & Vicsek, 2010; M. Nagy et al., 2013). Personality and dominance rank of the dogs were measured by questionnaires completed by the owner. Because the capacity to form dominance hierarchies is likely to vary from breed to breed (Mertens, 2004), we chose a group that contains multiple individuals of the same breed, the Hungarian vizsla. The studied group is composed of five vizslas (with two dam-offspring pairs) and one small-sized, mixed-breed dog.

14.3 Methods

Subjects

6 dogs (5 Hungarian vizslas and one mixed breed; labelled V1 to V5 and M, respectively) and their owner took part in the experiments. Demographic characteristics are shown in Table 20. Photos of the subjects are presented in Figure 22, vizsla kinship is depicted in Figure 23.

Figure 22. The owner and her dogs participating in the study (A). Dogs wore a harness equipped with a GPS and moved freely during the walks (B).

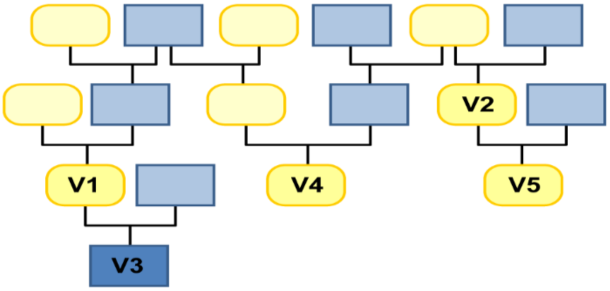

Figure 23. Genealogy of the vizslas. The colouring and shape of symbols indicate the sex of the individuals: yellow rounded boxes are females, blue rectangular boxes are males. The graph shows all relevant relationships between the subjects and their parents/offsprings.

Procedure

GPS data were collected during 14 daily walking tours, each lasting about 30-40 minutes between 2 May 2010 and 25 November 2010. We analysed 823,148 data points. The high-resolution GPS devices were attached to the dogs with ordinary harnesses (Figure 24), while the owner carried one device attached to her shoulder. The 5 Hz custom-designed GPS devices had a time resolution of 0.2 s and previous independent tests with the same devices showed a spatial accuracy of 1-2 m (4]. Weighing only 16 g, and with dimensions of 2.5 cm x 4.5 cm, it is reasonable to suppose that the devices did not hinder the dogs' movements.

The group always walked on the same open grassy field, with the approximate dimensions of 500 x 1000 m, near Budapest, Hungary (located 47^o 25' 17'' N latitude, 19^o 8' 45'' E longitude). The task of the owner was walk continuously and with a constant speed as far as possible during the walks. The dogs were allowed to walk and run freely, and the owner called the dogs back to herself only when she noticed some kind of danger, which happened on just a few occasions. Graphical summary of the Procedure is presented in Figure 24.

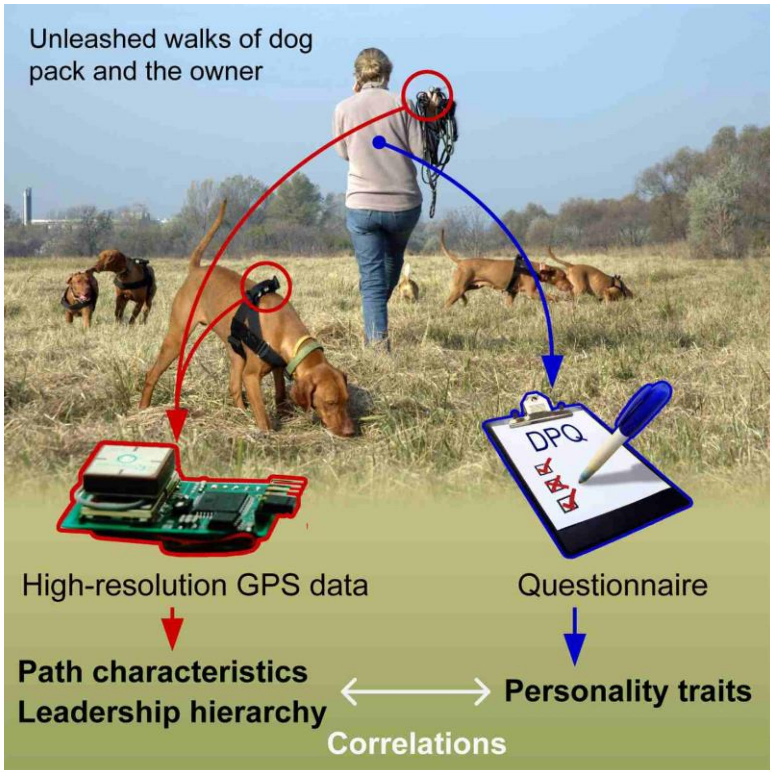

Figure 24. Graphical abstract of the study.

Questionnaire surveys

The personality of the dogs was quantified using two questionnaires that were completed by the owner at the end of the GPS measurements.

(1) The Dog Personality Questionnaire (DPQ) (Jones, 2008). DPQ was compiled from 1,200 descriptions culled from dog-personality literature, shelter assessments, and dog experts' input. A

narrowed list was administered to more than 6,000 participants. Items were evaluated in terms of factor- and facet-loadings, content validity, internal consistency, inter-rater reliability, test-retest reliability, and predictive validity. Convergent criteria favoured five factors, labelled as Fearfulness, Aggression towards People, Activity/Excitability, Responsiveness to Training and Aggression towards Animals. Narrower facets within each factor were also identified. The DPQ has a 75-item and a 45-item form, but we used the latter one (Table 20, for factors and facets also see Table 18).

(2) The dominance questionnaire (Pongrácz, Vida, Bánhegyi, & Miklósi, 2008), to our knowledge, is the only questionnaire available, which was developed with the aim of assessing dominance. The questionnaire quantifies agonistic interactions between pairs of dogs. The owner had to answer four questions concerning each dog pairs: usually which one barks first when a stranger comes to the house (in a competitive situation, dominant dogs bark more (Cafazzo et al., 2010), which dog licks the other's mouth more often (a submissive display, Schenkel, 1967), which one eats first when they get food at the same time and at the same spot (dominant animals have priority access to food, Drews, 1993), and which one wins fights (dominant animals are consistent winners, Drews, 1993). Dogs could receive 1 point for each question, and we summed up the points of each dog (Figure 26C, Table 20).

Dogs	**V1**	**V2**	**V3**	**V4**	**V5**	**M**
Breed	Vizsla	Vizsla	Vizsla	Vizsla	Vizsla	Mix
Sex	female	female	male	female	female	female
Neutered status	intact	neutered	intact	intact	intact	intact
Age at adopting (years)	2	0	0	0.2	0	2
Age at the end of measurement (years)	6.5	7	1.5	1	4	4
Weight (body mass) at the end of measurement (kg)	28	26	26	26	25	10
DPQ1. Fearfulness	2.00	1.08	2.67	1.25	4.08	3.42
DPQ2. Aggression towards people	2.17	2.83	2.17	1.50	2.00	1.33
DPQ3. Activity/Excitability	4.67	5.00	5.00	5.17	5.17	4.67
DPQ4. Responsiveness to training	5.83	6.83	5.50	5.50	5.50	2.17
DPQ5. Aggression towards animals	5.33	5.33	4.33	3.44	5.22	4.11
DOMINANCE Questionnaire	10	14	10	2	8	1

Table 20. Demographic variables and factor scores of dogs. (DPQ: Dog Personality Questionnaire, Jones, 2008)

Data Analysis

To extract information concerning the interactions between group members, we used a directional correlation analysis (Nagy et al., 2010) with a time window to quantify the fast, joint direction changes of pairs. Highly correlated direction changes of pairs are usually found only when two dogs interact by running a part of a loop together. The timescale of the owner's direction changes was much larger than that of the dogs, and – due to the short time window and the typically small time delays – it was not covered in the calculations. Therefore, interactions between the owner and the dogs were not detected with this method. However, we know that the owner was walking on a predetermined route, and clearly led the whole group on a longer time scale (Figure 25).

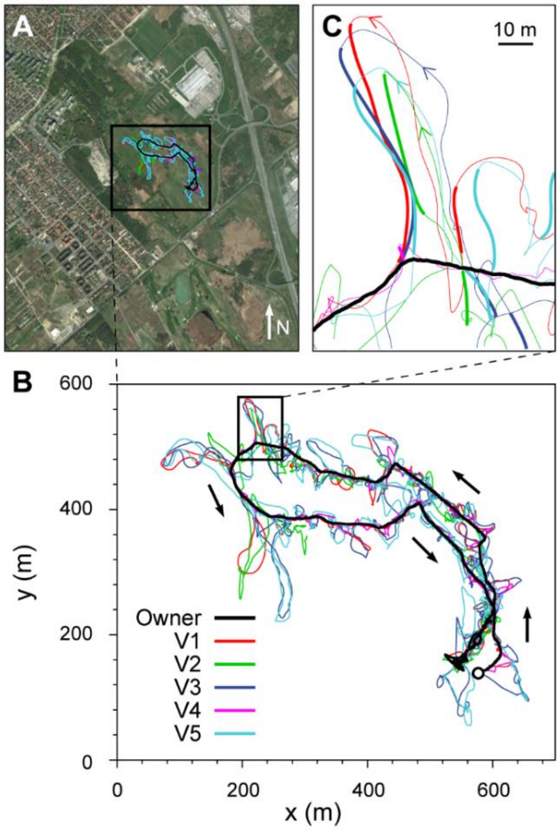

Figure 25. A typical walk of the group and the illustration of returning loops (A). Highly detailed trajectories of the dogs (only vizslas are shown) and of the owner during a 30-minute walk. Arrows indicate the direction of motion. ***(B)*** *The dogs run in loops and return from time to time to the owner. Thickened segments of the tracks show when a dog's return to its owner was found by our automated method (C).*

We calculated directional correlation values for all short trajectory segments that were in a 6 s time window (t_{win}; in other details the method was identical to (Nagy et al., 2010)), thus isolating short-term effects. We used t_{win}=6 s in the study, but the exact choice for the time window size has no substantial effect on the results. A local interaction event was defined to exist when corresponding trajectory segments had a higher correlation value than C_{min}=0.95.

To extract leading tendency differences between members of pairs, the temporal directional correlation delay times (τ_{ij}) were determined with the maximal correlation value. Positive τ_{ij} values correspond to leading events when dog *i* leads dog *j*, as the direction of motion of *i* is 'copied' by *j* delayed in time. For each pair, leading-following events corresponding to different τ_{ij} time delays were summed for each case in a walk, and for all 14 walks measured. For a detailed description of the applied method and a histogram of the found time delays between dog *i* and dog *j*, see Figure 26A.

If a clear maximum of the time delay histogram exists, it indicates frequent interaction between a dog pair at and near a well-defined time. In many cases it can be seen from the histograms of those dog pairs where interaction was found (*Figure 26* shows a typical example) that the leading and following roles (i.e. the sign of the time delay) are dynamically changing during a walk and also between walks. Significant deviation from zero in the location of the maximum value indicates that the dogs in the current pair have different leading propensities, suggesting a directed leader-follower interaction. The full width at half maximum of the histogram characterises how stable the leader-follower relationship between a pair is.

We constructed an interaction network based on the detected interactions and leading tendency differences (Figure 26B). An edge (or link) indicates detected interaction between a dog pair. In those pairs where there is a significant difference in leading tendency we defined a directed edge (pointing from the dog who was found to lead more frequently to the one who more often assumes the role of follower).

The result of the method using the directed edges of the leadership network to characterise active connections was confirmed in an independent way. From the positional data we determined whether members of a pair spend more time in the close vicinity of each other compared to a randomized case. This vicinity method does not require synchronised movement from interacting pairs. The resulting "social" network of the directional correlation and the vicinity method are in high correlation (two-tailed Pearson correlation, r=0.600, n=15 (number of possible pairs), p=0.018).

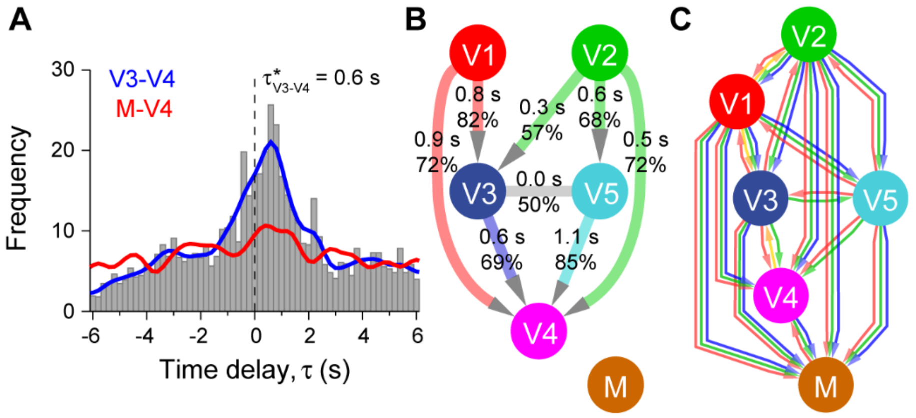

Figure 26. Directional correlations between tracks of dog pairs (A), the resulting leadership network (B). and the results of the dominance questionnaire C). In details: Directional correlation delay time (τ) values for a given pair (V3 and V4) when high correlation was found for a time window shown by the grey histogram, while the blue curve shows the function gained by Gaussian smoothing with σ=0.3 s. The distribution shows a clear peak at τ=0.6 s. For a comparison, the red curve shows a directional correlation delay time function for another pair (M and V4), where no connection was found between the two dogs in the absence of a significant peak.* ***(A)*** *Summarised leadership network composed of the directional delay time values. Each directed link points from the individual that plays the role of the leader more often in the given relationship toward the follower. The grey link shows a strong connection between V3 and V5 with an evenly matched relationship (τ*=0 s). The upper values on the edges indicate the mode of time delays in seconds and the lower values show the average portion that the leader of that pair was actually leading. Note that these modes are from wide distributions (as shown on panel A) with an average full width at half maximum of 3.7 s. The mixed-breed (M) is not connected to any vizslas, and so is not part of the network. This network is used to calculate leading tendency, which is the number of followers that can be reached travelling through directed links.* ***(B)*** *Dominance network between the dogs derived from the dominance questionnaire* (Pongrácz et al., 2008). *Each directed*

edge points from the dominant individual toward the subordinate one. The colours represent the context when dominance is evident: red: barking, orange: licking the mouth, green: eating and blue: fighting. The nodes were arranged in the vertical direction in such a way that more edges point downwards than upwards between all pairs ***(C).***

14.4 Results

Characteristics of the paths

A general overview of the GPS-logged trajectories (see Figure 25) shows that the dogs run away from the owner periodically, then turn back and return to her, in a loop. It can also be seen that they prefer running these loops or a part of them with one or more group. Given that the dogs' speed was significantly higher than that of the owner (1.5-3.7 times), this motion pattern allows dogs to cover a greater distance than the owner while also keeping the group together. We calculated several simple characteristics of the trajectories and performed an analysis concerning the returning events (Table 21).

The preferred running speeds of the dogs, the relative distances covered, and the distances from the owner were unique and consistent characteristics of an individual dog's path, while other characteristics (e.g, distance from dogs) were less consistent and/or distinctive.

Dogs	**V1**	**V2**	**V3**	**V4**	**V5**	**M**
Preferred running speed (m/s)	2.6 ± 1.0	3.0 ± 0.5	3.4 ± 0.6	1.5 ± 0.4	4.0 ± 0.5	1.4 ± 0.2
Relative distance covered	2.4 ± 0.7	2.3 ± 0.3	3.7 ± 1.0	1.8 ± 0.5	3.2 ± 0.7	1.5 ± 0.2
Distance from the owner (m)	10.3 ± 4.2	16.9 ± 4.0	20.2 ± 5.3	9.0 ± 2.4	23.3 ± 6.5	13.7± 4.4
Distance from dogs (m)	16.0 ± 3.3	17.0 ± 1.4	18.1 ± 2.6	15.9 ± 3.7	19.9 ± 2.6	19.0 ± 3.6
Time period of the returns (s)	52 ± 47	52 ± 47	40 ± 37	75 ± 74	52 ± 49	108 ± 94
Loop length (m)	16 ± 16	20 ± 14	20 ± 17	12 ± 10	24 ± 21	22 ± 20
Far-from-owner ratio	0.45 ± 0.17	0.56 ± 0.07	0.50 ± 0.12	0.56 ± 0.06	0.54 ± 0.08	0.59 ± 0.11

Table 21. Relevant variables describing the characteristics of dogs' paths and variables extracted from the returning event analysis for each subject (Vizslas; V1 to V5 and the mixed-breed dog; M). For each variable, averages over the walks and standard deviatio between the walks are shown (Mean ± SD), except for time period of the returns and loop length, where the SD of all data is indicated.

Interactions

To extract information about the interactions between group members, we used a directional correlation analysis (Nagy et al., 2010) with a time window to quantify the fast, joint direction changes for all possible pairings of the dogs (Figure 26A).

We detected frequent short-term interactions and leading tendency differences between dog pairs within the group. The leading and following roles between interacting pairs were often changed during walks and between walks. To check the robustness of the interactions, the directional delay times were calculated for the first 7 and the second 7 walks separately for all pairs. High correlation was found (two-tailed Pearson correlation: r=0.635, n=15, p=0.011), i.e.

significant differences in leading tendency were detected over longer timescales. Calculated from a Gaussian fit to the peak of the relevant distributions we found that dogs play the role of leader in a given pair about 50-85% of the time (57% to 85% when directed leader-follower relationships were found).

Based on the directional delay time values, we created a summarised leadership network (Figure 26B). In the network each directed link points from the individual, which played the role of the leader more often in that given relationship toward the follower. We used this network to calculate leading tendency, which is the number of followers that can be reached travelling through directed links.

We also calculated ‘active connections’, which shows the number of how many interactions a dog has (with the number of edges a dog is connected with in the network).

Relationships between the trajectory variables, leading tendency, dominance ranks, and personality traits

Correlations between trajectory-based variables, leading tendency, personality traits (Jones, 2008, Table 20) and dominance rank (Pongrácz et al., 2008, Table 20) were calculated using two-tailed Pearson correlation for the vizslas only ($n=5$) (Figure 27)) and also for all subjects ($n=6$). We tested our data for normality using a Shapiro-Wilk test ($p<0.05$), and where a significant deviation from a normal distribution was found, we used Spearman correlations (indicated as r_S).

Our main aim was to investigate whether the leadership we defined based on the motion patterns had any connection with the social dominance.We found that the leading tendencies calculated from the GPS data significantly correlated with the dominance ranks gained from the dominance questionnaire (Pongrácz et al., 2008) ($r=0.92$, $n=5$, $p=0.026$). To support this result, we performed a comparison with a randomisation using all possible permutations, and this correlation value proved to be significantly higher than it was for the randomised cases.

To find more correlations in our dataset of trajectory variables and personality traits, all 300 possible pairings were analysed. Note that due to the large number of variable pairs and the small number of dogs involved in the study, none of the p-values remain significant after correction for multiple comparisons (Bonferroni, Sidak or Benjamini–Hochberg procedure). But the correlations mentioned here were all significantly higher than the corresponding values of the randomly permuted cases.

The distance from other dogs correlated with the fear of dogs facet ($r_S=0.92$, $n=5$, $p=0.028$) and the excitability facet ($r_S=0.92$, $n=5$, $p=0.026$). Dogs that, according to the owner, avoid other dogs and seek constant activity maintained a longer distance from their group mates during the walks.

The time period of the returns (the average time duration between returning events) was found to be inversely correlated with the controllability facet ($r=-0.82$, $n=6$, $p=0.046$), and the dominance rank measure ($r=-0.84$, $n=6$, $p=0.036$). Dominant dogs who were more responsive to training returned to the owner more often.

The far-from-owner ratio (the time ratio of being relatively far from the owner) correlated negatively with companionability ($r=-0.87$, $n=6$, $p=0.024$). Dogs that, according to the owner, seek companionship from people also like staying in the owners’ proximity.

The preferred running speed correlated with the general aggression facet of the aggression toward people factor (*r*=0.95, *n*=5, *p*=0.015). More aggressive dogs ran faster during the walks.

In addition to being correlated with dominance rank (mentioned earlier), leading tendency was positively correlated with: age (*r*=0.91, *n*=5, *p*=0.032), responsiveness to training (r_S=0.92, *n*=5, *p*=0.028), controllability (*r*=0.98, *n*=5, *p*=0.003), and aggression towards people (*r*=0.95, *n*=5, *p*=0.013). These relations indicate that those dogs that have a tendency to take the leading role during walks are more aggressive and dominant, and they are also more controllable by the owner, based on the personality questionnaires (Figure 27).

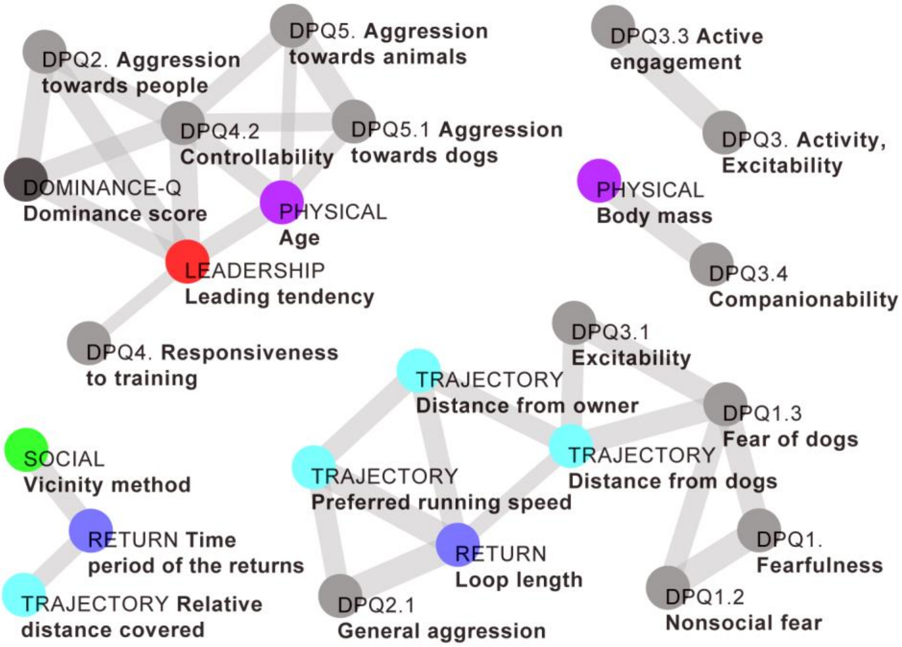

Figure 27. Significant correlations of variables calculated from trajectory data with the personality traits of the dogs measured by questionnaires. The figure shows the significant correlations (where p*<0.05) between the variables (edge width indicating the strength of correlation) calculated for the vizslas (*n*=5). The first term of each name and the colouring of the nodes show the origin of each variable: DPQ: Dog Personality Questionnaire ([51]; gray), Dominance-Q: dominance questionnaire ([29]; dark gray); Physical: physical attributes of the dogs (purple); Trajectory: simple characteristics from the trajectories (cyan); Return: relevant characteristics of the returns to the owner (blue); Social: number of social connection to other dogs calculated from trajectories (green); Leadership: leadership hierarchy from directional correlation delays (red). Only those questionnaire variables are shown which had significant correlation with any variable of another type. All connections are shown between the variables presented on the plot.*

14.5 Discussion

By analyzing the GPS trajectories of freely moving dogs and their owner during walks, we found significant differences in simple path characteristics of the individual dogs. The preferred running speed of vizslas ranged from 1.5 to 4.0 m/s (5.4-14.4 km/h), they covered a 1.8-3.7x longer distance than the owner during a walk, and the usual distances from the owner ranged from 16 to 20 m. These results might be useful for conservation managers in establishing areas where dog walking is prohibited (Banks & Bryant, 2007) and may also help in designing parks, as dog-walking is a popular method for increasing human physical activity (for a review, see Cutt, Giles-Corti, Knuiman, & Burke, 2007).

A directional correlational analysis (M. Nagy et al., 2010, 2013) revealed leader-follower interactions between the group members. We detected a loose but consistent hierarchical leadership structure. Due to the dynamic nature of the pairwise interactions, role reversals did occur during walks and an individual took the role of the leader in a given pair in about 73% (ranging from 57% to 85%) of their interactions, where directed leader-follower relationships were found. This ratio is of similar magnitude to the case of wild wolf packs with several breeding individuals, where leaders led for 78% of the recorded time, ranging from 58% to 90% (Peterson et al., 2002). The role of initiating common actions is also frequently interchanged between guide dogs and the owner (Naderi, Miklósi, Dóka, & Csányi, 2001) and between dogs during play (Ward, Uer, Rb, & Muts, 2008). But over a longer timescale, differences in leading tendency remained consistent; thus decision-making during the collective motion was not based on an egalitarian system in our sample.

Although the existence of an overall dominance hierarchy in dogs is debated (Bradshaw et al., 2009), and the vizsla is a “peaceful” breed, which, compared to other breeds, rarely fights with conspecifics (B Turcsán, Kubinyi, & Miklósi, 2011), we detected a dominance hierarchy via a questionnaire assessing agonistic and affiliative situations (Pongrácz et al., 2008). We found that dominance rank and leadership were strongly connected. Dogs who tend to win in everyday fighting situations, eat first, bark more or first, and receive more submissive displays from the others, and have more influence over the decisions made during collective motion.

The correlation between leadership and dominance is consistent with a trend in ‘despotic’ social mammals (King & Cowlishaw, 2009), but probably not characteristic in wolves with several breeding individuals (Peterson et al., 2002). In large wolf packs (with 7-23 individuals), breeding individuals lead during travels, independently from dominance status. But this situation is relatively rare, as the typical wolf pack is a nuclear or extended family, where the only breeding male leads the pack during travel (Mech, 2000). Unlike wolves, the dog is a promiscuous species, and in a group, there is usually no single pair of breeders (Cafazzo et al., 2010). In our family dog group, the highest ranking dog (V2) was neutered, which may suggest that both leadership and dominance have little or no relationship with reproductive behaviour in family dogs, consistent with observations in feral dogs in India (Pal, 2005, 2011; Pal, Ghosh, & Roy, 1999).

We also investigated the relationship between leadership and personality to reveal which personality types occupy particular positions in the leadership network. We found that leaders/dominants were more responsive to training, more controllable, and more aggressive than followers/subordinates. Other data also suggest that dominance cuts across different contexts and is correlated with boldness, extraversion, and exploratory tendencies in several taxa (Johnstone & Manica, 2011), and assertiveness in wolves (Peterson et al., 2002), but reported links between personality and leadership are rare (Krause et al., 2010).

Age was a reliable indicator of leadership and dominance. If rank acquisition is learnt at an early age with regular reassessments of dominance, younger dogs may remain subordinate, long after initial body weight differences have disappeared. In our group, both dams were dominant over their adult offsprings, and each adult vizsla dominated the juvenile vizsla, which supports the hypothesis that the acceptance of subordinate status within a dog group is probably mediated by conditioning. If rank acquisition is learnt at an early age with regular reassessments of dominance, younger dogs may remain subordinate, long after initial body weight differences have disappeared (Datta & Beauchamp, 1991).

Not only leadership and dominance, but movement characteristics were also related to personality. Fearful and excitable dogs maintained a longer distance from other dogs. More controllable and dogs returned to the owner more often, while less companionable dogs spent more time far from the owner. Surprisingly, more aggressive dogs ran faster during the walks. As male dogs harvest more game than females in preindustrial societies (Koster & Tankersley, 2012), and experimental evidence on mice suggests that testosterone increases persistence of food searching in rodents (Archer, 1977), higher speed might be related to testosterone levels. Note, however, that even the most “aggressive” score was relatively low in our sample (2.67 out of the maximum 8).

Social organization and social structure vary among populations (Kappeler & Schaik, 2002), and in the case of dogs, they vary among breeds and groups (Wright, 2004), thus group decision-making processes are expected to vary accordingly (Fischer & Zinner, 2011). The main limitation of our study is the low sample size. Observing other groups and breeds may provide different results. For example, the hierarchical network of sled dogs which work as a team with a lead dog (Huson, Parker, Runstadler, & Ostrander, 2010) is more robust than that of our sample. It would also be interesting to investigate what happens with the leadership network if the owner runs or rides a bike, and her speed is comparable to the dogs’ speed.

To summarise, by using GPS devices we found that the leader and follower roles are dynamically interchanged during walks, but are consistent over a longer timescale. The leader-follower network was hierarchical, and the dogs' positions in the network correlated with dominance order derived from everyday life situations. Leadership also correlated with age and personality traits such as trainability and aggression.

An important limitation of the study is the small sample size. However, our main aim was to develop a new method for tracking the motion patterns of terrestrial mammals. Our findings on the connection between variables extracted from GPS trajectory data, age, dominance rank, and personality traits could pave the way for automated animal personality and dominance measurements. As dogs are ideal models of human social behaviour (Miklósi, 2014; Topál et al., 2009), aging (Chapagain et al. 2018; Szabó et al. 2016), and social robots (Miklósi & Gácsi, 2012), the present study may also be applied to measure social interactions in humans, as in the case of parents walking with their children, or humans interacting with robots.

15 Dominance status and age in companion dogs sharing the same household[11]

15.1 Abstract

Dominance is well defined in ethology, debated in psychology, and is often unclear among the dog owning public and in the press. However, to date, no study has examined how owners perceive dominance in dogs, and what different behaviours and personality types are used to describe dominant and subordinate individuals. A questionnaire study was launched to investigate the external validity of owner-derived estimates of dominance in dog dyads sharing the same household (N=1151). According to the owners, dogs rated as dominant (87%) have priority access to resources (resting place, food, and rewards), undertake certain tasks (defend and lead the group, bark more), display dominance (win fights, lick the other's mouth less, and mark over the other's urine), share certain personality traits (smarter, more aggressive and impulsive), and are older than their partner dog (all $p<0.0001$). An age-related hypothesis has been suggested to explain dominance in dogs, but we found that dog age did not explain the occurrence of dominance-related behaviours over the owners' estimate of dominance status. Results suggest that owner-derived reports of dominance ranks of dogs living in multi-dog households correspond to ethologically valid behavioural markers of dominance. Size and physical condition were unrelated to the perceived dominance. Surprisingly, in mixed-sex dyads, females were more frequently rated as dominant than males, which might correspond to a higher proportion of neutered females in this subgroup. For future studies that wish to allocate dominance status using owner report, we offer a novel survey.

15.2 Introduction

The term dominant is often used by dog owners to describe dogs, however, there may be little agreement regarding its meaning, as dominance is defined differently in ethology, psychology, and among the public. In ethology, dominance describes long-term dominant-subordinate social relationships within a dyad or group (Clutton-Brock et al., 1979; Drews, 1993). Dominant individuals usually have priority access to key resources such as food and reproductive partners, due to the consistent winning of agonistic interactions or deference, during which one individual consistently gives way to another (Lorenz, 1966; Smith & Price, 1973). However, in psychology, dominance is often referred to as a personality trait (Gosling and John 1999) and describes the disposition of an individual to assert control in dealing with others. Finally, the word "dominance" is defined as having control, authority, and power or influence over others (Westgarth, 2016), and the general public may use this word to describe individuals who are more powerful, successful, or important than others. When we consider these three definitions, it is not surprising it is unclear what dog owners mean when they use the term 'dominance' in reference to their dogs.

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[11] Based on Kubinyi E, Wallis LJ. 2019. Dominance in dogs as rated by owners corresponds to ethologically valid markers of dominance. PeerJ 7:e6838.

In the next paragraphs we summarize the current knowledge about dominance in dogs and then we examine how scientific findings are related to the perception of dominance in the dog owning public. Although dominance hierarchies have previously been described in free-ranging dogs (Bonanni & Cafazzo, 2014; Bonanni et al., 2010; S. Cafazzo et al., 2010), in dogs living in packs in enclosures (Dale, Range, Stott, Kotrschal, & Marshall-Pescini, 2017; Range, Ritter, & Viranyi, 2015; Van Der Borg, Schilder, Vinke, De Vries, & Petit, 2015), and in neutered pet dogs at a dog day care centre (Trisko & Smuts, 2015; Trisko, Smuts, & Sandel, 2016), the existence and validity of linear dominance hierarchies in these animals is highly debated both by the public and some researchers, mainly because agonistic interactions are rare and contextual (Schilder et al., 2014). Data on kennelled dogs suggest that dominance is based on submission (signalled mostly by body tail wag and low posture) rather than on aggression (Van Der Borg et al., 2015). Therefore, it has been suggested that domestication has altered the social behaviour of dogs compared to wolves, and submissive behaviour is used to defuse conflicts (Bradshaw, Blackwell, and Casey 2009).

In addition, as van Kerkhove (2004) notes, although dominance hierarchies in dogs are often described through access to resources (or "competitive ability", (de Waal, 1986)), not all individuals are equally motivated (or physically able) to obtain them. Therefore the subjective resource value, in combination with associative learning (Bradshaw, Blackwell, and Casey 2016; Bradshaw et al. 2009) and personality (McGreevy, Starling, Branson, Cobb, & Calnon, 2012) can explain interactions between dogs more simply than dominance theory. Moreover, if researchers do not assume the existence of a dominance hierarchy, they seldom identify one, thus a more dynamic approach is needed in order to understand social organizations (Overall, 2016).

However, when a hierarchy was detected in a dog group, several parameters have been shown to covary with dominance status, such as age, sex, and personality. Older dogs were found to be more often dominant than young individuals (Bonanni et al., 2017, 2010b; Cafazzo et al., 2010; Mech, 1999; Peterson et al., 2002; Trisko & Smuts, 2015). Therefore Bradshaw et al. (2016) suggested that a simple rule of thumb could help to explain formal dominance in dogs: “in order to be allowed to stay in the group, perform affiliative behaviour towards all the members of the group older than you are”. However, in a group of domestic dogs, van der Borg and colleagues (2015) did not find correlations of rank with age, and it remains unexplored whether the age related hypothesis is a better predictor of formal dominance than dominance displays.

Concerning sex as a potential confounding factor of dominance, conflicts between dogs living in the same household are more common between dogs of the same sex, and female-female pairs are most often affected (Sherman, Reisner, Taliaferro, & Houpt, 1996; Wrubel, Moon-Fanelli, Maranda, & Dodman, 2011). Mixed-sex dyads are more likely to affiliate and less likely to show unidirectional displays of submissiveness and aggression than same-sex pairs (Trisko & Smuts, 2015). In wolves, separate male and female age-graded dominance hierarchies have been observed in captive packs (Packard, 2003). Overall, male wolves were found to be more often dominant and/or leaders of the pack (Clark, 1971; Haber, 1977; Mech, 1999). In one study on free-ranging dogs, a sex age graded hierarchy was found, such that males dominate females in each age class, and adults dominate over subadults, and subadults over juveniles (Cafazzo et al., 2010). However, sex had no clear effect on dominance in a family pack of captive arctic wolves, although sex-separated linear hierarchies showed a stronger linearity than female-male hierarchies (Cafazzo, Lazzaroni, & Marshall-Pescini, 2016).

Personality traits might also associate with dominance status. For example, aggression towards people and controllability was linked to dominance rank and leadership in pet dogs according to Ákos et al. (2014). Since some dog owners describe dogs that often show dominant behaviour towards other dogs as having a "dominant personality", studies linking personality traits to dominance status would be especially useful to help clarify the correct terminology to the public. Dog owners confusion regarding the term dominance can be partly explained by the fact that based on a literature review on canine personality, researchers have identified a broad dimension labelled as 'Submissiveness', and defined it as the opposite of dominance (Fratkin, Sinn, Patall, & Gosling, 2013). According to the authors, "Dominance can be judged by observing which dogs bully others, and which guard food areas and feed first. Submission can also be reflected by such behaviours as urination upon greeting people". Thus, even in the scientific literature some authors define dominance as a personality trait, and there is an ongoing debate in human, primate, and dog personality research on how to interpret certain traits. However, according to the majority of ethologists dominance is not a personality trait (Schilder et al., 2014). While personality is largely independent of context and is stable over time (Jones and Gosling 2005) dominance status depends on the interacting partners.

The popular media has also played a role in influencing owners' attitudes, by often describing dominant dogs as those with behavioural problems or a tendency towards aggression. A dog is often referred to as dominant when it "misbehaves", e.g. jumps up on or shows aggression towards the owner. The belief that such behaviours may signify that the dog is attempting to control the owners' behaviour, is based on erroneous models of wolf pack organisation, and has often been used to justify the use of abusive training techniques (Bradshaw et al., 2016). However, negative reinforcement and positive punishment training techniques can cause increased stress, fear and mistrust, and are associated with increased aggression towards other dogs in the household (Casey et al., 2013), and towards human family members (Casey et al., 2014). Positive punishment has obvious abusive connotations too, according to these studies.

Previously, several studies have attempted to determine the dominance rank of dogs living in multi-dog households by utilising owner questionnaires (Ákos et al., 2014; Pongrácz, Bánhegyi, & Miklósi, 2012; Pongrácz et al., 2008). Pongrácz et al. (2008) used a four item questionnaire to measure dogs' dominance status in dyads, and related them to differences in social learning in response to a human or dog demonstrator. Dogs were identified as dominant if they displayed at least three behaviours out of four (barked more/longer, licked the other dog's mouth less, ate first, and won fights). Dominant dogs were less likely to learn from observing other dogs, and more likely to copy a human demonstrator. They also performed better than subordinates in a problem solving task, but only when observing a human demonstrator (Pongrácz et al., 2012). Subordinate dogs showed better learning in the dog demonstrator condition. Results from both studies suggest that social rank affects performance in social learning situations, as dominant dogs tend to follow humans while subordinate dogs follow other dogs. Thus, owner questionnaires could be a valid method to determine the dominance rank of individuals within dog dyads, similarly to other dog behaviour studies, particularly as the quality of data produced by citizen scientists has proved to be satisfactory (Hecht & Spicer Rice, 2015).

To understand how the dog-owning public use the word "dominance", we evaluated what attributes they associated with dominance using a questionnaire study. We have to note here, that, as Westgarth suggests (2016), it is possible that a dominance hierarchy is not fundamental to the structure of the dogs' social system, but is rather the by-product of human observation. According to this view, dominance is simply the question of individual interpretation, and this is another reason to investigate how the public interprets "dominance" in dogs. In this study, we surveyed people that owned multiple dogs. We investigated the relationship between the dogs' ranks, behaviour, and demography. We were interested in finding out whether dogs that the owners have classified as "dominant" display certain behaviours more or less often than their subordinate partner. We also tested the age related hypothesis suggested by Bradshaw et al. (2016) by determining which factor best explained behavioural and demographic differences between the dyads, owner reported hierarchical status or age status.

15.3 Methods

Subjects

Between 25th June and 13th August 2017, 1156 owners of at least two dogs filled in a questionnaire in Hungarian, which was advertised in a social media Dog Ethology group. We identified the dogs using their given names, to ensure that no duplicate entries were included in the analysis. After data cleaning and deleting of duplicate entries, 1151 responses remained, which detailed owners' responses for unique individual pairs of dogs. Owners indicated the sex and reproductive status of each dog in the dyad, after allocating them to either Dog A or Dog B (based on their own choice). We have no information on how owners chose which dogs to compare if they had more than two dogs. Twenty-three percent of the dyads consisted of males only, 28% females only, and 49% were both sexes. The percentages of neutered individuals were 45% in males and 62% in females.

Procedure

The questionnaire consisted of 21 items (Table 22). In the case of items 1-19, owners indicated which of the two dogs best fitted the description: Dog A, or Dog B. Owners could also select "Similar" if both dogs fitted the description, or "N/A". When the owners marked "N/A" we assumed that they could not answer the question as the dog/dogs did not display that behaviour, or that situation did not occur (e.g. the dogs never fight with each other or they do not go for walks together), or owners were unsure/did not fully understand the question, or the answer was not known to them (e.g. they could not assess which of the dogs was in better physical condition). We chose the behaviours based on previous studies (Pongrácz et al., 2012, 2008), and included markers of agonistic (i.e. winner of fights) and formal dominance (i.e. licking the mouth of the partner, usually during greeting ceremonies (signalling the acceptance of lower social status) Bonanni et al., 2010), as well as resource-holding potential (obtains more food, (Vervaecke, de Vries, & van Elsacker, 2000), better resting places, etc.). In addition, we included other factors, which have previously been proposed to be relevant when measuring leadership and dominance, such as age, sex, size, physical condition, leadership and specific behavioural characteristics, including intelligence, obedience, aggressiveness and impulsiveness (Cafazzo et al., 2010; Conradt & Roper, 2003; Conradt & Roper, 2005; Drews, 1993). Items 2-4 and 6 were the same as those used in (Pongrácz et al., 2008). In the case of items 20 and 21, the owner could also indicate “both” or “neither” dogs (Table 22).

Item number	**Item name**	**Questions**
1	status	Which of the dogs is the “boss” (has a dominant status) to the best of your knowledge?
2*	bark	When a stranger comes to the house, which dog starts to bark first (or if they start to bark together, which dog barks more or longer)?
3*	lick mouth	Which dog licks the other dog’s mouth more often?
4*	eat first	If the dogs get food at the same time and at the same spot, which dog starts to eat first or eats the other dog’s food?
5	reward	If they got a special reward (e.g. a marrowbone), which dog obtains it?
6*	fight	If the dogs start to fight, which dog wins more frequently?
7	play ball	If you play with a ball with both dogs, which one retrieves it more frequently?
8	greet owner	When you enter your home, which dog greets you first?
9	walk first	Which dog goes in the front during walks?
10	resting place	Which dog acquires the better resting place?
11	overmark	Which dog marks over the other’s urination?
12	defend group	If the dog’s group is perceived as being under attack, which dog is in the front?
13	smart	Which dog is smarter?
14	obedient	Which dog is more obedient?
15	aggressive	Which dog is more aggressive?
16	impulsive	Which dog is more impulsive?
17	size	Which dog is heavier?
18	physical condition	Which dog is in a better physical condition?
19	age	Which dog is older?
20	sex	Which dog is male?
21	neutered	Which dog is neutered?

*Table 22. Questionnaire items. Owners were asked to fill out the questionnaire for two of their dogs (‘A’ and ‘B’) and indicate which dog corresponds better to the description. They could also select “Similar” if both dogs fitted the description or “N/A” if the question did not apply to the dog dyad. Items marked by * were adopted from Pongrácz et al. (2008).*

Statistical analysis

Analyses were performed in SPSS 22.0 and R 3.3.2. Descriptive statistics were calculated for the sample and summarised in the results section. Note that we did not have the opportunity to use dominance rating (dominant vs subordinate) as a response variable in a model directly, due to the design of the questionnaire, which collected information for dyads (resulting in one line of data per dyad), and not individual dogs. Therefore, individual binomial analyses were the best way to answer our question, "Do dogs which the owners classify as "dominant" show certain behaviours more or less often than their subordinate partner?" and to deal with missing values.

Binomial tests using Dominance Status on the full sample

To investigate the owners' responses for each item (1 to 21), we calculated the percentage allocation of the dogs to each possible category: "Differ" (the dogs in a particular dyad differed in that behaviour/characteristic), "Similar" (the dogs' behaviour was similar) and "N/A" (the owner was not able to determine if the dogs differed).

In order to answer the question "do dogs that the owners classify as "dominant" show certain behaviours more or less often than their subordinate partner", we used binomial tests to compare the distribution of observations between the dogs for each of the replies to items 2 to 21. We included only the dogs that were allocated a "dominant" or a "subordinate" status, based on the response of the owner to item 1 ("Which of your dogs is the boss/dominant). We did not consider dyads where owners indicated that their dogs were "Similar" in dominance status, or where they marked "N/A" (N=148). (Sample sizes are indicated in Figure 1 for each item). We lowered the p level to 0.0023 from 0.05 as suggested by a Bonferroni correction for the 22 comparisons.

Binomial tests using Age Status on the full sample and comparison with Dominance Status

We tested the age related hypothesis suggested by Bradshaw et al. (2016), by using the response of the owner to Age (item 19, "Which of your dogs is older?"), to assess differences between dogs allocated an "older" or "younger" status (dogs which were "Similar" in age, or that were marked "N/A", N=72, were excluded). Next, we used two-sample tests for equality of proportions with continuity correction in order to determine which factor (Dominance status or Age status) best explained the behavioural and demographic differences between the dogs. This test is used to compare two observed independent proportions. The test statistics analysed by this procedure assume that the difference between the two proportions is zero under the null hypothesis.

Binomial tests on the mixed-sex and same-sex dyads

In order to examine any effect of the dyad composition on dominance status allocation, we created subsets of data including mixed sex dyads (N=491), and same-sex dyads (N=512), and ran additional binomial tests to inspect possible associations for items 2 – 21. We again adjusted for multiple comparison using Bonferroni correction and lowered the significance level to 0.0025.

Correlation of the items

We used the binary behavioural categories: "which of your dogs' expresses the behaviour more", the dominant=1 or the subordinate=0, of the 18 different items: bark, lick mouth, eat first, reward, fight, play ball, greet owner, walk first, resting place, overmark, defend group, smarter, obedient, aggressive, impulsive, size, physical condition, and age, and correlated them using a Pearson Correlation. For this analysis we only used dyads where we had no missing information (N=215).

Differences in the number of dominance related behaviours expressed in dominants and subordinates

We created a “dominance score” by summing all the items that were significantly associated with a “dominant” status for each dog in every dyad. Then we created a “difference score” by subtracting the subordinates’ dominance score from the “dominants” for each dyad. The difference score was then used as the response variable in a general linear model to identify the key variables associated with the difference score. All possible interactions between dominant sex (male or female), subordinate sex (male or female), dominant neuter status (intact or neutered), subordinate neuter status (intact or neutered) and dominant age (older or younger) were entered into the model. We also included the main effect of the order the dogs were entered into the questionnaire (Dog A first or second). We included only the dyads where an asymmetry in dominance was detected by the owner (N=931).

15.4 Results

Descriptive statistics

Eighty-seven percent of owners indicated that their dogs differed in social status, 10% perceived them as similar, and 3% marked the question as “N/A” (Fig. 1). Some items were unreliable for differentiating between the dogs. For example, 30.1 - 35.1% of the dyads were reported to be similar in greeting the owner, smartness, and physical condition. Other items were relatively difficult for the owners to assess; 16.2 - 24.3% of owners did not specify a particular dog for the items lick mouth, fight, overmark, and aggressive, Figure 28).

Binomial tests using Dominance Status on the full sample

We tested which items (from items 2-21) were associated with the perceived dominance rank (item 1). Eleven different dog-dog or dog-owner oriented behaviours, five behavioural/personality traits and five demographic factors were examined. The binomial tests revealed that dogs rated as dominant usually (1) have priority access to certain resources such as food, rewards, resting places; (2) are perceived as undertaking specific tasks, such as “guard” the house through barking more, walk in the front during walks (i.e. “leading” the group), defend the group in case of perceived danger; (3) display dominance: win more fights, mark over the other’s urination, and more frequently accept that the other dogs lick their mouth; (4) have characteristic personality traits: are smarter, more aggressive and impulsive; and (5) are older than subordinates according to the owners. Physical condition, obedience, sequence of greeting the owner and retrieving balls were unrelated to perceived dominance ($z=>|5.03|$, $p<0.0001$; see Figure 29 and Table 23 for an overview of the results).

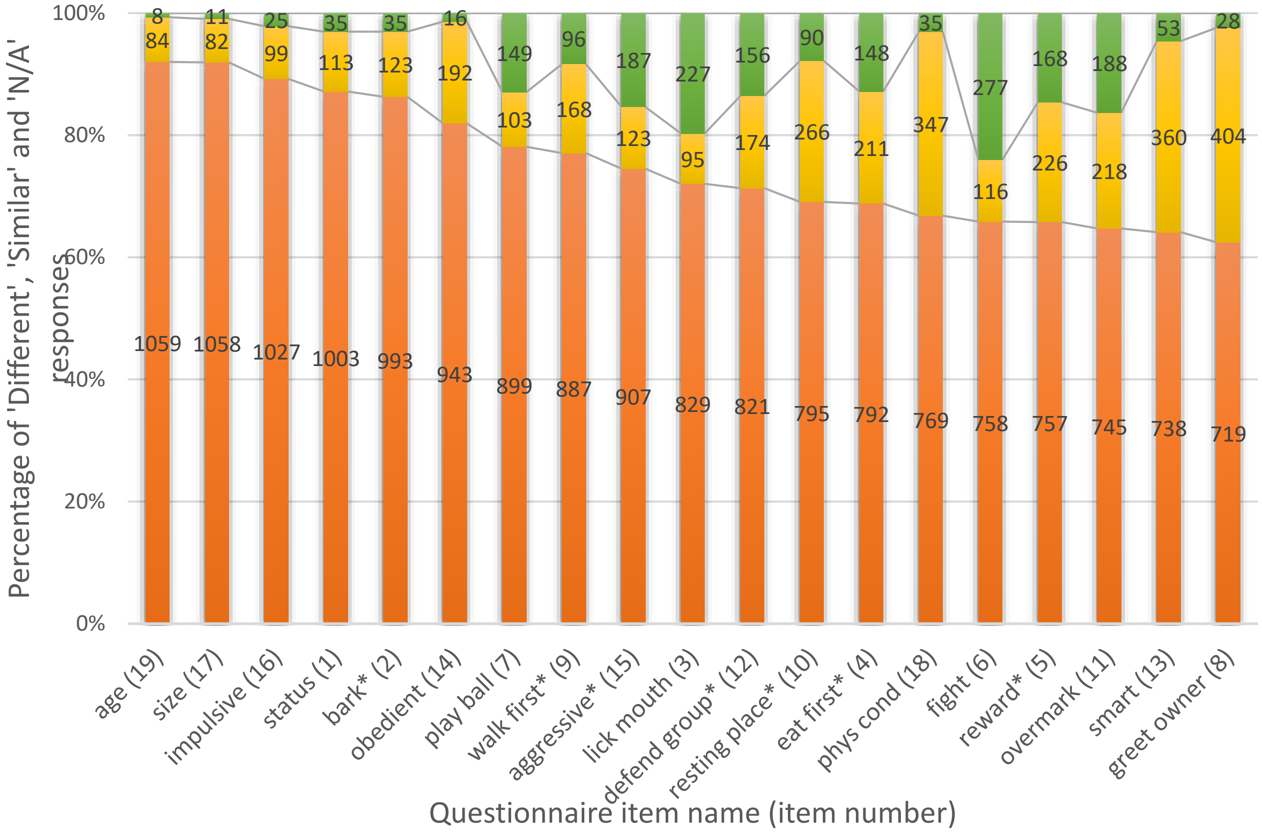

*Figure 28. Differences between characteristics in dog dyads. Colours: orange: dogs in a dyad differ in the characteristic, yellow: dogs are similar, green: N/A. Sample sizes are indicated on the columns, item numbers are in brackets. Seven items, highlighted by *, are suggested for future work based on both their significant link with dominance status (independently from the sex of the dogs) and their occurrence (% of 'Similar' responses were lower than 24.7 and % of 'N/A' responses were lower than 16.1, see Descriptive statistics).*

Binomial tests using Age Status on the full sample and comparison with Dominance Status

When we tested the age related hypothesis suggested by Bradshaw et al. (2016) we found that twelve items were associated with Age status. Six in the same direction as the “dominance” status (bark, lick mouth, fight, resting place, defend group, and smart), and one in the opposite direction, owners found older dogs to be less impulsive, but “dominant” dogs more impulsive. Age but not dominance was associated with five items. Older dogs bark more, play with the ball less, greet the owner less, are in worse physical condition, are larger, and are less often intact than their younger partner dog, according to the owners ($p<0.001$). Dominance status was more strongly linked with 11 items in comparison to age status (for statistical details see Table 23).

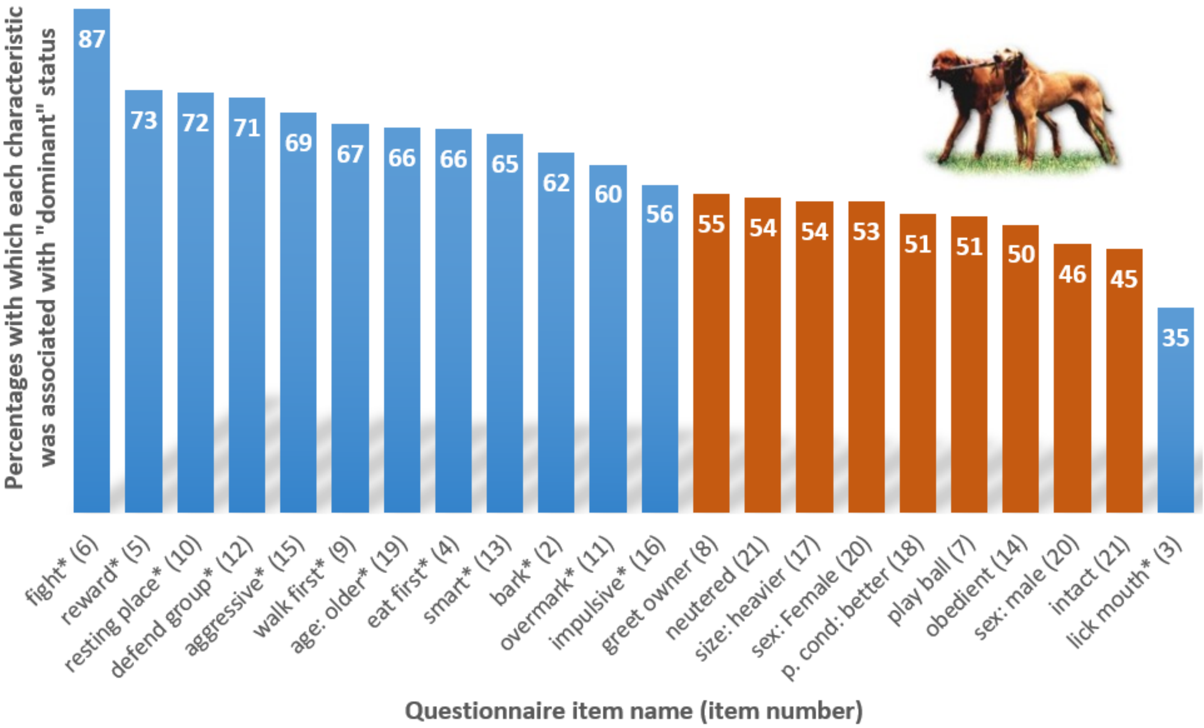

*Figure 29. Percentages with which each characteristic was associated with "dominant" status (numbers in white at the top of each column). The * next to the item name and blue coloured columns indicate that "dominant" status was associated with the item after Bonferroni correction (for the Binomial tests all p values are ≤ 0.0022), red columns indicate that the characteristic was not associated with the item. Item numbers are in brackets. See Table 2 for more statistical results.*

<u>Binomial tests on the mixed-sex dyad sample</u>

In mixed-sex pairs where a dominant was indicated (N=491), 51% of males and 67% of females were neutered. Females were more often reported as dominant over males (57% females, binomial test z=3.25, p<0.001). If we compare dominant females with dominant males in order to help determine what factors might explain why more females are dominant than males: results indicate that 56% of dominant females were older than their partner, in comparison to 66% of dominant males. Thus, older age does not explain the prevalence of dominant females. When a female was rated as the dominant individual, she was more often neutered than when the male was the "dominant" (female neutered 72%, male neutered 51%). "Dominant" males more often (90%) marked over their female partners (while 20% of "dominant" females marked over submissive male partners), defended the group in case of perceived danger, and they were often larger in size than the female "subordinate". Refer to Table 24, Table 25 for more information.

Binomial tests on the same-sex dyad sample

In same-sex pairs (N=512, 48.5% neutered) there was no significant difference between the number of neutered and intact "dominant" animals (z=1.86, p=0.063). “Dominant” individuals were again more often older than “subordinates” (N=319 dyads, 67% older, binomial test z=7.38 p<0.001).

The items that best described owner reported dominant individuals in the full sample remained significant in the same-sex pairs subsample, apart from the item impulsive, which did not differ between subjects rated as dominant and subordinate. Owners reported that 73% of "dominant" females and 64% of "dominant" males marked over their "submissive" same-sex partners. More results can be found in Table 25.

Difference in the number of dominance related behaviours expressed in dominants and subordinates

The dyad that showed the greatest relationship difference (difference score) between the “dominant” and “subordinate” individual (and therefore the clearest status difference) was in a mixed sex dyad when an intact male was considered as dominant over an intact female.

	A. Dominance Status					**B. Age Status**					**Prop. diff**	**C. 2-sample test for equality of proportions**			
Item	**Count**	**Total**	**Prop**	**Z**	**P**	**Count**	**Total**	**Prop**	**Z**	**P**		**X2**	**P**	**95% CI**	
Bark	547	884	0.619	7.03	<**0.0001**	512	920	0.557	3.40	<**0.0001**	0.062	6.953	0.008	0.016	0.109
Lick mouth	259	737	0.351	-8.03	<**0.0001**	218	779	0.280	-12.25	<**0.0001**	0.071	8.669	0.003	0.024	0.120
Eat first	473	717	0.660	8.51	<**0.0001**	400	746	0.536	1.94	0.0261	0.124	22.662	<**0.0001**	0.072	0.175
Reward	497	684	0.727	11.81	<**0.0001**	386	714	0.541	2.13	0.0164	0.186	51.141	<**0.0001**	0.135	0.237
Fight	606	700	0.866	19.31	<**0.0001**	443	703	0.630	6.86	<**0.0001**	0.236	101.920	<**0.0001**	0.190	0.281
Play ball	404	793	0.509	0.50	0.7150	349	835	0.418	-4.71	<**0.0001**	0.091	13.330	<**0.0001**	0.042	0.141
Greet owner	352	644	0.547	2.32	0.0100	295	674	0.438	-3.20	<**0.0001**	0.109	15.194	<**0.0001**	0.054	0.164
Walk first	532	795	0.669	9.50	<**0.0001**	430	824	0.522	1.22	0.1114	0.147	35.819	<**0.0001**	0.099	0.196
Resting place	517	716	0.722	11.85	<**0.0001**	425	754	0.564	3.46	<**0.0001**	0.158	39.352	<**0.0001**	0.109	0.208
Pee	400	669	0.598	5.03	<**0.0001**	372	697	0.534	1.74	0.0407	0.064	5.465	0.019	0.010	0.118
Defend group	527	739	0.713	11.55	<**0.0001**	437	760	0.575	4.10	<**0.0001**	0.138	30.545	<**0.0001**	0.089	0.187
Smart	433	665	0.651	7.76	<**0.0001**	410	692	0.592	4.83	<**0.0001**	0.059	4.710	0.030	0.651	0.593
Obedient	415	838	0.495	-0.24	0.6221	477	879	0.543	2.50	0.0063	-0.048	3.679	0.055	-0.096	0.001
Aggressive	524	762	0.688	10.32	<**0.0001**	392	780	0.503	0.11	0.4572	0.185	53.997	<**0.0001**	0.136	0.235
Impulsive	512	908	0.564	3.82	<**0.0001**	313	952	0.329	-10.53	<**0.0001**	0.235	103.120	<**0.0001**	0.190	0.280
Size: heavier	497	929	0.535	2.10	0.0178	575	999	0.567	5.43	<**0.0001**	-0.032	3.051	0.081	-0.086	0.005
P Cond: Better	353	687	0.514	0.69	0.2461	209	734	0.285	-11.63	<**0.0001**	0.229	76.941	<**0.0001**	0.175	0.280
Age: Older	615	931	0.661	9.77	<**0.0001**										
Sex: Male	427	927	0.461	-2.36	0.0090	503	990	0.508	0.48	0.3168	-0.047	4.128	0.042	-0.093	-0.002
Sex: Female	576	1078	0.534	2.22	0.0131	556	1128	0.493	-0.45	0.6936	0.041	3.621	0.057	-0.001	0.080
Neutered	580	1073	0.541	2.63	0.0043	613	1133	0.541	2.73	0.0031	0	0.000	1.000	-0.043	0.042
Intact	423	933	0.453	-2.82	0.0024	446	985	0.453	-2.93	**0.0017**	0	0.000	1.000	-0.045	0.046

Table 23. Results of the binomial tests using A) the owners' allocation of the dogs to "dominant" or "subordinate" status (item 1) and B) "older" or "younger" status (item 19) as the predicted variables and the 21 items. Bold type indicates that status was assoc associated with the characteristic after Bonferroni correction (for the Binomial tests all p values are ≤ 0.0022). Two-proportion z-tests were used to determine whether the proportion of "dominant" and "older" dogs were equal for each item. C) Two-sample tests for equality of proportions with continuity correction in order to determine which factor (Dominance status or Age status) best explained the behavioural and demographic differences between the dogs. P Cond=Physical condition, Prop=Proportion, Prop Diff=Proportion difference, and 95% CI=95% Confidence intervals.

	***A.* Mixed-sex Dyad**					**B. Same-sex Dyad**						**C. Proportion comparison**	
Item	**Count**	**Total**	**Prop**	**Z**	**P**	**Count**	**Total**	**Prop**	**Z**	**P**	**Prop. Diff**	**Z**	**P**
Bark	268	424	0.63	5.39	**<0.0001***	279	460	0.61	4.52	**<0.0001**	0.03	0.7815	0.4354
Lick mouth	227	356	0.64	5.14	**<0.0001***	251	381	0.66	6.15	**<0.0001**	-0.02	-0.6011	0.5485
Eat first	214	348	0.61	4.23	**<0.0001***	259	369	0.70	7.70	**<0.0001**	-0.09	-2.456	0.0139
Reward	234	329	0.71	7.61	**<0.0001***	263	355	0.74	9.02	**<0.0001**	-0.03	-0.8678	0.3843
Fight	289	338	0.86	13.00	**<0.0001***	317	362	0.88	14.24	**<0.0001**	-0.02	-0.8011	0.4237
Play ball	192	383	0.50	0.00	0.5000	212	410	0.52	0.64	0.2605	-0.02	-0.4438	0.6599
Greet owner	182	314	0.58	2.77	0.0028	170	330	0.52	0.50	0.3102	0.06	1.6426	0.1010
Walk first	253	375	0.67	6.71	**<0.0001***	279	420	0.66	6.68	**<0.0001**	0.01	0.3105	0.7566
Resting place	246	340	0.72	8.19	**<0.0001***	271	376	0.72	8.51	**<0.0001**	0.00	0.0831	0.9362
Overmark	177	346	0.51	0.38	0.6857	223	323	0.69	6.79	**<0.0001**	-0.18	-4.7143	**<0.0001**
Defend group	255	362	0.70	7.73	**<0.0001***	272	377	0.72	8.55	**<0.0001**	-0.02	-0.5127	0.6101
Smart	205	321	0.64	4.91	**<0.0001***	228	344	0.66	5.98	**<0.0001**	-0.02	-0.6532	0.5157
Obedient	202	404	0.50	0.00	0.5198	213	434	0.49	-0.34	0.6671	0.01	0.2666	0.7872
Aggressive	240	359	0.67	6.33	**<0.0001***	284	403	0.70	8.17	**<0.0001**	-0.04	-1.076	0.2801
Impulsive	252	435	0.58	3.26	**0.0005***	260	473	0.55	2.12	0.0172	0.03	0.8994	0.3681
Size: Heavier	234	450	0.52	0.80	0.2115	263	479	0.55	2.10	0.0177	-0.03	-0.6973	0.4839
P Cond: Better	168	325	0.52	0.55	0.7471	185	362	0.51	0.37	0.6819	0.01	0.1538	0.8808
Age: Older	296	455	0.65	6.38	**<0.0001***	319	476	0.67	7.38	**<0.0001**	-0.02	-0.6319	0.5287
Neutered	310	491	0.63	1.97	0.0488	270	512	0.53	1.86	0.0629	0.10	3.3347	**0.0009**
Sex: Female	282	491	0.57	3.25	**0.0006***	294	512	0.57	3.31	**0.0004**	0.00	0.0038	1.0000

Table 24. Results of the binomial tests using the owners' allocation of the dogs to "dominant" or "subordinate" status (Item 1) as the predicted variable and the 20 items in A) mixed-sex and B) same-sex dyads. Bold type indicates that status was associated with the characteristic after Bonferroni correction (for the Binomial tests all p values are ≤ 0.0022). Two-proportion z-tests were used to determine whether the proportion of "dominant" dogs in mixed-sex and same-sex groups were equal for each item. C) We compared the "dominants" proportion of each item of each group using a z score calculation with Bonferroni correction for multiple comparisons. Dominant individuals in same-sex dyads mark over subordinate urinations more often than dominants from mixed-sex dyads (same-sex 69% and mixed-sex 51%). Dominant individuals were more often neutered in mixed-sex dyads in comparison to same-sex dyads (mixed-sex 63%, same-sex 53%). P Cond=Physical condition, Prop=Proportion and Prop Diff=Proportion Difference.

	Dominant female			**Dominant male**				**Proportion comparison**	
Item	**Count**	**Total**	**Prop**	**Count**	**Total**	**Prop**	**Prop Diff**	**Z**	**P**
Bark	162	248	0.65	106	176	0.60	0.05	1.07	0.2846
Lick mouth	125	201	0.62	102	155	0.66	-0.04	-0.70	0.4839
Eat first	128	201	0.64	86	147	0.59	0.05	0.98	0.3271
Reward	137	187	0.73	97	142	0.68	0.05	0.98	0.3271
Fight	177	205	0.86	112	133	0.84	0.02	0.54	0.5892
Play ball	111	220	0.50	81	163	0.50	0.01	0.15	0.8808
Greet owner	96	175	0.55	86	139	0.62	-0.07	-1.25	0.2113
Walk first	137	222	0.62	116	153	0.76	-0.14	-2.87	0.0041
Resting place	154	197	0.78	92	143	0.64	0.14	2.82	0.0048
Overmark	39	193	0.20	138	153	0.90	-0.70	-12.93	**<0.0001**
Defend group	127	212	0.60	128	150	0.85	-0.25	-5.22	**<0.0001**
Smart	118	183	0.64	87	138	0.63	0.01	0.27	0.7872
Obedient	114	228	0.50	88	176	0.50	0.00	0	1.0000
Aggressive	126	206	0.61	114	153	0.75	-0.13	-2.66	0.0078
Impulsive	158	255	0.62	94	180	0.52	0.10	2.03	0.0424
Size: heavier	106	257	0.41	128	193	0.66	-0.25	-5.27	**<0.0001**
Physical Condition	89	184	0.48	79	141	0.56	-0.08	-1.37	0.1707
Age: Older	159	258	0.62	137	197	0.70	-0.08	-1.75	0.0801
Neutered	203	282	0.72	107	210	0.51	0.21	4.78	**<0.0001**

Table 25. Comparison of male and female "dominants in mixed-sex dyads. In order to determine whether there were differences between the dominant males and females in each item measured, we compared the dominants proportion of each group (dominant male and dominant female in mixed-sex group) using a z score calculation. Results are displayed for mixed-sex dyads by the sex of the dominant. Bold type indicates that social status was associated with the characteristic after Bonferroni correction (for the Binomial tests all p values are ≤ 0.0026). Prop=Proportion and Prop Diff=Proportion difference.

15.5 Discussion

Our aim was to understand how owners interpret dominance in dog dyads living in their households and to determine whether our psychometric tool measures dominance as defined in ethology. We found that the majority (87%) of owners labelled one of their two dogs as dominant. Perceived dominance status was characterised by fighting ability, submission, competitive ability, subjective resource value, personality, specific roles, and older age. Only thirteen percent of owners were unable to determine a clear rank order between their dogs. This may be because: (1) the dogs have a non-interactive relationship (the partners co-exist without social interactions, i.e. they avoid each other), or an ‘egalitarian’ relationship (the partners affiliate regularly, e.g. play with each other, without agonistic behaviour or exhibiting dominance, Trisko, Smuts & Sandel, 2016); (2) the dogs may not have lived together long enough to form a clear rank order; (3) the owner might actively work against the dogs displaying dominance behaviour (e.g. chasing away the dominant dog from the better resting place, not allowing the dominant to feed first, preventing fights, and favouring the loser dog, etc.); (4) the owner does not accept/understand the concept of dominance; and finally, (5) the survey design encourages that the owner makes a selection.

We found that the results from the questionnaire show external validity. Items associated with the perceived dominance corresponded to behavioural markers of dominance identified by Pongrácz et al. (2008), such as fighting, barking, eating first and receiving mouth licking, and markers related to priority of access to resources such as food, rewards and resting places (Schjelderup-Ebbe, 1922). However, items that examined control over a ball and the owner (greeting) did not differ between “dominant” and “subordinate” dogs, which suggests that the subjective resource value (Bradshaw et al. 2009; van Kerkhove 2004) has probably a greater effect than the perceived rank. Owners also indicated that dogs higher in status overmark lower ranking dogs, similarly to the findings of Lisberg & Snowdon (2011). In the current study "dominants" marked over same-sexed dogs more often than different-sexed dogs, suggesting higher intra- than inter-sexual competition. Dominance was also associated with items theoretically concerning behaviours viewed by humans as responsibilities, such as defending and leading the group. Similarly, Ákos et al. (2014) found that during off-leash walks, dogs rated as dominant by the owners are more often followed by their group-mates that were rated as submissive. Finally, our results confirmed that some personality traits (aggression, impulsivity, and smartness) are associated with reported dominance, as was suggested by Ákos et al. (2014), who found that aggression towards people and controllability was linked to dominance rank and leadership in pet dogs.

According to the literature, dominance ranks are influenced by several confounding factors, such as age and sex. As predicted, older individuals were more often allocated a higher status by owners in the full sample, and in both mixed and same-sex pairs. However, in contrast to the age related hypothesis, which suggests that age better explains the social structure in dog groups (Bradshaw et al., 2016), we found that dominance status, as perceived by the owner, was more strongly associated to 11 of the items than age status. Thus, dog age did not explain the occurrence of dominance related behaviours over and above the owners' estimate of dominance status. Sex was also linked to dominance; in mixed-sex dog dyads, females were perceived by owners as dominant more often than males, even though in 59% of the dyads they were smaller in size than their male partner. This could be related to the fact that dominant females were more often neutered than dominant males. Previous studies have determined that hormonal activity influences inter-dog aggression (Sherman et al., 1996) and aggression has been found to be more frequent in neutered females compared to intact females and neutered males (Wright & Nesselrote, 1987, Scandurra et al., 2018).

Our study has several limitations: (1) We did not measure the dominant behaviour of the dogs, only the dominance perceived by the owner. Therefore, we have no information about convergent validity, whether the ratings of dominance and behaviour reflect actual rates of behaviour. (2) Only relationships between single dyads were examined. Previous work has determined that individuals can and do establish different types of relationships including “friendships”, when paired with different individuals, and these relationships can also change over time, suggesting high social complexity in dogs (Trisko et al., 2016). Future studies should examine how individuals’ relationships differ within multi-dog households. (3) We also did not include items on affiliative behaviour in the questionnaire, so it was not possible to classify the dominance relationships further into formal (affiliation and dominance) and egalitarian (affiliated with no dominance) types (Trisko et al., 2016). (4) We were not able to examine breed differences in dominance relationships. Dog breeds and breed groups differ greatly in morphology and typical behaviour (P. D. McGreevy et al., 2012; Starling et al., 2013; Borbála Turcsán et al., 2011), therefore the types of relationships between dogs may also be highly dependent on the breed composition of the group (van der Borg et al., 2015). (5) Due to time constraints, we applied single item statements to describe personality traits. (6) We investigated the interpretation of dominance only in Hungary, although there could be significant cultural differences (Wan et al., 2009). (7) We asked owners to compare two dogs to each other, which was difficult for some owners as reflected in a large amount of missing values in the dataset. Future studies should aim to collect data for subordinate and dominant dogs separately using for instance Likert scales, which would allow the use of statistical modelling. (8) Finally, future studies should also investigate inter-observer reliability, thus multiple people should rate the dogs in order to reduce the chance that the answers are reflective of one individual's views and biases.

Whether dominance as perceived by owners is just a by-product of human observation remains to be answered. However, if we assume that our questionnaire measured actual dominance relationships within the dyads, our results show that the age, sex and neuter status of the dyad influences the relationship between the subjects, which has broader implications for the management of dogs within households. Since humans are ultimately responsible for choosing the social partners (human and conspecific) of their dogs, they have a duty to try to ensure that social relationships are as amicable as possible, in order to keep chronic stress levels, and therefore welfare at an acceptable level. For example, in mixed sex dyads, neutered females were often seen as dominant, and showed behaviours that might increase conflict (reflected in the reduced difference score), regardless of age or body size. Competition in the dyad could be reduced, and any possible increase in dominance motivation in females (caused by neutering) would be avoided, if females could be kept intact and the male neutered if necessary (to prevent breeding). Previous research has indicated that a sex/age graded hierarchy is present in dogs (Cafazzo et al., 2010), and as such, owners could reinforce the position of older individuals in the hierarchy in order to reduce competition in the household. Additionally, to prevent conflicts, owners should try to avoid keeping multiple dogs of the same sex and age. Finally, future studies are necessary to determine how owners perceive their own relationships with their dogs. For example, whether owners have different types of relationships with the dogs within their household, and how this might influence the intraspecific relationships between their dogs; a topic which is currently hotly debated. For instance, one study has determined that dogs form similar relationships with both humans and dogs, and that the quality of the bond varies more with the individual partner than between dog vs. human partners; indicating that relationships cannot be entirely attributed to an individual's personality (Cimarelli, Marshall-Pescini, Range, & Virányi, 2019).

In sum, owner estimates of dominance rank corresponded to previously established behavioural markers of dominance displays, which supports that dominance relationships are robust and well-perceivable components of companion dog behaviour and owner-derived reports about dominance ranks have external validity (in pre-schoolers see Hawley, 2002 for similar results). However, the results lack convergent validity, because no simultaneous measure of behaviours were taken, and the data represents only one culture.

We conclude that owners of multiple dogs interpret dominance based on specific behaviours, obtaining resources and certain personality traits. We suggest that future studies that wish to allocate dominance status using owner reports should include the following seven items: which dog starts to bark first, eats first, obtains the reward, walks at the front, acquires the better resting place, defends the group, and is more aggressive. Asking which dog wins fights or which dog licks the mouth of the other might also be useful, as both were highly predictive of owner perceived social status if they did occur, in approximately 70% of cases.

16 Age related differences in the spindling activity of the sleeping brain[12]

16.1 Abstract

The mapping of where aging processes in dogs and humans converge and diverge is an important step in fully understanding dog's potential as a model of cognitive aging. The aim of the investigation was to test if the detection method can reproduce relationships between sleep spindles (short trains of EEG waves) and aging known from the human literature. Non-REM bursts of activity in the sigma range (9-16 Hz) typical of sleep spindles predict learning in dogs, similar to humans and rats. Little is known, however, about the age-related changes in amplitude, density (spindles/minute) and frequency (waves/second) of canine spindles. We investigated a large sample (N=155) of intact and neutered pet dogs of both sexes, varying in breed and age, searching for spindles in segments of non-REM sleep. We recorded EEG from both a frontal midline electrode (Fz) and a central midline electrode (Cz) in 55.5% of the dogs, in the remaining animals only the Fz electrode was active (bipolar derivation). A similar topography was observed for fast (≥ 13 Hz) spindle occurrence as in humans (fast spindle number, density on Cz>Fz). Density of fast spindles increased with age on Fz. These effects were more pronounced among intact animals and on Fz. Slow spindle density declined and fast spindle frequency increased with age on Cz, while on Fz age-related amplitude decline was observed. Our findings support the argument that sigma bursts in the canine non-REM sleep are analogous to human sleep spindles, and suggest that in dogs, slow and fast spindles display different trajectories related to age.

16.2 Introduction

The sleeping brain shows various patterns of activity that predict awake performance in the domains of memory and cognitive ability (Genzel, Kroes, Dresler, & Battaglia, 2014). Several of these oscillatory activities are also altered in the aging process (Crowley, Trinder, Kim, Carrington, & Colrain, 2002), which makes sleep physiology an attractive target for studying cognitive aging in humans and animal models. The dog has been suggested to be a suitable model for human cognitive aging (Beth Adams, Chan, Callahan, & Milgram, 2000; Chapagain et al., 2018; B J Cummings, Head, Ruehl, Milgram, & Cotman, 1996). Recently sleep physiology in dogs has received growing attention (Bunford et al., 2018; Kis et al., 2017; Kis, Szakadát, et al., 2014; Varga, Gergely, Galambos, & Kis, 2018) due to the development of a non-invasive polysomnographic method (Kis, Szakadát, et al., 2014) which opens up a possibility for integrating the study of cognitive aging and sleep in pet dogs.

Electroencephalography (EEG), a commonly used neuroimaging technique, transforms the activity of different brain areas or networks into signals visible as repetitive, rhythmic waves of varying shape, size, duration and frequency. The EEG

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[12] Based on: Iotchev, B. I., Kis, A., Turcsán, B., de Lara, D. R. T. F., Reicher, V., Kubinyi, E. (2019) Age-related differences and sexual dimorphism in canine sleep spindles. *Scientific Reports, 9,* 10092.

signal during non-REM, the deepest phase of sleep in mammals is characterized by large and slow waves (less than 4 waves per second). During the non-REM phase sleep spindles emerge as short (ca. 0.5-5 seconds) trains of smaller waves, 9-16 waves/second (De Gennaro & Ferrara, 2003; Jankel & Niedermeyer, 1985). These bursts originate in the thalamus, where sensory information is processed. Sleep spindles are believed to support cognitive development, (Hahn et al., 2018; Ujma, Sándor, Szakadát, Gombos, & Bódizs, 2016), sleep stability (Dang-Vu, McKinney, Buxton, Solet, & Ellenbogen, 2010) and overnight memory consolidation (Genzel et al., 2014; K. Whalley, 2017). Although related research is intensive in humans, rodents and even cats, sleep spindles were mostly neglected in dogs. However, our research group confirmed that a higher incidence of spindle-like bursts in sleeping dogs predict better performance on a novel task (Iotchev, Kis, Bódizs, van Luijtelaar, & Kubinyi, 2017).

In humans age affects spindle activity (Crowley et al., 2002), which makes sleep physiology an attractive target for studying cognitive aging in humans and animal models. Age-related changes are characterized by decreased density (spindles/minute) and amplitude (Crowley et al., 2002; Latreille et al., 2015; Martin et al., 2012; Rauchs et al., 2008; Smirne et al., 1977). A subset of studies also found an increase in spindle frequency with age (Crowley et al., 2002).

Studies often separate fast (> 13 Hz) and slow (< 13 Hz) spindles, because in humans some spindle-associated findings are sensitive for this distinction, e.g. a rise in spindle density during adolescence appears to be specific for fast spindles (Hahn et al., 2018). Fast spindles are predominant in central and posterior derivations, in both humans and rats (Jobert, Poiseau, Jähnig, Schulz, & Kubicki, 1992; Terrier & Gottesmann, 1978; Zeitlhofer et al., 1997), whereas slow spindles in frontal derivations.

In the present investigation, we aim to compare spindle features across neutered and intact dogs from both sexes varying in age, relying on a large sample (> 150 dogs) of subjects, which underwent ca. 3 hours of polysomnographic recordings with no additional experimental manipulation. Currently the literature on canine spindles is sparse and little is known about the development of spindling in dogs across their life span. Early work by Pampiglione (1963) is quoted to conclude that spindles are rare in young dogs (see Jankel & Niedermeyer, 1985). Other works that mentions spindles in the dog did not quantify the events (P. Ákos, Thalhammer, Leschnik, & Halász, 2012; Jeserevics et al., 2007). A notable exception in terms of developmental insight is the comparative work of Petersen et al. (Petersen, Di Perri, & Himwich, 1964) which showed a later postnatal expression of sleep spindles in dogs, compared to cats and rabbits (in dogs the first spindles appeared about a month after birth, while in rabbits between one and two weeks, and in cats two weeks to a month). Even this study, however, does not quantify spindles, but merely compare the time between species they become visually detectable.

In the absence of an equally well-developed body of findings in the dog, yet emerging evidence for an analogy between human and canine spindles (Iotchev et al., 2017), we base our expectations on the human literature. It should be noted, however, that the most confirmed analogy between humans and other mammals to date remains the association between spindle expression and learning (Eschenko, Molle, Born, &

Sara, 2006; Iotchev et al., 2017; Mölle, Eschenko, Gais, Sara, & Born, 2009). As far as dog-specific findings are concerned, the literature only contains information on the development of sleep spindles in young dogs compared to the young of other species. Sleep spindles appear later in dogs compared to rabbits and cats (Petersen et al., 1964), but not humans (Olbrich, Rusterholz, LeBourgeois, & Achermann, 2017). Pampiglione (1971) suggested that dog brains generally mature slower in comparison with pigs. A previously observed increase in frontal spindles with age in the dog (Iotchev et al., 2017) fits with this late onset of sleep spindles, but the change in spindle occurrence across a dog's lifespan remains an open question, to be investigated in the present study. In humans age-related increases in spindle occurrence last only until adolescence (Bódizs, Gombos, Ujma, & Kovács, 2014; Hahn et al., 2018). A lifelong increase in spindle density does therefore not seem likely, especially since in humans a decrease is observed among the eldest (Crowley et al., 2002; Latreille et al., 2015; Martin et al., 2012; Rauchs et al., 2008; Smirne et al., 1977). Among the three spindle features we expect amplitude-related findings to be most robust, as a recent meta-analysis suggests that findings associated with spindle amplitude are the overall most reliable (Ujma, 2018).

16.3 Methods

Subjects

155 dogs (age range 1-16 years, 7.6 ± 4 (M ± SD); 76 females; 107 neutered and 11 of unknown reproductive status; 96 purebred from 39 different breeds) were taken from our Family Dog Project database consisting of ca. 3 hour long, first-time polysomnographic recordings with no additional experimental manipulations. Dogs that did not sleep during the recording (N=8 in the full sample, N=2 in the subsample with an active Cz electrode) were excluded from all analyses, while dogs that slept but did not express spindles (N=1 in the full sample, none in the subsample with active Cz electrode) were also excluded from analyses of amplitude and frequency. For analyses focusing on fast spindles ($\geq$ 13 Hz) more dogs were excluded from amplitude and frequency comparisons (additional N=20 in the full sample, 7 in the subsample with an active Cz electrode) due to a higher proportion of dogs displaying no fast spindles. One additional dog was excluded in the amplitude and frequency analyses for slow spindles, as it only showed detections $\geq$ 13 Hz.

Polysomnographic method and spindle detection

We used the non-invasive polysomnographic method (Kis et al., 2017; Kis, Szakadát, et al., 2014). Dogs were invited in the sleeping laboratory where they were given three hours to sleep on a mattress with their owner (Figure 30). EEG data were obtained with surface attached electrodes in the same way as is done with humans, thus the procedure was fully non-invasive. All dogs except eight slept during the three hours. In all dogs, electrodes were placed on the skull midline. The frontal midline electrode (Fz) was active in all dogs, but the central midline electrode (Cz) was active in only 55.5% of the total sample (Figure 31). Six or seven other electrodes aided sleep stage identification or were used as reference or as ground. Technical arrangements are described in (Iotchev et al., 2019). The EEG signal was divided in sleep-stages using visual inspection (Kis et al., 2017) with *Fercio's EEG Plus* program. Spindle detection was run only in the non-REM sleep phase because of higher occurrence and higher functional relevance. The spindle detection program is presented in detail in (Iotchev et al., 2017, 2019).

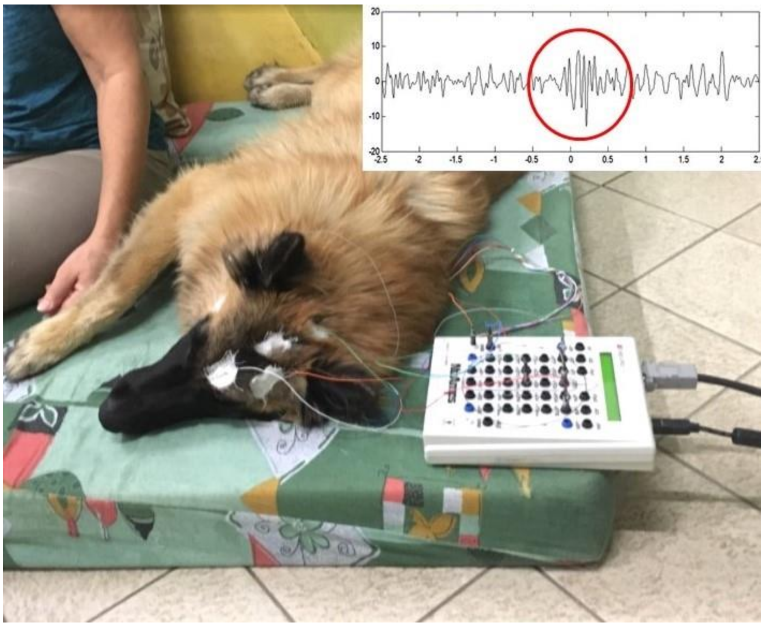

Figure 30. A dog sleeping with electrodes attached on the head and the body and sleep spindle (up right, highlighted with a red circle).

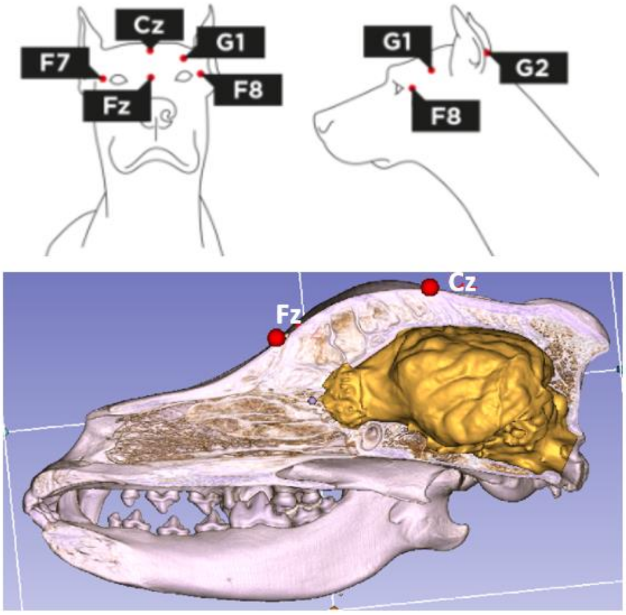

Figure 31. Placement of the midline electrodes (Fz: frontal midline, Cz: central midline), eye-movement electrodes (F7, F8), ground (G1) and reference (G2) electrodes. (Figure: Vivien Reicher and Kálmán Czeibert).

Statistical analyses

Independent samples t-tests were used to inquire if dogs of different sex and reproductive status were of significantly different age (in years). This was done to later exclude the possibility that age-effects are potentially explained by sex or reproductive status. Topographic differences in spindle features between Fz and Cz were tested using paired t-tests on the sub-sample of dogs (N=84) that have data from both derivations. To test how spindle features (density, amplitude, frequency) might differ across age we used Generalized Linear Models (GLM) with robust model estimation, using age (in years) as a covariate, adding sex and reproductive status as fixed factors, and testing for the interactions sex × age and sex × reproductive status. The models were optimized with backwards elimination, excluding the least significant factors first (starting with interactions and keeping factors that are involved in significant interactions), until reaching the lowest absolute value for the Akaike criterion of model evaluation. The last factor removal was reversed if it resulted in a worse Akaike value and the final model is reported. Prior to testing, the residuals obtained for the initial model were examined for deviations from a normal distribution. If normality assumptions were violated (Kolmogorov-Smirnov test of normality P<0.05) the distribution assumptions were adjusted to Gamma for spindle amplitudes and frequencies (recommended for variables with no possibility for negative values) and Tweedie for spindle density (recommended for variables with the possibility for zero values, but not negative values). All analyses were repeated for the sub-sets of slow and fast spindles, separated as previously(Iotchev et al., 2017) using the criterion applied in studies by Schabus and colleagues (Hahn et al., 2018; Schabus et al., 2006) (fast spindles: spindles oscillating in a frequency ≥ 13 Hz, slow spindles: ≤ 13 Hz). We also repeated all analyses for detections in Fz and Cz. The need for outlier control analyses was determined visually (it appeared necessary in two analyses concerning amplitude) upon which outliers were identified based on standard scores (cases with a standard score above or below 2.68 for the variable in question were excluded). All analyses were performed with SPSS version 22.0.0.0.

16.4 Results

We report here only the significant findings associated with age. For associations with sex and neutered status see Iotchev et al. (2019).

Density: Slow spindle density on Cz declined with age (GLM, Wald Chi-Square=7.4, P=0.007). Fast spindle density on Fz significantly increased with age (GLM, Wald Chi-Square=8.107, p=0.004).

Amplitude: Slow spindle amplitude on Fz declined with age (GLM, Wald Chi-Square=4.169, P=0.041). Slow spindle amplitude on Cz was significantly rising with age in females (GLM, Wald Chi-Square=7.006, P=0.008), but not in males (GLM, Wald Chi-Square=0.109, P=0.741).

Frequency: Fast spindle frequency on Cz was found to rise with age (GLM, Wald Chi-Square=5.666, P=0.017). Slow spindle frequency on Cz the did not change with age for females (GLM, Wald Chi-Square=0.99, P=0.32), but was rising in males (GLM, Wald Chi-Square=7.262, P=0.007). These results are summarized in Figure 32 and

Table 26.

Spindle characteristic	Type of spindle	Recording site	Observation
density	slow	Cz	younger>older
	fast	Fz	younger<older
amplitude	slow	Fz	younger>older
	slow	Cz	younger<older (only in females)
frequency	slow	Cz	younger<older (only in males)
	fast	Cz	younger<older

Table 26. Summary of all significant associations with age, dependent measure (density, amplitude, frequency), type of spindle (fast, slow), and recording site (Fz: frontal midline, Cz: central midline).

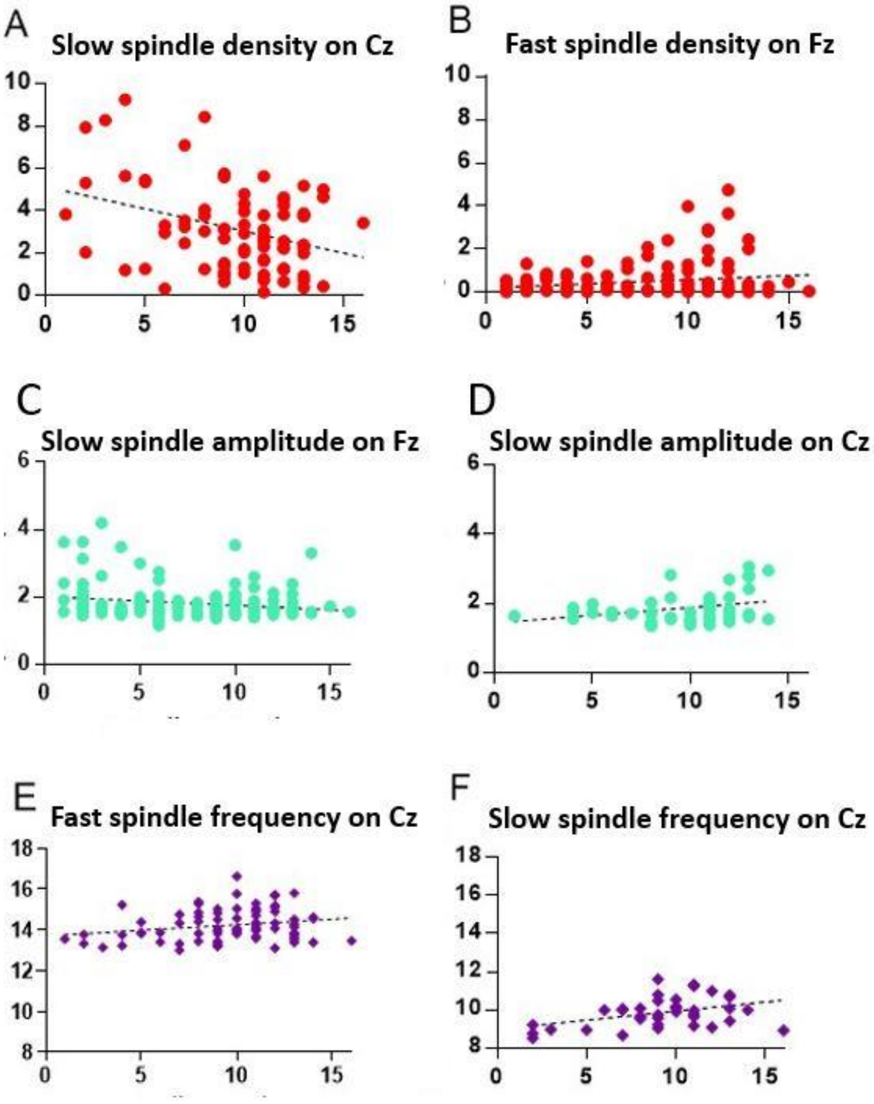

Figure 32. Spindle density as a function of age for slow spindles (≤13 Hz) on Cz (N = 84) (A), and fast spindles (≥13 Hz) on Fz (N = 147) (B), age is in years displayed on the x axis of all figures. Slow spindle amplitude (≤13 Hz) on Fz (N = 145) (C). Slow spindle amplitude, in females and on Cz, excluding one outlier (N = 45) (D). Fast spindle frequency for all dogs on Cz (N = 77) (E) and frequency of slow spindles in male dogs on Cz (N = 38) (F).

16.5 Discussion

The aim of the present investigation was to test in dogs if the sleep spindle detection method can reproduce relationships associated with aging known from the human literature. We have found that aging follows a different course with regard to slow and fast sleep spindles and placement of EEG electrodes in the dog. On Cz, slow and central spindles appear to follow similar trends to what has been observed in humans (Crowley et al., 2002; Latreille et al., 2015; Martin et al., 2012; Rauchs et al., 2008): older dogs have lower spindle occurrence. However, older dogs display a higher occurrence of frontally detected fast spindles, which was not observed in any other species. However, it is interesting to note that in humans fast spindle occurrence increases from childhood to adolescence (Hahn et al., 2018) and that in rats spindle occurrence does not change with age at all (van Luijtelaar & Bikbaev, 2007).

Age-related decrease in spindle amplitude in humans is specific to the transition from middle to old age and associated with cognitive decline (Latreille et al., 2015; Martin et al., 2012; Rauchs et al., 2008; Smirne et al., 1977). During aging the number of cortical interneurons and inhibitory synapses decreases (Bu, Sathyendra, Nagykery, & Geula, 2003) and such loss limits the ability of the thalamus to excite inhibition in the cortex which decreases spindle amplitudes (Sitnikova, 2010). In harmony with the human literature, the amplitude of slow canine spindles decreased with age on Fz; in particular Martin et al. (Martin et al., 2012) and Landolt and Borbély (2001) also find stronger age-related amplitude decline in frontal derivations. However, it increased in females on Cz, suggesting that in dogs the decline of amplitude and density are topographically dissociated and affected by sexual hormones (Bódizs, 2017).

Increase in (fast) spindle frequency with age, similar to humans' sleep spindle frequency (Crowley et al., 2002; Ktonas et al., 2007) was observed on Cz. Slow spindle frequency increased only in males on Cz. While age-related increases in frequency are small in magnitude (Crowley et al., 2002) and more seldom observed (De Gennaro & Ferrara, 2003), they have been specifically associated with risk of dementia (Ktonas et al., 2007).

A limitation of the study is that dogs of different weights and sizes have different life expectancies. Despite of this we have not used correction factors on subjects' age, because we are not aware of any theory in the literature on how differences in life expectancy might affect the aging trajectory of spindles. For the sake of generalizability, we decided to use a heterogeneous dog population.

In sum, we found evidence for a different course of aging in the fast and slow canine spindles. Slow and central spindles appear to follow human-like aging trends, while frontal fast spindles continue to increase in density, similarly to human adolescents (Bódizs et al., 2014; Hahn et al., 2018). Our findings support the argument that sigma bursts in the canine non-REM sleep are analogous to human sleep spindles and the canine brain could be a good model for human brain aging, regarding sleep spindle activity.

17 The genetic background of longevity based on whole-genome sequence data of two methuselah dogs[13]

17.1 Abstract

A deeper understanding on extreme longevity and its background is fundamental for biomedical research in order to develop efficient medicine against age-related diseases. In many aspects, the companion dog is an ideal model to study aging. We used the whole-genome sequence of two extremely old dogs, which lived 22 and 27 years (90-135% longer than the average lifespan of dogs), to investigate the genetic background of longevity and determine why these dogs were successful in aging. We identified more than 7500 novel SNP mutations in the two dogs when compared to 3 publicly available canine databases with SNP information from 850 dogs. Most novel mutations were in noncoding regions, while about 92% of the remaining SNPs were at introns. In each dog, more than 400 of the novel SNPs were missense variants, out of which 76 overlapped between the two animals. When analyzing a pre-defined set of 1062 genes presumably linked to aging in human, a small proportion of them included missense mutations in the analyzed samples. We identified 12 disruptive mutations (i.e. mutations, which might result in non-functioning proteins) in the samples, although their actual effect is unclear. Approximately 100 thousand new indel mutations were also identified in the two individuals, ~62 thousand of which overlapped between them. Based on *in silico* analysis, we identified 670 missense mutations across 472 genes and several genetic pathways that are primary candidates for age-related research in dogs (and their homologs in humans) in future studies. Based on their gene ontologies, these genes were related – among others – to immune response and the nervous system in general. A link between extreme longevity and the regulation of gene transcription/translation suggests that one crucial genetic requirement of extreme longevity lies within the *fine-tuning* – i.e. the superior calibration – of RNA (and thereof protein) production of an organism. This phenomenon defines an interesting direction for future research aiming to better understand longevity.

17.2 Introduction

The genetics of aging and longevity has been studied in multiple species, including *C. elegans* (e.g. Gems and Riddle, 2000), fruit fly (e.g. Lehtovaara et al., 2013), mice (e.g. Piper et al., 2008), dogs (see Hoffman et al., 2018 for a summary) and humans (e.g. Herskind et al., 1996). Based on these studies, longevity is known to be influenced by both genetic and environmental factors (López-Otín et al., 2013), with an estimated heritability of 15-30% in humans (e.g. Herskind et al., 1996). However, more recently a study showed that heritability of lifespan might have been overestimated in the past and an upper limit estimate of ~7% was proposed (Ruby et al., 2018). Extreme longevity (i.e. longevity of centenarians) was reported to have a higher heritability than longevity itself (Sebastiani et al., 2016). Several mechanisms of aging are either directly

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[13] Based on Jónás, D., Sándor, S., Tátrai, K., Egyed, B. Kubinyi, E., (2019). The genetic background of longevity based on whole-genome sequence data of two methuselah dogs. Submitted.

(genomic instability, telomere attrition and epigenetic alterations) or indirectly related to genetics (loss of proteostasis and stem cell exhaustion; López-Otín et al., 2013). Furthermore, multiple genetic pathways were already identified to be linked to longevity, such as the insulin/insulin-like growth factor signaling pathway, the telomere maintenance pathway or the DNA damage response and repair pathway (Deelen et al., 2011; Debrabant et al., 2014). All of these pathways are crucial to sustain normal cell functions and are related to the previously mentioned genetic hallmarks.

For many reasons, the companion dog is an especially promising model organism for human age-related research (see General Discussion in this thesis and Sándor and Kubinyi, 2019 about our *state of the art* knowledge about the genetic pathways involved in aging in dogs).

A deeper understanding on extreme longevity and its background is fundamental for biomedical research to develop efficient medicine against age-related diseases (both for treatment and prevention). More specifically in dogs, the identification of genes and other genetic loci linked to longevity allows breeders to select more efficiently for longevity within the breeds. Furthermore, it was highlighted before in humans, that the prevalence of multiple age-related diseases was lower among the offspring of centenarians and that longevity-related genes provide a certain level of ‘protection’ against cognitive decline and neurodegeneration (e.g. Sanders et al., 2010 after Han et al., 2013). As a result of selection on such genetic loci in dogs, the proportion of the beneficial alleles can be increased within the breed under selection, increasing the average life expectancy of the given breed and simultaneously improving the quality of life of the companion pets and their owners alike.

Han et al. (2013) studied 6 centenarians (105-109 years old) to investigate the genetic background of extreme longevity in humans. These centenarians lived ~50% longer compared to the average human lifespan (72 years, WHO, 2018). Given this definition of extreme longevity and the average lifespan of companion dogs (10-13 years; Adams et al., 2010; Leroy et al., 2015; Inoue et al., 2018), dogs older than ~17 years can be considered as dogs of extreme age. Mixed-breed dogs are known to live longer: Inoue et al. (2018) studied the lifespan of more than 12,000 dogs and found the average length of lifespan of mixed-breed individuals to be 15 years. Therefore, extreme longevity in their case corresponds to ~22.5 years of age. In the data published by Inoue et al., 13 dogs lived 22-25 years (no dogs above the age of 25 were recorded in their study), corresponding to 0.1% of the studied population, or 1.16% of the mixed-breed individuals, assuming that all individuals of age 22-25 were mixed-breed. These numbers suggest that there is a sufficiently large population of dogs with an extreme longevity to be included in age-related studies.

The main aim of this study is to investigate the genetic background of longevity in two dogs, who lived an extremely long life, which is the first such study in canines. The dogs studied here lived 22 and 27 years, or approximately 50-80% longer, than the average lifespan of a mixed-breed dog (90-135% longer, than the average lifespan of all dogs). Our secondary aims are to compare the results to that of Han et al. (2013), to extend our understanding of extreme longevity and to promote the companion dog to be used in age-related research.

17.3 Methods

The canine reference genome (*CanFam 3.1* version) as well as all relevant information related to it (e.g. gene annotations) were downloaded from ENSEMBL (version 94, released in October, 2018; Hunt et al., 2018). Since the canine reference genome excludes the Y-chromosome, this chromosome was not included in the analysis.

Whole-genome sequence data

DNA was collected from either buccal swab or blood samples of two mixed-breed individuals of extreme age (i.e. methuselah dogs): from a 27 years old mixed-breed intact male (Buksi, lived in Sárrétudvari, Hungary; ID: old_rep1; buccal swab sample collected at the age of 26) and from a 22 years old mixed-breed neutered female dog (Kedves, lived in Ócsa, Hungary; ID: old_rep2; blood sample collected at the age of 22; Figure 33).

Figure 33. The two dogs participating in this study: Buksi (left) and Kedves (right).

DNA samples were isolated and sequenced by Omega Biosciences (Norcross, Georgia, USA). Sequencing was performed on an Illumina HiSeq 2500 instrument, producing 150 basepairs long paired-end sequences. A total of ~2x481 and ~2x473 million reads were sequenced for the two samples. In spite of the similar sequencing depth, depth of coverage differed significantly between the two samples after alignment to the reference genome (average depth of coverage across the whole genome was 46.1 and 60.1 for old_rep1 and old_rep2, respectively).

On-line databases

Our working hypothesis was that the likelihood of common variants (i.e. variants segregating in dogs with an average lifespan) to positively affect longevity was lower than that of the variants uniquely present in individuals with extreme longevity. Therefore, our primary focus was on the short genetic variations that are unique in the methuselah dogs sequenced within the framework of this study. In order to exclude the most common variants, all SNPs and indels previously identified and published in at least one of three on-line databases were excluded. These databases included the Dog Genome SNP Database (DoGSD), which is "a data container for the variation information of dog/wolf genomes" (quote from the DoGSD website accessed on 25/06/2019; http://bigd.big.ac.cn/dogsdv2/); the database created by the [American] National Human Genome Research Institute (NHGRI) based on the whole-genome sequence of 722 dogs and the Broad Institute's dog SNP database, which was created as part of the Canine Genome Sequencing Project. The last database was created for the *CanFam 2.0* genome version and therefore positions were lifted over to the current (*CanFam 3.1*) version, which was used in this study; 16,388 SNPs (15,951 of which were on autosomes) were removed in the process, out of the 2,544,508 from the original study (Table 27).

The NHGRI's database included indel mutations as well (n=12300815), which were used to remove the common indels. It is important to note that since indels were not included in two of the databases, the common indel variants were detected in a much smaller pool of individuals and therefore indels that are otherwise common among dogs with an average lifespan might have still remained in the dataset.

Database	**38 autosomes+X chromosome**[1]	**38 autosomes**
DoGSD database (Bai et al., 2014)	54,644,335	52,318,004
Broad Institute database (Lindblad-Toh et al., 2011)[2]	2,528,120	24,66,855
NHGRI database (Plassais et al., 2019)	20,269,614	19,693,593
Total number of non-redundant SNPs	61,180,804	58,623,548

1: +MT in case of the DoGSD database. 2: after lift-over from *CanFam v2.0* to *v3.1*.

Table 27. Number of SNPs in three, previously published databases.

Candidate gene set

In a previous study, Han et al. (2013) discovered 89 novel non-synonymous SNPs via exome sequencing in six centenarians by targeting a predefined set of 988 genes. These genes were selected from pathways that are known to be involved in either aging or longevity. In this study we included an additional 157 genes from autophagy pathway (adding up to 1,145 genes in total) and identified their canine homologues (in total, 1,062 homologues were found). Although the related genetic pathways were already associated with aging, only limited information is currently available regarding the individual genes.

WGS-data processing

The general outline of the analysis is shown on Figure 34. Following sequencing, a quality control step of the raw reads using the FastQC program (Andrews, 2010; RRID:SCR_014583) was implemented. Alignment was performed with the mem command of the BWA aligner (Li and Durbin, 2009; BWA, RRID:SCR_010910), using the standard parameter settings except for the "-M" option, which was used to make the output files compatible with the Picard software toolkit (Broad Institute, 2009). Alignment quality control was assessed by calculating alignment statistics with Samtools (Li, 2011; SAMTOOLS, RRID:SCR_002105) and Picard (Picard, RRID:SCR_006525).

Short variants (SNPs and short insertions-deletions) were then identified with the GATK software (Van der Auwera et al., 2013; GATK, RRID:SCR_001876). Short variants were called using the HaplotypeCaller command of GATK and separately for each chromosome to accelerate the SNP calling step. The standard parameter values were used during variant calling as well, except for the number of allowed processors, which was increased from 1 to 8. Files containing the variants from different chromosomes were then merged with the MergeVCFs command (Picard) and the different types of variants (SNPs and indels) were separated with the SelectVariants command of GATK. This latter step was necessary, because different filtering options were applied for the different types of mutations.

Variants were filtered based on quality scores using the VariantFiltration tool (GATK). In case of both SNPs and indels, the recommended hard-filtering options were used. For SNPs, the applied filtering options were: QD < 2.0; FS >60.0; MQ < 40.0; MQRankSum< 12.5; ReadPosRankSum< -8.0; SOR >3.0. For indels, the recommended filtering options were used, except for the inbreeding coefficient, which parameter was excluded, as this option requires ten or more individuals in the analysis (the applied filtering options in case of indels are: QD < 2.0; FS > 200.0; ReadPosRankSum< -20-0; SOR > 10.0). After quality-based variant filtration, the SNP and indel variants that were identified in both individuals were determined, as these variants are of greatest interest. The overlap category in the tables hereinafter will refer to this set of SNPs.

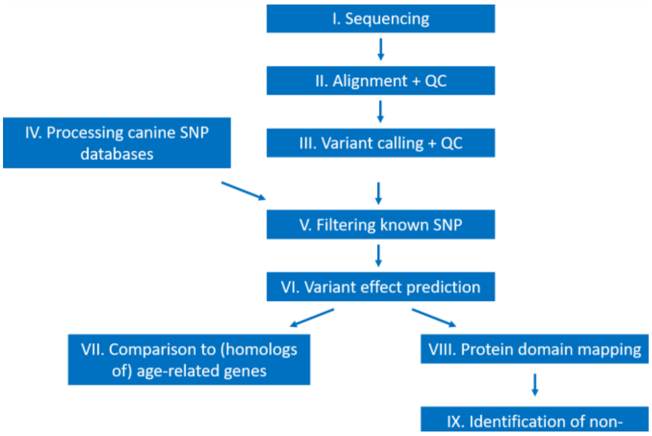

Figure 34. Outline of the study.

Downstream analysis

All SNP and indel mutations published in at least one of the three canine databases were excluded from the analysis. Ensembl's *Variant Effect Predictor* software (McLaren et al., 2016) was used to identify mutations with a potentially high impact on the phenotype and the genes incorporating one or more such mutations were identified. These genes were then compared with the age-related gene set defined above and in parallel, the non-synonymous SNPs mutations located within annotated protein domains were analyzed as well. For this analysis, the known protein domains were located on the protein-coding genes.

17.4 Results

In spite of the similar sequencing depth between the two individuals, depth of coverage differed significantly between them after alignment to the reference genome. The average depth of coverage across the whole genome was 46.1 and 60.1 for old_rep1 and old_rep2, respectively.

Table 28. shows the number of SNPs and indels identified in this study before and after filtering out the previously published mutations (n=61,180,804; see Table 27). The two methuselah dogs were very similar in these numbers and a large proportion of both SNPs (64%) and indels (52%) overlapped between the individuals. The number of unpublished SNPs was also similar between the two individuals (1.4% of all detected SNP), but only ~17% of those SNPs were shared between them (
Table 28). Indels showed a similar picture as SNPs, except for the unpublished indels (on average 17% of the indels were novel indels) and their overlap between the two individuals (62%), which were considerably higher.

	old_rep1	**old_rep2**	**Overlap**
Number of SNP	4,754,086	4,817,227	3,038,929
Number of SNP (autosomes)	4,648,426	4,688,907	2,973,573
Number of unpublished SNP	41,099	46,375	7,505
Number of indels	552,996	578,712	295,412
Number of indels (autosomes)	521,840	556,968	280,773
Number of unpublished indels	97,826	99,698	62,288

Table 28. Number of variants discovered in the two individuals with extreme longevity as well as the number of overlapping mutations.

Most of the SNPs located in genes were found in introns (92%), while only a small proportion of them were located in exons (~4.6%), out of which ~2.4% were missense variants (Table 29) Disruptive mutations were detected in 12 known genes, none of which overlapped between the two individuals:

- 3 genes had a start codon lost mutation: ENSCAFG00000007858, ENSCAFG00000011630 and ENSCAFG00000030632

- 9 genes had a stop codon gain mutation in the gene body: ENSCAFG00000000433, ENSCAFG00000008061, ENSCAFG00000010498, ENSCAFG00000017946, ENSCAFG00000024414, ENSCAFG00000002007, ENSCAFG00000004279, ENSCAFG00000029250 and ENSCAFG00000032497

Annotation category	**old_rep1**	**old_rep2**	**Overlap**
5’ UTR variant	323	379	121
Start lost	1	4	0
Intron variant	15,507	17,277	2,798
Missense variant	422	411	76
Synonymous variant	387	394	86
Stop gained	5	5	0
Stop lost	1	0	0
Stop retained variant	199	248	30
3’ UTR variant	323	379	121

Table 29. Number of SNPs for different annotation categories. The table also includes the unknown genes.

Although intron variants usually do not affect protein expression levels directly, they might still influence gene expression by modifying transcription factor binding sites. However, since no information was available on the location of transcription factor binding motifs on the canine genome, this could not be addressed in this study.

Table 30. shows the number of genes that include SNP variants in either exons or regulatory regions (defined as 5 Kb upstream and downstream of every protein coding gene) for the two animals and the shared set of SNPs between the two dogs, as well as the number of SNPs. 327 novel exonic SNPs were detected in both dogs, while an additional 2505 exonic SNPs were identified in either one of the two methuselah dogs. Among the 327 SNPs, there were 67 missense mutations, overlapping with 37 genes. In total, 13,334 SNPs were identified in regulatory regions, 1861 of which were shared between the 2 individuals, overlapping with 887 protein-coding genes.

We previously identified from the literature a set of 1,062 genes that were likely related to aging and longevity. Out of these, 19 had novel missense mutations in the studied companion dogs, one of which (ENSCAFG00000014403) was found in both individuals. Based on its gene ontology (biological process category, inferred from electronic annotation by Ensembl), it is related to intracellular protein transport and long-term synaptic depression. Some genes are listed below:

- ENSCAFG00000024527: ion transport, chemical synaptic transmission,
- ENSCAFG00000001710: nervous system development, synaptic transmission – cholinergic, excitatory postsynaptic potential
- ENSCAFG00000018622: positive regulation of protein phosphorylation, leading edge cell differentiation, negative regulation of apoptotic process

	old_rep1	**old_rep2**	**Overlap**

Number of longevity-related genes in dogs[1]	1062		
Number of exonic SNP	86,621	86,521	55,159
Number of novel exonic SNP	1,371	1,461	327
Number of novel, missense SNP mutations	376 (257)	361 (261)	67 (37)
Number of age-related genes with missense mutation(s)	9 (8)	16 (12)	1 (1)
Number of genes with novel SNP in regulatory regions[2]	7,014 (4370)	8,181 (5005)	1,861 (887)
Number of age-related genes with mutations in regulatory regions[2]	411 (159)	464 (168)	200 (31)

1: the definition of longevity-related genes is provided in the materials and methods section; 2: defined as 5 Kb upstream and downstream of gene body

Table 30. Number of SNPs located within known age-related genes and in their promoter regions. Number of affected genes are shown in parenthesis.

We also examined the possible effects of missense SNPs on the protein level. The missense SNPs located in protein domains were identified and evaluated across all protein-coding genes. 180 genes hosting 248 missense mutations were identified, out of which 19 SNPs at 9 genes were shared between the 2 individuals. Interestingly, the missense SNPs were close to each other in the case of the 4 genes that included multiple shared SNPs between the two methuselah dogs (SNPs were located within 3-60 bp).

Analysis of short insertions and deletions

In case of both individuals, 75% of the indels were 5 bp or shorter and 86% were shorter than 10 bp. Indels longer than 100 bp were extremely rare (~0.36%).

Table 31. shows the number of protein coding genes with indels, grouped by impact category. Surprisingly, 524 genes hosted at least 1 short insertion/deletion of a measurable effect (according to Ensembl-VEP's prediction) and most of these genes were part of gene families. High impact categories in

Table 31. included mutations such as stop codon lost mutations (leading to elongated transcripts), indels resulting in frameshift mutations or insertions/deletions affecting coding regions. This group was the most numerous from the 3 impact categories.

	Impact category[1]	Protein-coding genes	Protein-coding genes (excl. protein families)
old_rep1	High	367	56
	Moderate	85	27
	Low	1	0
old_rep2	High	397	60
	Moderate	95	27
	Low	0	0

1: Impact category groups were defined by Ensembl as follows: HIGH – The variant is assumed to have high (disruptive) impact in the protein, probably causing protein truncation, loss of function or triggering nonsense mediated decay; MODERATE – A non-disruptive variant that might change protein effectiveness; LOW – Assumed to be mostly harmless or unlikely to change protein behavior

Table 31. Number of genes including at least 1 indel per impact category.

17.5 Discussion

Here we presented the first study on extreme longevity in companion dogs. We observed approximately 4.8 million SNPs and ~550 thousand indels in the two subjects. A large part of these short genetic variants overlapped between the two samples. This can be explained by the origin of the reference genome: DNA sample for the reference genome originated from a female boxer and therefore it is not surprising that the mixed-breed individuals analyzed in this study carried different alleles at many loci, compared to the reference genome. Therefore, the majority of the SNP and indel variants (and most likely other types of variants as well) were shared between our two old subjects. The difference in the depth of coverage between the individuals did not influence the variant calling, suggesting that even the lower coverage (46x) was sufficient to detect SNP and indel variants. This is in accordance with the literature, where ~30x coverage was proposed to be sufficient for SNP and indel calling (Sims et al., 2014).

Compared to SNP mutations, both the total number of unpublished indels and their overlap between the two individuals were considerably higher. This is because compared to the literature, more novel indels were detected (~17% of all indels) than novel SNPs. This might be for several reasons: first, only one of the three databases used to filter out known indels included short insertions/deletions and consequently many, otherwise common indels in the dog species might have remained in the dataset after filtering. Furthermore, the number of previously published indels was also ~40% less numerous than the number of SNPs published in the same study and ~80% less than the number of SNP in the combined SNP databases. Finally, misalignment of reads e.g. from different copy-number variant regions (including genes of the same gene family) might also have contributed to the higher number of shared indels. Such errors could have also occurred in the reference sequence, resulting in the detection of false positive indels.

Due to the disruptive nature of the mutations that occurred in 12 genes, we examined these genes individually. In the case of the ENSCAFG00000008061 gene, the average depth of coverage at the SNP position was significantly lower (by ~70%) when compared to the average depth of coverage of the whole genome of the same individual. The depth of coverage was such low for a ~440 bp long segment surrounding the mutation, with a short segment completely missing. This is probably because this particular region contains repetitive sequences with a high GC content (77% on average). The segment also partially overlaps with the first exon of the gene. In addition, the sequence coverage pattern of this particular region was very similar in the old_rep2 individual as well, in which the mutation was not detected. Therefore, this particular SNP is most likely a false positive, probably due to an incorrectly annotated gene on the reference genome. Out of the remaining 11 genes, 2 genes were without a human homolog and both of them were categorized as novel genes in ENSEMBL (i.e. only protein and cDNA sequence alignment support is available for the gene; these genes are: ENSCAFG00000017946 and ENSCAFG00000030632); currently no information is available on their functions that could link them to longevity and/or aging. The remaining nine genes had homologs in human. In three of these genes (ENSCAFG00000011630, ENSCAFG00000007858 and the already mentioned ENSCAFG00000008061), the close proximity (50-500 bp regions) of the disruptive mutations was not conserved between dogs and humans based on the reference genome sequences. Furthermore, two genes with a human homolog (the previously mentioned ENSCAFG00000011630 and the ENSCAFG00000032497 gene) had a relatively low cDNA sequence similarity between the reference genomes of the two species (40-60% as compared to 80-97% in case of the remaining seven genes). The remaining seven genes included six known (ENSCAFG00000000433, ENSCAFG00000002007, ENSCAFG00000004279, ENSCAFG00000007858, ENSCAFG00000010498, ENSCAFG00000029250) and a novel (ENSCAFG00000024414) gene and are most likely true positive hits. Some of the stop codon gain mutations were located at the beginning of the genes (the ENSCAFG00000029250, ENSCAFG00000024414 genes, where the 28th or the 390th nucleotide positions, out of 3,390 and 1,755, respectively were affected) having most likely a major, disruptive effect, while in the ENSCAFG00000010498 gene the mutation was located near the 3’ end of the gene (at the 3116th nucleotide out of 3261) and might not be influential regarding protein functionality. Finally, it is worth mentioning that the potential disruptive effect of these mutations might be alleviated, if the genes are present in multiple copies in the canine genome, although this is unlikely in case of the genes with missing segments.

In our dataset, only a small proportion of the exonic mutations located in the pre-defined set of age-related genes were missense. In comparison, Han et al. (2013) found 710 non-synonymous mutations (previously known and novel SNP combined) at the 988 investigated genes when studying six human centenarians, out of which 89 SNPs were novel. We identified 670 novel missense mutations at genes in total, 24 of which were at the pre-defined age-related genes. The difference is probably due to the smaller sample size in our study, but partly also because our analysis was not limited to a pre-defined set of genes and because less data was available from canine databases as compared to human databases used by Han et al. (2013). Although the genetic pathways linked to these genes were already associated with aging and longevity, none of these genes (or their homologs) were found to be linked to longevity by Han et al. (2013) and therefore, these are novel candidate genes for longevity in dogs.

Most of the genes with exonic SNPs were not among the set of genes, which were previously linked to longevity in the literature. Three example genes with some related GO terms are listed below:

- ENSCAFG00000003004: negative regulation of cell growth, negative regulation of apoptotic process
- ENSCAFG00000004892: regulation of apoptotic process, positive regulation of extrinsic apoptotic signaling pathway
- ENSCAFG00000019380: calcium ion transport, regulation of heart contraction, regulation of blood pressure, modulation of chemical synaptic transmission.

Therefore, these genes are candidates for longevity in dogs and their association with longevity should be further investigated once more methuselah dog samples become available. Additionally, we identified a total of 887 genes with SNP mutations in their regulatory regions, which SNPs also overlapped between the two methuselah dogs. 31 of these genes were included in the age-related gene set as well. However, the investigation of the potential effect of these SNPs is not feasible from WGS-data only. Most of the genes with human homologs linked to longevity did not include novel SNPs in the studies dogs. The main reason could be the lack of statistical power to detect quantitative trait loci in our study. Another possible explanation could be that mutations located in other genes may indirectly affect the function of longevity related genes by altering regulatory mechanisms. We identified several missense mutations in genes that had a function in translational processes (e.g. ENSCAFG00000005572 or ENSCAFG00000000104) and thus may affect the synthesis of proteins in general, including possible aging-related regulatory factors.

Based on the publicly available SNP databases, the missense SNP mutations at protein domains were mainly located in SNP-poor regions: within a 200 bp symmetric window around these missense SNPs, ~71% of the windows contained 5 SNPs or less in the SNP database we created by merging the 3 public databases (which contained a total of 61,180,804 SNPs) and 87% of them had 10 SNPs or less within the same window. The highest SNP counts within these windows were 23 and 35 for old_rep1 and old_rep2, respectively. Therefore, it is likely that the SNPs we identified in the methuselah dogs are mostly functional. These closely located SNPs also influenced the same protein domains. Two of these genes (ENSCAFG00000002366 and ENSCAFG00000009496) and two others with 1 single SNP at protein domains (ENSCAFG00000023076 and ENSCAFG00000003564) are directly related to (the regulation of) gene transcription or translation, suggesting a link between gene regulation and longevity. The remaining gene with more than 1 SNP mutation at a protein domain is related to immune response (ENSCAFG00000018626). Genes including more than 1 missense SNP mutation in either one of the 2 individuals revealed mostly the same pattern in SNP distribution with only a few exceptions.

Regarding genetic pathways, 6-6 genes were part of the “Wnt signalling pathway”, “inflammation mediated by chemokine and cytokine signaling” and the “nicotinic acetylcholine receptor signaling” pathways and 5-5 genes were part of the “Alzheimer disease-presenilin” and the “cadherin signalling” pathways. These pathways define an interesting direction for future longevity research. In addition to the named genetic pathways, the genes themselves (as well as their homologs in other species) are principal candidates for association studies aiming to identify linkage between genes and long life expectancy.

Main limitation of the study

The primary limitation of this study is the small sample size: for this study, two dogs of extreme age were sequenced, analyzed and compared to a set of 850 dogs of a presumably averagely long lifespan.

Aging is a complex trait with many genes, regulatory regions, environmental factors, etc. involved. Consequently, it is possible that dogs with an averagely long life also carry mutations that have a positive impact on the length of their lifespan and it is the combined effect of all loci and environmental factors that results in an overall shorter lifespan. Therefore, when we removed the previously published SNPs from the data, we might also have removed relevant mutations and focused only on the most promising mutations. This was done because with only two extremely old dogs, we did not have the statistical power to find all genomic loci influencing longevity. However, in the future, when more data become available, the mutations with smaller effects can also be investigated.

Conclusions

With a comprehensive analysis of all canine genes, we detected rare variants linked to canine longevity. Several genes were identified as potential longevity-associated genes. Based on their gene ontologies, these genes were related – among others – to gene transcription/translation and its regulation, to immune response and the nervous system in general. A link between extreme longevity and the regulation of gene transcription/translation suggests that one crucial genetic requirement of extreme longevity lies within the *fine-tuning* – i.e. the superior calibration – of RNA (and thereof protein) production of an organism. This phenomenon defines an interesting direction for future research aiming to better understand longevity.

We also identified several disrupted genes, which were not functioning in either of the two methuselah dogs, although none of these were shared between the individuals. The dogs sequenced for this study were unrelated mixed-breeds, suggesting that the mutations detected in both of them are relevant. Therefore, the SNPs we found in the two individuals (and the related genes) could be targeted in age-related research in the future. This is further supported by the fact that none of these mutations were found in databases containing information from a total of 850 dogs with an average lifespan, representing a wide range of breeds as well as mixed-breed individuals from multiple countries.

III. General discussion

18 Theses: Novel scientific achievements[14]

I. Demography and health

1) The prevalence of age-related cognitive decline in companion dogs across the entire adult lifespan (Chapter 7)

We tested the internal consistency of the most widely used *cognitive dysfunction questionnaire's* domains. We found that five out its eight scales have not shown reliable internal consistency in a Hungarian sample. This warrants caution when the diagnosis is based on the number of signs shown within a domain. The result prompted us to create a short, seven-items scale with high internal consistency assessing cognitive decline based on the items' positive correlation with relative age. With this survey we found that signs of cognitive decline were already detectable in dogs at 50-75% of the expected lifespan, independently from the weight and size of the dog, indicating that large dogs age faster. Visual, auditory and olfactory impairments all resulted in significantly higher occurrence of cognitive decline associated behaviours, suggesting that it is crucial to collect information about the sensory functions of aged dogs when evaluating cognitive decline with online questionnaires or behavioural tests. Participating in dog training had beneficial effects on cognitive aging suggesting cognitive reserve or altered aging curve in trained dogs.

2*) Demographics of companion dogs across age groups and identifying the key variables associated with health status (Chapter 8)*

Dogs go through similar stages of development as humans, however, many owners do not consider their dog's life stage when e.g. selecting a diet. We examine the link between the age and health of the dog, and owner and dog demographics in a cross-sectional sample. The oldest age group (>12 years old) was classified by owners more frequently as unhealthy, less often have a "normal" body condition score. There were more male and neutered dogs among them, received less activity/interaction/training with the owner, and were more likely to have experienced one or more traumatic events such as spending time in a shelter, changing owners, prolonged disease. Purebred dogs suffered from health problems at a younger age and died at an earlier age than mixed breeds. The youngest age group was more often fed raw meat (e.g. BARF), and had owners aged under 29 years, reflecting new trends. Our results partially contradict our prediction that dogs that are heavier in weight are more likely to suffer from health problems. However, they implicate a new factor, which has an impact on health regardless of breed and age, that of experiencing traumatic events during the lifespan. The prevalence of dogs that had experienced one or more traumatic events in their

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[14] For the hypotheses see Chapter 5.

lifetime was high (over 40% of the sample). Our results corroborate previous research indicating that age is the strongest predictor of health status regardless of breed, height and weight, as it is in humans.

II. Cognition, emotion and face processing

3) Developing a behaviour test for assessing discrimination and reversal learning (Chapter 9)

This study aimed to develop a reversal learning task that could detect age-related changes in the learning abilities of family dogs in a short time-frame (about 1 hour). We have met this goal, as both the reversal learning and the preceding discrimination learning tasks detected age differences, old dogs learned significantly slower than young dogs. In the reversal learning, training based on the location of stimuli was easier for the dogs than training based on the size, shape, colour of stimuli. In the latter condition, we observed a ceiling effect, as most old dogs did not learn the task in 50 trials. Therefore, we suggest that training based on objects' location is more informative for reversal learning tasks. Overall, these results suggest that our discrimination learning and reversal learning tasks could successfully be used to investigate differences in spatial function between young and old dogs. This test has the potential for clinical state examinations, which are widely used among human clinicians but currently nonexistent in veterinary praxises.

4) Positivity effect in dogs: do old dogs experience less negative emotions? (Chapter 10)

Older people experience fewer negative emotions as their attention shifts from negative stimuli due to age-related changes in the brain, which is known as the "positivity effect". We found that compared to young dogs, old individuals reacted slower only to the negative sounds, suggesting that an age-related positivity effect is present in dogs, similarly to humans. There was no significant difference in the latency to recover between groups. Similarities in emotional processing between humans and dogs may imply analogous changes in subcortical emotional processing in the canine brain during aging. Studying age-related differences in the processing of emotional stimuli in animals gives us more insight into the biological changes of the aging brain, affecting how older individuals perceive and process their social environment.

5) Age-related effects in looking at faces of humans and conspecifics (Chapter 11)

Responsiveness to faces changes with age, either through change in perceptual processes or age-related differences in processing social stimuli. We found that independent from age, dog portraits attracted longer looking times than human profiles and short-headed dogs were more attentive to faces. In a subsequent experiment older dogs took longer to approach food placed in front of the images, due to changes in facial perception or due to a decline in sensorimotor functions, and mesocephalic dogs were faster than dogs of other skull length types. Because dogs, at least in some contexts, rely on our faces to understand our behaviours and intentions it is important to consider for the refinement of dog-human communication, training, and welfare, how the animal's age and other characteristics impact facial perception. Moreover, a comparison between humans and dogs might uncover previously unforeseen factors regarding age-related changes in orientation to socially relevant visual cues and explain socially inappropriate behaviour in the elderly.

III. Personality and intraspecific relationships

6) Age-related changes in human-based personality traits and associations with owner and dog demographics (Chapter 12)

Human personality traits change across the lifespan, and that significant life events and educational experiences can influence personality traits. People tend to show increased self-confidence, warmth, self-control, and emotional stability with age. We found on a large sample with more than 10,000 individuals that older dogs were calmer, less trainable, less social, and less bold than younger dogs. These traits are analogous to the human traits Emotional Stability, Openness/Intellect, Agreeableness, Extraversion. Other variables, including the dog's sex and neutered status, owner's gender, age, education, previous experience with dogs, the number of people and dogs in the household, and purpose of keeping the dogs also had detectable effects on the traits.

7) Interventions to increase play and training motivation may alleviate the negative effects of aging (Chapter 13)

Inconsistencies about the canine personality traits may be due to the different methods were used to obtain the trait scores. The Dog Personality Questionnaire has been shown to demonstrate reliability and validity, therefore it is a suitable means for investigating the replicability of previous results. We found that three of the five factors showed significant age effects. Activity/Excitability decreased with age. Aggressiveness towards animals showed a quadratic developmental trajectory peaking in dogs aged 6 to 10 years. Whilst Responsiveness to training also decreased, only dogs older than 12 years differed significantly from the other groups. When the models were re-run including the other explanatory variables, the age group was no longer significant for this trait. The amount of time spent interacting/playing with the owner partially mediated the relationship between age and Responsiveness to training, implying that interventions to increase play and training motivation may alleviate the negative effects of aging on dogs' trainability.

8) The relationship between age, personality, dominance and leadership in a group of dogs (Chapter 14)

We obtained high-resolution spatio-temporal GPS trajectory data (823,148 data points) from six dogs belonging to the same household and their owner during fourteen, 30-40 min unleashed walks. A directional correlation analysis quantified interactions between pairs of dogs that run loops jointly. We found that dogs play the role of the leader about 50-85% of the time, i.e. the leader and follower roles in a given pair are dynamically interchangeable. However, on a longer timescale, tendencies to lead differ consistently. The network constructed from these loose leader-follower relations is hierarchical. According to our results, the collective motion of a dog group is influenced by age and the underlying hierarchical social network. Leader/dominant dogs are older, more trainable, controllable, and aggressive than follower/subordinate dogs. Leader dogs exert a disproportionate influence on the decisions about running away and turning back to the owner during walks. Findings could pave the way for automated animal personality and human social interaction measurements.

9) Chapter 15. Dominance status and age in companion dogs sharing the same household

We examined how owners perceive dominance in dogs, and what different behaviours and personality types are used to describe dominant and subordinate individuals with a questionnaire study. According to the owners, dogs rated as dominant (87%) have priority access to resources (resting place, food, and rewards), undertake certain tasks (defend and lead the group, bark more), display dominance (win fights, and mark over the other's urine, the other dog licks their mouth), share certain personality traits (smarter, more aggressive and impulsive), and are older than their partner dog. In 66% of the dog dyads, the older individual was dominant. The newly developed questionnaire is more reliable in assessing dominance than previous attempts, therefore a useful mean for assessing age-related changes in social ranks.

IV. Steps towards understanding the mechanisms of aging

10) Age related differences in the spindling activity of the sleeping brain (Chapter 16)

The dog brain is still relatively unknown compared to the brains of other model animals such as rodents. We found a different course of aging in the fast and slow canine sleep spindles (brief trains of rhythmic activity, 0.5-6 seconds long, 9-16 Hz, which appear in the EEG signal of humans and other mammals during non-REM sleep). Slow and central spindles follow human-like aging trends. Frontal fast spindles continue to increase in density with age, which seems to be unique to dogs, although human adolescent development is also characterized by increased density. Our findings support the argument that sigma bursts in the canine non-REM sleep are analogous to human sleep spindles and suggest that in dogs, slow and fast spindles display different trajectories related to age.

11) The genetic background of longevity based on whole-genome sequence data of two methuselah dogs (Chapter 17)

We identified more than 7500 novel SNP mutations in two methuselah dogs (with a lifespan of 22 and 27 years) when compared to three publicly available canine databases with SNP information from 850 dogs. Based on *in silico* analysis, we identified 670 missense mutations across 472 genes and several genetic pathways that are primary candidates for age-related research in dogs (and their homologs in humans) in future studies. Based on their gene ontologies, these genes were related – among others – to immune response and the nervous system in general. A link between extreme longevity and the regulation of gene transcription/translation suggests that one crucial genetic requirement of extreme longevity lies within the *fine-tuning* – i.e. the superior calibration – of RNA (and thereof protein) production of an organism. This phenomenon defines an interesting direction for future research aiming to better understand longevity.

19 Putting things together[15]

Aging is considered as one of the largest risk factors for both diseases and mortality (Flatt and Partridge, 2018). Age-related diseases include sensory changes (e.g. hearing loss), chronic- (e.g. high blood pressure) and neurodegenerative disorders (e.g. Alzheimer's disease) among others (Jaul and Barron, 2017). Furthermore, both the number- and proportion of elderly people are expected to increase at an accelerating rate in the future (the United Nations estimated a 230% increase – from 901 million to 2.092 billion – in the population of people of 60 years or above from 2015 to 2050; United Nations, 2015). Both longevity and *healthspan* (defined as the "period of time during which humans and non-human animals are generally healthy and free from serious chronic illness"; quote from Wallis et al., 2018) are direct consequences of (a healthy) aging and are equally as important from both social and economic points of view.

Firstly, dogs have a much shorter life expectancy than humans – 10-12 years on average in dogs (Adams et al., 2010; Proschowsky et al. 2003; Leroy et al., 2015) vs. 72 years in humans (2016 estimation; WHO, 2018) – making aging-related experiments much shorter in time. Secondly, dog breeds show a huge phenotypic and genetic variability (Leroy et al., 2009; Ostrander et al., 2017), which can also be observed in longevity (e.g. Inoue et al., 2018; Jimenez, 2016). Dogs also have more shared ancestral sequences with humans than rodents do (Lindblad-Toh et al., 2005). Furthermore, they also share the same, rich environment that humans live in (unlike laboratory animals, which are kept in a highly controlled environment) and therefore near-identical environmental factors influence their everyday life, aging and life expectancy (Hoffman et al., 2018) than those influence the aging of humans. Finally, dogs have multiple, spontaneously developing age-related diseases late in their lives that can be considered as analogues of multiple human diseases (e.g. the canine cognitive dysfunction, which is similar to Alzheimer's disease; see Chapagain et al., 2018 for

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[15] Partly based on Jónás, D., Sándor, S., Tátrai, K., Egyed, B. Kubinyi, E., (2019). The genetic background of longevity based on whole-genome sequence data of two methuselah dogs. *Submitted.*

more details). The undeniable relevance of this final point and the previously mentioned other advantages of the companion dog make this species an ideal candidate model animal for the study of the process of aging. In addition, it is also expected that the obtained results are more applicable to humans than those conducted on other laboratory animals.

The study of aging and longevity is of great importance. It enables us to better understand the process of aging itself, which in turn allows the promotion of a healthy lifestyle among the general population and allows mankind to successfully cope with the long-term socio-economical consequences of an aging population. These studies have recently gained a lot of attention (e.g. Chapagain et al. 2018; Mongillo, Araujo, et al. 2013; Szabó et al. 2016; Wallis et al. 2016), both due to the dog's potential as a model animal (Adams et al. 2000; Araujo, Studzinski, and Milgram 2005; Cummings et al. 1996) and to expand veterinary applications (Landsberg, Hunthausen, & Ackerman, 2003). Our results support the increasing amount of evidence (e.g. Cotman and Head 2008; Cummings et al. 1996; Head 2013; Kaeberlein, Creevy, and Promislow 2016; Neus Bosch et al. 2012; Schütt et al. 2016) that describes the domestic dog as a good translational model for the study of aging. Both dogs and humans spontaneously develop similar medical conditions that increase the risk of death, as they age, such as cancer (Hoffman, Creevy, Franks, O'Neill, and Promislow 2018). We have observed similar changes in behaviour as well in a convenient sample of pet dogs (Szabó et al 2018, Chapter 7). This body of evidence may reflect a naturally occurring and age-related cognitive decline, which follows similar pathways in dogs as it does in humans (Head et al., 1995; Head, 2013), and results for example in decreased capacity of emotional processing (Smit et al, 2019, Chapter 10) and reversal learning (Piotti et al 2018, Chapter 9).

The brain mechanisms behind these changes have been also suggested to be similar in dogs and humans (see age-related changes in characteristics of EEG sleep spindles, Iotchev et al. 2019, Chapter 16). Beta-amyloid accumulation has been observed in dogs as early as 9 years of age in the brain region of the prefrontal cortex and from 14 years on in the entorhinal cortex (Head et al. 2000). Atrophy of the cerebral cortex and enlargement of the brain ventricles have also been described in MRI (Magnetic Resonance Imaging) scans of a group 16 years old dogs (Kimotsuki et al., 2005) and in morphometry studies of the cerebral ventricles of both young and old dogs (2-5 years vs 10-12 years, González-Soriano et al. 2001). One study looked at MRI scans of a group of 18 dogs aged between 4 and 15 years (with age treated as continuous variable), observing a non-linear relationship between age and brain ventricles enlargement, cortical atrophy. Interestingly, individual differences were also detected, as one 6 year old dog in the sample was as severely affected as the 14 year old dogs in the group; this suggests that some individuals can develop age-related brain degeneration before others and this can be as early as 6 years of age (Su et al., 1998). The same dogs had been tested for their cognitive performance in a different study (Head, Callahan, Muggenburg, Cotman, & Milgram, 1998), but no relationships between cognitive findings and brain changes were reported (Su et al., 1998). The dogs had been tested for cognitive performance up to 2 years prior to the MRI scanning (Head, Callahan, Muggenburg, et al. 1998; Su et al. 1998) and the results of the cognitive tests were reported according to 3 distinct age groups (young: < 5 years, middle aged: 5-10 years, old: 10+ years which made any comparison difficult to interpret. Studzinski et al. (2006) also investigated cognitive decline in dogs; they observed spatial deficits starting from the age of 6 and reported that age alone predicted 48.2% of the variability in learning in a task to assess memory. Overall, a large body of evidence indicates that functional decline in cognitive domains, such as learning, memory, executive function, and spatial function, occurs similarly in dogs and humans as they age (for reviews, Cotman & Head, 2008; Head et al., 2013). It should however be noted that, in this body of research, it is difficult to evaluate the effect of age alone as these findings are rarely adjusted to the lifespan variability that is due to factors such as breed, size, or weight (Szabó et al., 2016), effects we were aware of and controlled for it with equation (Szabó et al 2018, Chapter 7) or specifically chosen samples (Smit et al, 2019, Chapter 0; Piotti et al 2018, Chapter 9, Bognár et al 2018, Chapter 11).

In addition to the physiological reasons described above, the use of family dogs as models for aging research is also supported by ecological reasons. Through their unique domestication history, dogs have adapted to a specific niche, i.e. the human social environment (Topál et al. 2009). However, a large proportion of the research on dog cognitive decline involves purpose-bred and raised laboratory animals (Head, 2013 for a review). It should be noted that laboratory conditions do not mimic the animals' natural environment (Wood, Desjardins, & Fernald, 2011). There is some evidence that family dogs (i.e. dogs living with humans as pets) and purpose-bred research dogs (i.e. kennel reared domestic dogs) diverge in their performance during some cognitive tasks (Lazarowski & Dorman, 2014). Indeed, it has been argued that dogs residing long-term in kennel environments may be affected by cognitive deficits, due to the lack of stimulation provided by their living environment (Miklósi & Topál, 2011; Mongillo, Araujo, et al., 2013; Turcsán et al., 2019). Thus, results from canine cognitive tests, when performed in conditions similar to those of the human environment, are likely to have strong ecological validity. Family dogs, which share the same living environment as humans, are promising subjects for research that is both clinically relevant and provide the necessary vertical integration of findings originated from invertebrate and rodent models (Waters, 2011). Consequently, studies on family dogs' cognitive aging have begun to emerge (e.g. Chapagain et al., 2017; González-Martínez et al., 2013; Heckler et al. 2014; Mongillo et al., 2013; Piotti et al., 2017; Wallis et al., 2016; Wallis et al., 2017). One remaining issue is that some of these tests still require prolonged and complex procedures (discussed below); this limits the replicability of such studies, especially outside of the laboratory setting (Heckler et al., 2014 for a discussion). The importance of replicability is being discussed in psychology research, as replications and data reproducibility are necessary to generalise research findings to the general population (Westfall, Judd, & Kenny, 2015) and further efforts should be made to increase the replicability of research. We have succeeded in developing a simple, relatively quick reversal learning test for family dogs in two versions, facilitating future longitudinal tests (Piotti et al 2018, Chapter 9).

In our studies we found numerous demographic and environmental differences between dog age groups based on the owners' reports, emphasizing the importance of taking into account these age-related changes in future studies. We have reported that over 40% of a convenient sample of pet dogs had experienced one or more traumatic events in their lifetime that still has an impact on their behaviour (Wallis et al 2018, Chapter 10). Since stress affects the physical, mental and social health of the animal, managing an animal's stress after a traumatic event, as well as attempting to prevent the occurrence of the event in the first place, is essential in order to improve healthspan and wellbeing in dogs. Owners should be made aware of the personality trait characteristics of their dog, and the methods they use to cope with stressful experiences, as well as the most common types of trauma and their risk factors. In this way, they can better support their dog when stress is unavoidable, but also can attempt to reduce their dog's exposure to stress in order to diminish any negative impacts on their healthspan. Since personality and coping style can also change with age within an individual (Kubinyi et al 2009, Chapter 12, Wallis et al 2018, Chapter 13.1), owners should learn how to read their dogs behavior to better understand their specific needs at all life stages, as well as how to prevent the development of unwanted negative behaviors in their dogs for example by using positive training techniques or by adopting older dogs that show less problematic behaviour compared to young individuals (Turcsán et al 2017).

Not only cognition and personality but also social behaviours were found to be affected by age (Howse et al., 2018; Rosado et al., 2012; Szabó et al., 2016). Social rank is likely to change with age (Kubinyi et al 2019, Chapter 15; Bonanni et al. 2010, 2017; Cafazzo et al. 2010; Pal et al. 1998) which might affect at least some cognitive abilities such as observational learning (Pongrácz, 2014; Pongrácz et al., 2008) and also leadership (Ákos et al. 2014, Chapter 14).

20 Perspectives

The experimental studies of Part II in this dissertation investigated the functions of the aging canine brain mainly from an ethologist's perspective, but several investigations are rooted in neuroscience and genetics. The Senior Family Dog Project at the Department of Ethology Eötvös Loránd University (Fgure 37) addresses economical, societal, mental health, animal welfare, and educational aspects of the canine aging. The recently finished, ongoing and future projects are summarized briefly below.

20.1 Cross-sectional and longitudinal behavioural investigations

Behavioural studies mainly looked at the effect of age-related changes in general, by comparing young adults to healthy aged dogs. These studies provided contradictory results in connection with activity and spatial orientation (Mongillo, Pitteri, et al., 2013; Rosado et al., 2012; Vas et al., 2007). However, the performance of an old individual in a cognitive test depends on the previous maximum level of the skill and age-related change too, which means that only *longitudinal studies* can separate the two effects. In the framework of the Senior Family Dog Project a specifically chosen dog population is followed over a 5 year period since 2016. We perform tests to measure learning and memory, inter-specific communication, activity levels, motor skills and impulsivity, attachment, and sensory performance (to check dogs general vision, hearing and olfactory abilities, labeled as “Cognitive Battery” Piotti et al, in prep, Figure 35). We use a psychometric approach to measure cognitive phenotypes and look for associations with age and other demographic variables using multivariate statistical methods. We re-test the dogs three months after their first test for short-term comparisons, then further test sessions are conducted one year apart for long-term analyses.

With the Cognitve Battery we test whether a three month-long physical and/or cognitive intervention is effective when applied to family dogs aged above 8 years. We are interested to observe whether transfer effects (i.e. improved performance on related but untrained tasks or improvement in tests measuring abilities which were not trained directly) took place in the sample. The candidates are pre-screened before enrollment to flag individuals which suffer from sensory or physical impairments. Baseline and post treatment tests have been completed in three cohorts. (Szabó et al. in prep).

We also seek age-related changes in vocalisation as a potential biomarker for cognitive decline (Marx et al, in prep).

In collaboration with the Clever Hans Lab in Vienna we analyse personality trait-age associations in a personality test battery test, and investigate the re-testing of dogs 4 years later. We confirmed the long-term consistency of the personality traits measured, found both linear and quadratic associations between these traits and age across the age range of 0.5-15 years, and also identified three individuals who showed behavioural markers of cognitive decline (Turcsán et al, in prep). Behaviours related to sociability and frustration were stable over ca. 4 years in Border collie dogs. Activity decreased over repeated testing, mostly due to systematic age effect. Novelty seeking also decreased, while Problem orientation increased from Test1 to 2, both partly due to age effect and partly due to random effect (e.g. experience with the task which led to lower interest in the ‘novel’ object, but higher success in solving a familiar problem). This is one of the first studies that shows behavioural consistency over such a long time in dogs.

Previous studies have documented the benefits of physical activity and cognitive enrichment on the performance of dogs in cognitive tasks. We described in detail a touchscreen apparatus, software and training methods that we have used to facilitate dog computer interaction (DCI). We compared dogs of different ages in their performance of learning different tasks on the touchscreen, and found that young dogs learn the tasks twice as fast as old dogs. However, feedback from the owners suggested that the learning experience is enjoyable for both the owner and the dog. DCI has the potential to improve the welfare of older dogs in particular through cognitive enrichment (Wallis et al, 2017).

We have assigned unfamiliar, nonaggressive dogs randomly to three types of dyads defined by sex, and observed their unrestrained, spontaneous behaviours in an unfamiliar dog park. Sex, dyad composition, neuter status, and age influence different aspects of the interactions in dyads. The time companion dogs spent in proximity to each other and number of approaches decreased with age. Indicating decreased intraspecific sociality and activity in older dogs (Iotchev et al., 2019).

Figure 35. Collage about the subtests of the Cognitive Battery.

20.2 Neural correlates of non-pathological aging: (f)MRI- EEG studies, and brain atlas

In humans, disturbances in memory and executive function during senescence (Beth Adams, Chan, Callahan, & Milgram, 2000; Cotman & Head, 2008) are accompanied by specific neural changes, like ventricle volume growth due to cerebral atrophy, the reduction in neuronal cell formation and the accumulation of beta-amyloid plaques (Head, 2011). Several studies have been carried out to reveal more specific alterations at the neural level that could be related to mental decline in dogs. A detailed descriptive study (Borras, Ferrer, and Pumarola 1999) found various age related changes in the dogs' brain, involving a wide variety of tissue types in the central nervous system (e.g. neurons, glia cells, vascular endothelial cells), such as retraction of the cerebral gyri, cerebral haemorrhages and lipofuscin accumulation, but they found no age effect with regard to cerebrovascular amyloidosis. Only laboratory dogs (beagle breed) were involved in these analyses so far (eg. Head, 2013). However, the laboratory beagle populations exhibit significant behavioural differences affecting socio-cognitive functions compared to the family beagle dogs (Turcsán et al. in prep.), so the results have only limited applicability in dogs and humans living in various conditions.

We aim to identify neural markers that are associated with cognitive aging using non-invasive methodologies (fMRI, EEG) on family dogs. First, functional magnetic resonance imaging (fMRI) studies are applied to dogs that have been trained to remain lying motionless for 6-8-minute-long intervals (a globally unique sample of dogs, Figure 36). Second, the polysomnography method provides spectral data (EEG) from the dogs, which is directly comparable with that of humans. In parallel, we also collect behavioural data, including cognitive and memory performance, personality ratings, as well as sensory data for each dog. We expect to find that brain-related changes correspond to cognitive performance.

Figure 36. Dogs participating in fMRI tests.

We successfully set up the methodological background to study rs-fMRI networks of aging dogs, describing multiple human analogous network. In the first study, we collected resting state fMRI data from 22 dogs (Szabó et al., 2019). Based on the results of the first study, and the experience gained from it, we continued data collection for the second study with an improved protocol. Data collection for the second study is nearly finished. We will analyse the age-related changes in connectivity, to describe in which regions the reduction of connectivity is most pronounced.

We used fMRI in order to measure brain activity while processing auditory and visual stimuli. We analysed multilevel fMRI adaptation which revealed a human-analogue lexical processing hierarchy in the dog brain (Gábor et al., 2019).

Voxel-based morphometry of the aging to the changes in the size of the lateral cerebral ventricles. The volumetry we performed revealed an interesting fact, namely that despite the sometimes remarkable (and statistically significant) ventricular size increase in aged dogs, no negative effect was shown in association with this on the performance in a highly specialised fMR task (sustained attention and self-inhibition, Gunde et al, in prep).

We have established a novel method for obtaining high-resolution macro-anatomical images of an in-situ dog brain, which, together with the structural images (CT and MRI) gained from the same study, could form the basis of a comparative canine brain atlas (Czeibert, Baksa, et al., 2019). We also summarized the existing terms of the canine gyriation nomenclature to show the inconsistencies in their usage regarding sulci of the brain. Based on this summary, we clarified their definition to provide a common base for future terminology (Czeibert, Piotti, Petneházy, & Kubinyi, 2019) and made an MR-normalization protocol for the fMRI studies to provide the means for a more reliable statistical analysis, and also included the creation of an individual label-based MRI-atlas for research purposes (Czeibert, Andics, Petneházy, & Kubinyi, 2019).

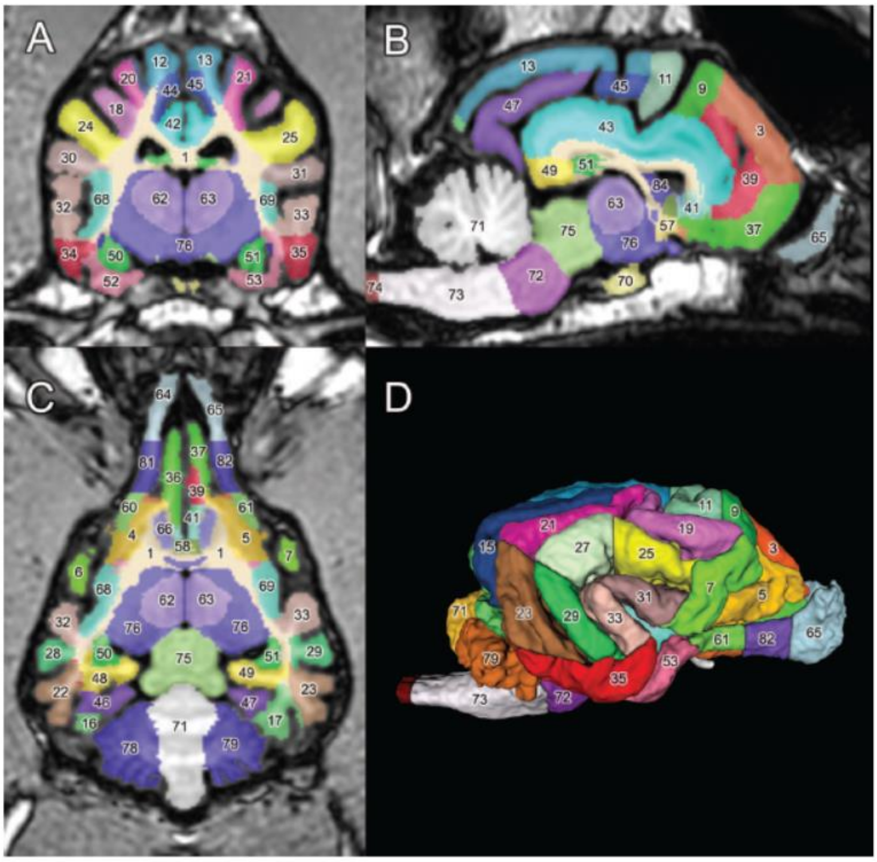

Figure 37. Overlaying the labels on the individual template with 50% opacity (for reference of the numbers, see the original paper). (A) Transverse plane. (B) Sagittal plane. (C) Dorsal plane. (D) Three-dimensional surface view of the labels from the right lateral aspect. Picture from Czeibert, Andics, Petneházy, & Kubinyi, 2019.

20.3 Canine Brain and Tissue Bank

We expected that some old dogs participating in our different studies would die due to natural causes during the 5 year long course of the project. This offered the possibility of comparing their behavioural performance to those dogs that live/lived longer. We also expected that some of our subjects' owners will offer the brain of their deceased dog for post-mortem analysis. Therefore, we have established the Canine Brain and Tissue Bank in Budapest at the Eötvös Loránd University in 2016 (https://familydogproject.elte.hu/canine-tissue-bank/). We developed a unique pet dog body donation system for those owners, who, in agreement with their veterinarian, voluntarily offer their dog's body after a medically reasoned euthanasia (Sándor et al, submitted). This donation system is in harmony with the international and national guidelines and laws. As an integral part of this, we established a state-of-the-art communication, transportation, and sampling system. The samples, together with thorough documentation of the dogs' previous, cognitive performance and medical history allow us to correlate the post-mortem physiological data with the behavioural measurements. So far we have obtained more than 100 dog brains and other tissues (e.g. liquor cerebrospinalis, lymphoid tissue, ganglia, muscle and skin; Sándor, Tátrai, et al. 2019). We evaluated the histological quality of the first canine brains donated to the Canine Brain and Tissue Bank by hematoxylin-eosin (HEMA) staining. Together with HEMA staining we tested the applicability of these tissue samples in immunohistochemistry analysis using three different antibodies against autophagy related proteins (SQSTM1, MAP1LC3B and a microtubule associated protein). These studies were only intended as a methodological set-up and yielded positive results.

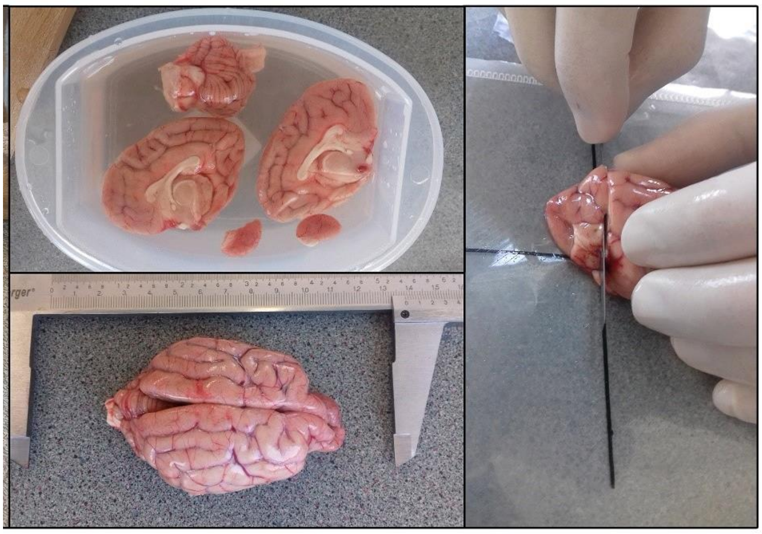

Figure 38. The Canine Brain and Tissue Bank collects samples from euthanised pet dogs whose bodies have been formerly donated by their owners. Credit : Kálmán Czeibert

We also tested several available methods for the proteomic analysis of dog cerebrospinal fluid (CSF) that was collected post mortem and we have evaluated the levels of autophagy-related genes (SQSTM1, MAP1LC3B) in dog cortical samples by western blot and Real-Time qPCR analysis. In the case of MTMR14, which has been shown to down-regulate autophagy, our results were in accordance with the findings of our collaborators utilising *Drosophila melanogaster* as a model species, regarding the age-related changes in the expression of this gene (Kovács et al., 2019).

An analysis of the associations between gene expression levels (set of all RNA molecules, transcriptome) and cognitive performance is also possible using suitable brain tissues. In this project, we aim to quantify the genes that are differentially expressed in the brains of old and young dogs, which will provide information about the genetics of cognitive aging in dogs. The total RNA set will be isolated from the brains of old and young dogs (brain tissue samples are available from the Dog Brain-bank) and the RNA samples will be sequenced. Old-vs-young sample comparisons will be performed according to the literature (Jónás et al, in prep.).

20.4 Genetics and molecular biology

Age-related cognitive decline, either being normal or pathological, is a fairly unique attribute of humans. Even in the case of neurodegenerative diseases, which can usually be easily characterized by certain behavioural or physiological changes, the underlying genetic factors often remain barely understood (Sándor and Kubinyi, 2019). In addition, the role of these factors in the development of disease is also strongly influenced by environmental context. For example, the average heritability of late-onset AD was estimated to be 0.58 (Gatz et al., 2006), although one genetic variant, the *ε4* allele of the *APOE* gene were shown to outmatch any other variants as the main risk factor for developing late-onset AD (Brousseau et al., 1994; Corder et al., 1993; Lambert et al., 2013; Strittmatter et al., 1993). Interestingly, the *APOE* e4 was also associated with cognitive function both in subjects suffering from Alzheimer’s disease and in non-impaired old individuals (Wisdom, Callahan, & Hawkins, 2011). A GWAS study confirmed that the *APOE* region is significantly associated with non-pathological cognitive aging due to a functional, regulatory non-protein-coding effect (Davies et al., 2014). This finding also pinpoints to the necessity of considering genetic factors that can modify the course of non-pathological cognitive aging, when we want to get a better picture of human aging. Our goal is to identify age-related genetic markers.

Some genes which have been implicated in gene-behaviour associations in dogs show associations with aging in humans, which makes them good prospective candidates for further research. In humans, the major cognitive domains, memory, in particular, show high heritability (Harris & Deary, 2011; Reynolds & Finkel, 2015). Several candidate genes were tested for association with cognitive ability and cognitive decline during aging in older people. The *brain-derived neurotrophic factor* (*BDNF*) gene, which is implicated in hippocampal-dependent memory (Egan et al., 2003), depression (Martinowich, Manji, & Lu, 2007) and susceptibility to psychiatric disorders (Hall, Dhilla, Charalambous, Gogos, & Karayiorgou, 2003; Notaras, Hill, & van den Buuse, 2015), showed that it was associated with reduced cognitive function in elderly people (Payton, 2009; Tapia-arancibia, Aliaga, Silhol, & Arancibia, 2008). Many studies reported that the *catechol-O-methyl-transferase* (*COMT*) gene, which is involved in the dopaminergic pathways of neurotransmitters, is associated with cognition, but a meta-analysis have not found strong evidence for this link (Barnett, Scoriels, & Munafò, 2008). Variation in the *disrupted in schizophrenia 1* (*DISC1*) gene was linked to cognitive aging specifically in women (Thomson et al., 2005). However, the findings did not replicate in a follow-up study on a different sample (Palo et al., 2007). On the other hand, for example, the *dystrobrevin binding protein* (*DTNBP1*) gene has been associated with cognitive function in multiple studies (Zhang, Burdick, Lencz, & Malhotra, 2010). Polymorphisms in the *dopamine receptor D4* (*DRD4*) gene were found to be linked to activity-impulsivity trait in dogs (e.g. Hejjas et al., 2007), and alleles of the human homolog are associated with longevity (Grady et al., 2013; Szekely et al., 2016). Furthermore, *BDNF* was shown to be differently expressed in dogs fed with an antioxidant cocktail or exposed to environmental enrichment (Fahnestock et al., 2012; Sechi et al., 2015), thus it may have a similar function in neuroprotection as its human counterpart. Oxytocin receptor gene (*OXTR)* polymorphisms in dogs were linked to human directed social behaviour (Kis, Bence, et al., 2014), while in humans, together with *BDNF*, it was linked to old age depression (Chagnon, Potvin, Hudon, & Préville, 2015). *COMT*, which has already been mentioned in connection with human cognitive aging above, is a suggestive marker of human contact seeking in laboratory beagles (Persson, Wright, Roth, Batakis, & Jensen, 2016). In summary, it is also possible that age-related canine personality changes (Fratkin et al., 2013) are also affected by these genes, however, this question requires further research efforts to get answered.

We found associations between specific gene (oxytocin receptor, OXTR; opioid receptor, OR) polymorphisms and the greeting behaviour of dogs, but we did not find an age effect in the test population (Kubinyi et al., 2017).

We designed experiments to detect the activity of mobile genetic elements in the dog genome. Specifically, we aimed at measuring the activity of the LINE-1 retrotransposon in the brain and its relation with aging. We have developed a western blot method to measure the amount of the ORF2 protein in the brain and muscle samples. Currently, we are collecting samples from euthanized dogs to compare age groups. We have also developed a quantitative real time PCR (RT-qPCR) method to measure the LINE-1 mRNA level in brain and muscle samples. For this purpose, we have designed a primer pair on the most conservative 200 bp range in the LINE-1 ORF2 sequence. Currently, we are looking for the best control genes and are focusing on collecting more samples to compare age groups (Tátrai et al, in prep).

We aimed to investigate how cognitive aging in dogs is associated with the composition of their intestinal microbiota. We collected faecal samples from old and young dogs (N=50) and analysed the compositions of their intestinal microbiota by 16S RNA sequencing using an Ion Torrent platform from Life Technologies. We have already performed PCR for 36 samples and sent them for sequencing to the University of Michigan (USA). Currently, we are collecting more faecal samples and are continuing with the PCR and sequencing analyses.

20.5 Closing remarks

The dog has grown to be one of the most important animals for researchers who aim to understand the biological background of complex traits. The significance of our best friend as a model animal originates in its unmatched morphological and behavioral variability and the unique population structure of purebred dogs being selected for various purposes through centuries. Dog breeds show a huge phenotypic, genetic and longevity variability and have more shared ancestral DNA sequences with humans than rodents do. Dogs have six-twelve times shorter lifespan than humans, making aging-related investigations feasible. Companion dogs live closely with humans thus share the same, varied environment, unlike laboratory animals. Importantly they also have multiple, naturally developing age-related diseases as humans, for example, the Alzheimer-like canine cognitive dysfunction. Therefore, it is expected that research on dogs is more applicable to humans than those conducted with laboratory animals. The results of the present thesis showed age-related differences in brain activity, cognition, personality, and social status in several aspects similar to human aging, supporting the dog's potential as a model of cognitive aging and providing knowledge for increasing the quality of life of dogs and owners alike.

Figure 39. Members of the Senior Family Dog Project in 2017 (above) and 2019 (below).

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22 List of the experimental studies in the dissertation[16]

1) Ákos, Zs., Beck, R., Nagy, M., Vicsek, T., Kubinyi, E. (2014). Leadership and path characteristics during walks are linked to dominance order and individual traits in dogs. *PLoS Computational Biology, 10,* e1003446.
2) Bognár, Z., Iotchev, I. B., & Kubinyi, E. (2018). Sex, skull length, breed, and age predict how dogs look at faces of humans and conspecifics. *Animal Cognition, 21,* 447-456.
3) Iotchev, B. I., Kis, A., Turcsán, B., de Lara, D. R. T. F., Reicher, V., Kubinyi, E. (2019). Age-related differences and sexual dimorphism in canine sleep spindles. *Scientific Reports, 9,* 10092.
4) Kubinyi E, Wallis LJ. (2019). Dominance in dogs as rated by owners corresponds to ethologically valid markers of dominance. *PeerJ, 7,* e6838.
5) Kubinyi, E., Turcsán, B., Miklósi, Á., (2009). Dog and owner demographic characteristics and dog personality trait associations. *Behavioral Processes, 81,* 392-401.
6) Piotti, P., Szabó, D., Bognár, Z., Egerer, A., Hulsbosch, P., Carson, R. S., & Kubinyi, E. (2018). Effect of age on discrimination learning, reversal learning, and cognitive bias in family dogs. *Learning & Behavior, 46*, 537-553.
7) Smit, I., Szabó, D., Kubinyi, E. (2019). Age-related positivity effect on behavioural responses of dogs to human vocalisations. *Submitted.*
8) Szabó, D., Miklósi, Á., & Kubinyi, E. (2018). Owner reported sensory impairments affect behavioural signs associated with cognitive decline in dogs. *Behavioural Processes, 157,* 354-360.
9) Wallis, L., Szabó, D., Kubinyi, E. (2019). Cross-sectional age-differences in canine personality traits; influence of breed, sex, previous trauma, and dog obedience tasks. Submitted.
10) Wallis, L. J., Szabó, D., Erdélyi-Belle, B., & Kubinyi, E. (2018). Demographic change across the lifespan of pet dogs and their impact on health status. *Frontiers in Veterinary Science, 5,* 200.
11) Jónás, D., Sándor, S., Tátrai, K., Egyed, B. Kubinyi, E., (2019). The genetic background of longevity based on whole-genome sequence data of two methuselah dogs. *Submitted.*

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[16] For a summary of the results of these studies (Theses) see Chaper 18.

23 Publications related to the theses

1) Ákos, Zs., Beck, R., Nagy, M., Vicsek, T., Kubinyi, E. (2014). Leadership and path characteristics during walks are linked to dominance order and individual traits in dogs. PLoS Computational Biology, 0(1): e1003446, IF=4.62
2) Bognár, Z., Iotchev, I. B., Kubinyi, E. (2018). Sex, skull length, breed, and age predict how dogs look at faces of humans and conspecifics. Animal Cognition, 1-10. IF=2.448
3) Bognár, Z., Piotti, P., Szabó, D., Le Nézet, L., & Kubinyi, E. (2019). A novel behavioural approach to exclude auditory and visual impairment before cognitive testing of family dogs. Submitted.
4) Corrieri L, Adda M., Miklósi, Á. Kubinyi, E. (2018). Companion and free-ranging Bali dogs: Environmental links with personality traits in an endemic dog population of South East Asia. *PloS One*, *13*(6), e0197354. IF=2.776
5) Czeibert, K., Andics, A., Petneházy, Ö., Kubinyi, E. (2019). A detailed canine brain label map for neuroimaging analysis. *Biologia Futura*, *70*(2), 112-120. IF*=0.696
6) Czeibert, K., Baksa, G., Grimm, A., Nagy, S. A., Kubinyi, E., Petneházy, Ö. (2019). MRI, CT and high resolution macro-anatomical images with cryosectioning of a Beagle brain: Creating the base of a multimodal imaging atlas. *PloS one*, *14*(3), e0213458, IF*=2.776
7) Czeibert, K., Piotti, P., Petneházy, Ö., & Kubinyi, E. (2019). Sulci of the canine brain: a review of terminology. Submitted. https://doi.org/10.1101/374744
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11) Iotchev, I. B., Egerer, A., Grafe, S., Adorján, A., Kubinyi, E. (2019). Encounters between pairs of unfamiliar dogs in a dog park. *Biologia Futura*, *70*(2), 156-165. IF*=0.696
12) Iotchev, I. B., Kis, A., Bódizs, R., van Luijtelaarm G., Kubinyi, E. (2017). EEG Transients in the sigma range during non-rem sleep predict learning in dogs. Scientific Reports. *7*(1), 12936. IF=4.259
13) Jónás, D., Sándor, S., Tátrai, K., Egyed, B. & Kubinyi, E. (2019). Study of the genetic background of longevity based on whole-genome sequence data of two methuselah dogs. Submitted.
14) Kovács, T., Szinyákovics, J., Billes, V., Murányi, G., Bjelik, A., Légrádi, Á., Szabó, M., Sándor, S., Kubinyi, E., Paracky, C., Lőke, J., Gulyás, B., Mulder, J., Gulya, K., Maglóczky, Z, Vellai, T. (2019). EDTP/MTMR14 lipid phosphatases promote brain ageing by progressively downregulating autophagy during lifespan. Submitted.
15) Kubinyi, E., Sasvári-Székely, M., Miklósi, Á. 2010. „Genetics and the social behaviour of the dog" revisited: Searching for genes relating to personality in dogs. In: From genes to animal behaviour: social structures, personalities, communication by color. Eds: Inoue-Murayama, M., Kawamura, S., Weiss, A. Springer.
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20) Miklósi, Á., Turcsán, B., Kubinyi, E. (2014) The Personality of Dogs. The Social Dog: Behavior and Cognition (eds: Kaminski, J., Marshall-Pescini, S) Elsevier
21) Piotti, P., Szabó, D., Bognár, Z., Egerer, A., Hulsbosch, P., Carson, R. S., Kubinyi, E. (2018). Effect of age on discrimination learning, reversal learning, and cognitive bias in family dogs. *Learning & Behavior*, 1-17. IF*=1.481
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23) Sándor, S., Czeibert, K., & Kubinyi, E. (2019). Establishing a canine brain and tissue bank – molecular validation by RT-qPCR targeting three reference genes 5. Submitted.
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27) Szabó, D., Czeibert, K., Kettinger, A., Gácsi, M., Andics, A., Miklósi, A., & Kubinyi, E. (2019). Resting-state fMRI data of awake dogs (Canis familiaris) via group-level independent component analysis reveal multiple, spatially distributed resting-state networks. Submitted. https://doi.org/10.1101/409532
28) Szabó, D., Miklósi, Á., Kubinyi, E. (2018). Owner reported sensory impairments affect behavioural signs associated with cognitive decline in dogs. *Behavioural Processes, 157*, 354-360. IF=2.008
29) Turcsán, B., Miklósi, Á., Kubinyi, E. (2017). Owner perceived differences between mixed-breed and purebred dogs. PLOS ONE, 12:e0172720. IF=2.806
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32) Wallis, L.J. Range, F., Kubinyi, E., Chapagain, D., Serra, J., Huber, L. (2017). Utilising dog-computer interactions to provide mental stimulation in dogs especially during ageing. In *Proceedings of the Fourth International Conference on Animal-Computer Interaction*(ACI2017). ACM, New York, NY, USA, Article 1, 12 pages.
33) Wallis, L.J., Iotchev, I. B., & Kubinyi, E. (2019). Dominant dogs as perceived by their owners are more assertive, trainable and older; results from a pilot survey. Submitted.