Scientific analysis of the effectiveness of special diet on the aging of attentiveness in pet dogs

Diploma thesis

University of Veterinary Medicine Vienna

submitted by
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Table of contents

1. Introduction 1
   1.1. Aim of the study 5
   1.2. Hypothesis 5

2. Material and Methods 7
   2.1. Subjects 7
   2.2. General methods 12
       2.2.1. Treatment and testing 12
       2.2.2. Video Coding and statistical analysis 13

3. Clicker training for eye contact (selective attention) 14
   3.1. Procedure 14
       3.1.1 Variables coded 14
       3.1.2 Hypothesis for the clicker training task 15
   3.2. Statistical analysis 15
   3.3. Results 16
       3.3.1. Latency to get into eye contact 16
       3.3.2. Latency to find food 18
   3.4. Discussion 20

4. Attention task (sustained attention) 24
   4.1. Procedure 24
       4.1.1. Variables coded 24
       4.1.2. Hypothesis for the sustained attention task 25
   4.2. Statistical analysis 26
   4.3. Results 26
       4.3.1. Latency to orientate at the stimulus 26
       4.3.2. Duration of looking at the stimulus (human/toy) 27
       4.3.3. Duration of looking at the owner in the social and non-social task 29
   4.4. Discussion 31

5. General Discussion 36

6. Conclusion 40
Table of contents

7. Summary 42
  7.1. Summary (English) 42
  7.2. Summary (German) 42
8. References 44
9. List of Tables and Figures 55
10. Statutory declaration 57
1. Introduction

Our pet animals are getting older than they did 20 years ago. As the life expectancy of pet dogs and cats is increasing, age-related changes are also increasing (Youssef et al. 2016). These changes relate to behaviour as well as cognitive abilities (Rosado et al. 2012, Snigdha et al. 2012a, Tipper 1991, Vas et al. 2006, Youssef et al. 2016) and are usually not life-threatening, but can lead to an impairment of the dogs’ welfare and dog-owner-relationship (Szabó et al. 2015). Due to the greater awareness among owners, the proportion of older dogs with behavioural problems coming to veterinary clinics is actually rising, especially the ones requiring therapeutic approaches (Szabó et al. 2015).

One of the most important changes that need to be addressed is aging of attentiveness as paying attention towards conspecifics, humans or other different stimuli can lead to benefits in social learning, cooperation and communication (Mongillo et al. 2010, Range et al. 2008). In accordance, due to its essential role for goal-directed behaviours and other perceptual and cognitive functions, a decline in attention has been found to be very critical for everyday life (Staub et al. 2012, Tipper 1991).

Attention is defined, as the ability of an animal to concentrate on specific aspects of the environment while ignoring others (Mongillo et al. 2016). It is affected by the animal’s ability to distribute attentional resources, the level of expertise in the given context, the quantity and quality of surrounding stimuli, the animal’s motivation, cognitive ability to pay and maintain attention and the type of exercise (Mongillo et al. 2016). As a consequence, attention span differs between different species and individuals of the same species, which may lead to individuals differing in the amount and type of information that they can deduce from a situation (Range et al. 2008). Moreover, attentiveness also depends on the closeness of the animal to animal or animal to human-relationship, age and the availability of characteristic features of a social stimulus (Mongillo et al. 2010, Mongillo et al. 2016, Range et al. 2008, Wallis et al. 2014) and diet (Dodd et al. 2002, Larsen and Farcas 2014).

Different forms of attention are distinguished, like focused, sustained, selective, alternating and divided attention (Sarter et al. 2001, Wallis et al. 2014).
Sustained attention is the ability to catch and retain the focus on a given stimulus or task. In humans, we know that it’s a very important factor that influences general cognitive functions and goal-directed behaviours (Sarter et al. 2001, Staub et al. 2012). Similar to humans, sustained attention is important also for animals as it is essential for communication, cooperation and learning (Wallis et al. 2014).

Another form of attention - selective attention - is equally important. Animals living together in a group of many individuals have to be selective to whom and what they pay attention to, in order to benefit from the interactions and behaviours of others (Mongillo et al. 2010, Range et al. 2008). To focus attention on relevant stimuli and ignoring irrelevant stimuli, animals have to learn how to attend selectively (Wallis et al. 2014).

All mentioned forms of attention have been shown to be affected by age (Tipper 1991, Vas et al. 2006, Snigdha et al. 2012a, Wallis et al. 2014). In humans the forms of attention have been studied very well (see Wallis et al. 2014 for a review), however in veterinary medicine we still do not know a lot about the different forms of attention and how they are influenced by age (Youssef et al., 2016), except a few studies on dogs (e.g. Mongillo et al. 2010, Mongillo et al. 2016, Range et al. 2008, Youssef et al. 2016). Similar to humans (Tipper 1991), in older Beagles and Border Collies it was shown that the ability to focus attention and inhibit distracting stimuli decreases with age, as it could have been shown, by different authors like Snigdha et al. (2012a) and Wallis et al. (2014), that the mechanisms for inhibition are very susceptible for breakdowns (e.g. schizophrenia, children with attentional deficit disorders, dogs with canine cognitive dysfunction) and are affected by age (older people/dogs). Humans and dogs are showing very similar patterns in different behavioural changes. For example, Vas et al. (2006) mentioned that the changes in attention skills, impulsivity and motor activity in aging dogs (e.g. inattention, separation anxiety, abnormalities in social behaviour, enhanced aggression etc.), are very similar to the changes occurring in children suffering from the attention deficit hyperactivity disorder (ADHD).
These similarities in deficits have led researchers to claim that dogs are a very good model for the research of some aspects of human behaviour and coherent diseases (e.g. ADHD, Alzheimer´s disease (AD)) (Rosado et al. 2012, Siwak et al. 2005, Vas et al. 2006, Wallis et al. 2014, Youssef et al. 2016).

As dogs are living close together with humans, are involved in complex social interactions with humans and are sharing the same experiences and environment as humans, investigating the effects of aging on attentiveness is not only important for medical research, but also due to the effects it is having on the living together with humans and the dog-owner relationship (Szabó et al. 2015).

Different studies in animals (e.g. rats and dogs) and in humans have shown that individuals with age-related cognitive decline significantly benefit from an enriched environment and/or an enriched diet (Bamidis et al. 2014/2015, Christie et al. 2009, Head et al. 2005, Manteca 2011, Milgram et al. 2004/2005, Opii et al. 2008, Pop et al. 2010, Snigdha et al. 2011/2012b). As dietary interventions are a form of treatment that is not invasive, easy to administer (e.g. through a fortified diet or supplements), can be produced easily and used for different species (e.g. rats, dogs, humans), many studies in the last years focused on dietary interventions as treatment or prophylaxis against cognitive decline.

According to the oxidative stress hypothesis, aging is shaped by the damage of various tissues caused for instance by oxidative stress. Limited mitochondrial function and the reduction of endogenous metabolic strategies are leading to the accumulation of reactive oxygen species (ROS) which are the main trigger for oxidative stress (Araujo et al. 2005, Christie et al. 2009, Head et al. 2005, Ikeda-Douglas et al. 2004, Snigdha et al. 2016). Due to its high metabolic rate, reduced content of antioxidants, limited ability of neuronal cells for regeneration and it’s high content of polyunsaturated fatty acids (which are highly susceptible to oxidative damage), the brain is very sensitive to oxidative stress and it’s negative effects on proteins, lipids and DNA/RNA, which may result in limited cognitive functions and associated neuropathology (e.g. the accumulation of β-Amyloid) (Araujo et al. 2005, Christie et al. 2009, Cotman et al. 2002, Head et al. 2005, Ikeda-Douglas et al. 2004, Larsen and Farcas 2014, Manteca 2011, Opii et al. 2008).

Antioxidants (e.g. Vitamin C, Vitamin E, polyphenols (for instance flavonoids), beta carotene, selenium) can significantly reduce oxidative damage and neuropathology (as they are neutralizing free radicals and preventing damage to cells and cell membranes) and are improving motor, neuronal and mitochondrial function (Fragua et al. 2017, Head et al. 2005, Landsberg 2005, Milgram et al. 2002a, Opii et al. 2008). Additional antioxidants also have anti-inflammatory effects, which may be important in cognition as it’s suggested that inflammation may contribute to neurocognitive decline (Cotman et al. 2002, Ikeda-Douglas et al. 2004, Landsberg 2005, Milgram et al. 2002a).

Mitochondrial co-factors (e.g. alpha-lipoic acid and acetyl-l-carnitine) are inducing antioxidant enzymes and leading to a up-regulation of the endogenous cellular antioxidant defence. They are also effective in reducing age-related mitochondrial dysfunction (as they are enhancing mitochondrial functions) and can lead to decreased oxidative damage of neurons (as they are reducing the production of ROS) and thus improving cognitive deficits (Christie et al. 2009, Head et al. 2005, Milgram et al. 2004, Snigdha et al. 2016)

Omega-3 fatty acids (e.g. docosahexaenoic acid) are an important nutrient for brain health (Hadley et al. 2017, Heath et al. 2007). As omega-3 fatty acids (especially docosahexaenoic acid) are a main component of neuronal cell membranes and therefore play a vital important role in receptor activation, signal transduction and membrane order (Araujo et al. 2005, Hadley et al. 2017).

The use of a mixture of fruits and vegetables additionally is on the one hand useful as many of them are rich in flavonoids (which are acting as antioxidants) and on the other hand are they containing different phytochemicals which are influencing membrane fluidity and reducing the inflammatory response (Ikeda-Douglas et al. 2004, Landsberg 2005).

Next to the above mentioned substances, Fragua et al. 2017 and Manteca 2011, mention that medium-chain triglycerids in the diet may be useful as an alternative energy source for the brain, which is recommended by Larsen and Farcas 2014, as they mention that the glucose...
metabolism of the brain becomes less efficient with ongoing age, leading to an increased production of ROS.

It could have been shown that a diet enriched with the above mentioned substances, when administered to dogs, can for instance improve clinical signs of cognitive dysfunction (which are related to recognition, sleep patterns, social interactions and house soiling), positively influences agility, recognition, learning, memory, the ability to perform in specific cognitive tasks (e.g. delayed non-matching to position task, oddity discrimination learning task, size discrimination learning task, landmark discrimination task, reversal learning task ), and has a positive impact on the immune system (Larsen and Farcas 2014, Milgram et al. 2002a/2002b/2005, Snigdha et al. 2012a/2016).

Therefore we wanted to test if we can validate the above mentioned findings by conducting a double blind study on pet dogs fed with a control or a enriched diet (which were similar in composition, except that the enriched diet was fortified with antioxidants, docosahexaenoic acid, phosphatidylserine and a higher amount of tryptophan) for the period of one year and testing them afterwards in two different cognitive tasks.

1.1. Aim of the study
1. Investigate the effect of aging on attentiveness in pet dogs of different breeds using the Vienna Canine Cognitive Battery.
2. Measure if an enriched diet has an effect on retaining attentiveness in old dogs.

1.2. Hypothesis
We hypothesize that we will be able to find an effect of age on the measures of attention, using the Vienna Canine Cognitive Battery. Older dogs will show a longer latency to orientate to the stimuli, a shorter duration of looking and a higher latency for eye contact and finding food.

We also predict that there will be differences between the group fed with the enriched diet and the control food. Dogs fed with the enriched diet will show a shorter latency for orientation, longer duration of looking and shorter latency for eye contact and finding food.
The findings of this study may provide the basis to show that the tested diet is effective in retaining attentiveness or can help to slow down or decrease aging of attentiveness and that, with the conducted test, as part of the Vienna Canine Cognitive Battery, we are providing a good tool for examining the aging of attentiveness in pet dogs.
2. Material and Methods

2.1. Subjects

In this study 92 pet dogs of different breeds, including mixed breeds, of 7-42 kg were part of the experiments. The dogs ranged from six to fourteen years of age. All of the participating dogs were living together with their respective owners in one household. The dogs were recruited using the dog-database of the Clever Dog Lab at the University of Veterinary Medicine, Vienna and through distributing flyers. All dogs used in this study were recruited for the project ‘Cognitive aging in pet dogs’. The dogs went through a full health screening; including physical, neurological, orthopedic and ophthalmological tests and a blood test, to rule out that any of the dogs suffered from other diseases and severe mobility problems or sensory impairments instead of canine cognitive dysfunction.

Tab 1. The following table lists the 92 dogs participating in both attention tasks

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<th>dog</th>
<th>age (month)</th>
<th>weight (kg)</th>
<th>sex</th>
<th>neutered or intact</th>
<th>breed</th>
<th>lifelong training score</th>
<th>clicker experience</th>
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This Table (Tab.) lists the 92 dogs participating in both tasks (except dog number 63 which only participated in the attention task), including the dogs age, weight, sex (f as female and m as male), if neutered or intact, breed, lifelong training score, if it had clicker experience or not and the allotted food group (square was the fortified diet and round the control diet), NA = no data available; Mix = crossbreed

2.2 General methods
2.2.1. Treatment and testing
The dogs were divided into two groups balanced for age, sex, training level, weight and breed. Each group received a test (enriched) or control food for one whole year. The test food was in composition similar to the control food, except that it was enriched with antioxidants, Docosahexaenoicacid (DHA), Phosphatidylserine and a higher amount of tryptophan. The diets were manufactured by a private pet food company and supplied to the Clever Dog Lab, Vienna. As the exact composition is protected by a confidential clause we cannot reveal the exact composition here. The caloric density of both diets was 3864 kcal/kg. Based on the dogs age, weight and body condition score, the nutritive intake was calculated separately for each
dog. The owners were instructed to exclusively feed their dogs the given dry dog food, with no more than 10% of other treats.

As this study was a double-blind study neither the experimenter nor the owner knew which food was the control or the enriched food (the dog food company labeled the dry dog food bags with diet 1 or diet 2), until all analyses had been conducted.

One year after feeding the distributed food, testing started. All of the dogs, which were fed with one of the two diets, were tested in two tasks:

1) **Clicker training for eye contact and**
2) **Attention task**

All of the testing was done in the red testing room, at the Clever Dog Lab at the University of Veterinary Medicine, Vienna. The room was empty except for one chair for the owner in one corner of the room. Four cameras were fixed to record the testing and could be adjusted according to the need for each test. The four cameras were connected to the computer outside of the testing room.

### 2.2.2. Video Coding and statistical analysis

All of the videos were recorded by using four cameras in the testing room. After the testing was finished, the videos were coded frame by frame using Solomon Coder (Version: beta 16.05.16, by Andras Peter). The reliability coding was done by an independent coder, using 20% random videos. We were interested in five main parameters, which measured attentional capture, sustained attention and selective attention.

In this diploma thesis all statistical analysis were carried out using SPSS (Version 24.0.0.0, IBM SPSS statistics), for the basic statistical analysis and graphs, and R 3.2.2. (RStudio 2015) for further models (e.g. linear mixed effect models).
3. Clicker training for eye contact (selective attention)

3.1. Procedure
In this task, we were measuring the dogs’ selective attention towards the experimenter and towards food by means of clicker training for eye contact. The task started when the owner with the dog on leash and the experimenter entered the room. The experimenter was carrying a food pouch on it’s belt, depending on the dogs preference containing slices of sausage or cheese (about 1 cm³) and holding a clicker in her left hand. The owner unleashed the dog to move freely in the room, and sat down on a chair facing the door, pretending to read a protocol. The experimenter stood at the center of the room. After calling the dog by its name, the experimenter threw a piece of sausage/cheese on the floor. Afterwards, the experimenter waited for the dog to establish eye contact. When the dog made eye contact with the experimenter, the experimenter immediately clicked and threw a slice of sausage/cheese on the floor. The experimenter directly stopped using the clicker, when the dog seemed to be scared of it’s noise and went on with a verbal confirmation. The experimenter stood at it’s position and repeated the actions for five minutes.

3.1.1. Variables coded
In this task we used

1.) the latency to establish eye contact and
2.) the latency to find food

as measurements for selective attention.

The latency to eye contact was measured as the time between the intake of a piece of sausage/cheese (when the dog lifted it’s head) and the moment the dog established eye contact with the experimenter (marked by the sound of the clicker or the experimenters voice). On the other hand the latency to find food was measured as the period between throwing the piece of sausage/cheese (when the sausage/cheese left the experimenters hand) until it was eaten by the dog (the moment before the dog lifted its head).
The test was run for five minutes, each dog had to complete as many trials as possible. Each trial consisted of establishing eye contact with the experimenter, throwing the piece of sausage/cheese, finding and eating the piece of sausage/cheese and establishing eye contact again.

### 3.1.2. Hypothesis for the clicker training task

In this task we hypothesized that older dogs are going to show a higher latency for establishing eye contact with the experimenter and also for finding the piece of food (sausage/cheese).

Additional we also predicted that dogs which were fed with the enriched diet are going to outperform the dogs fed with the control diet. Shown in a shorter latency for eye contact and finding food.

### 3.2. Statistical analysis

The minimum requirement to include a dog in the analyses were three correct trials within five minutes, which was reached by every participating dog. We used the average of the first three trials of the latency to eye contact and the latency to find food as our main measurement. Additional we also calculated the average of the last three trials for the latency to eye contact and the latency to find food to test for learning across trials. For the latter calculation, we only included data of dogs, which at least completed six trials and used a maximum of 20 completed trials, equal to the analysis by Chapagain et al. (2017a). If a dog completed more than 20 trials within five minutes, we used trial 18, 19 and 20 for the calculation. Two dogs had been excluded from this analysis, since they did not perform more than three (dog number 14) or four (dog number 11) trials.

We calculated a difference score between the data of the average of the first three trials and the average of the last three trial, to examine if there has been any task specific learning across trials.

20 randomly chosen dogs were double coded by a second coder to check for inter observer reliability by calculating the intra-class correlation coefficient for each variable. The
Reliability was excellent for the latency to establishing eye contact (ICC = 0.96) and good for the latency to find food (ICC = 0.72).

We analyzed the available data using linear fixed effect models, in R 3.2.2. (RStudio 2015). With age, sex, weight, food, lifelong training score and clicker experience as fixed effects, to check for any significant effect on selective attention parameters. Additional, we included the two-way interactions age with food and food with lifelong training score in our statistics. To meet the assumption of homogeneity of variance and normality, we inverse square root transformed the latency to eye contact and inverse transformed the latency to find food.

Additional we used the Spearman’s Rank Correlation test for the calculation of the correlation between latency to eye contact and latency to find food and the Wilcoxon Signed Ranks Test for the analysis of learning across trials.

3.3. Results

3.3.1. Latency to get into eye contact

For the first three trials the average of the latency to eye contact was 8.52 sec (range: 1.57-38.13 sec, SD: 7.80 sec). We found a significant effect on the latency to eye contact for age, weight and clicker experience (see Tab. 2 for statistical results, Figure (Fig.) 1-3 for graphic representation), suggesting that older dogs need more time to establish eye contact with the experimenter than younger dogs, heavier dogs longer than lighter dogs and dogs that were clicker trained were faster than inexperienced ones.

We found no effect of food, lifelong training score, sex, nor effects of the two-way interactions age with food and food with lifelong training score on the latency to eye contact.
Fig. 1-2. These scatter plots show the correlation between latency to eye contact and age (blue dots, left), and between latency to eye contact and weight (green dots, right), in a task for selective attention. A significant effect of age (Spearman: $r = 0.244$, $p = 0.02$) and weight (Pearson: $r = 0.334$, $p < 0.001$) on the latency to eye contact could have been detected.

Fig. 3. This boxplot illustrates that clicker experience positively influences the latency to eye contact in a selective attention task, as it leads to a decreased latency for establishing eye contact. The difference between the two groups was significant ($p < 0.001$).
3.3.2. Latency to find food

The average latency to find food in the first three trials was 2.11 sec (range: 0.90-23.30 sec, SD: 2.84 sec). Similar to the findings for latency to eye contact, we also found significant effects for age, weight and clicker experience (see Tab. 2 for statistical results, Fig. 4-6 for graphs). Older and heavier dogs needed longer for finding food than younger and smaller dogs. Dogs with clicker experience performed better in finding the food than dogs with no experience.

Fig. 4-5. These scatter plots demonstrate the effect of age (blue dots, left) and weight (green dots, right) on the latency to find food, after removing one outlier (for better illustration) in each case (dog number 60 for age and weight). A significant effect for age (Spearman: $r = 0.368$, $p < 0.001$) and weight (Pearson: $r = 0.322$, $p = 0.002$) was present on the latency to find food.

Fig. 6. This boxplots shows the effect of clicker experience on the latency to find food. The difference between the two groups was significant ($p < 0.001$). For the better illustration, three extreme outliers (dog number 60, 14 and 11, from the no experience group (blue)) had been removed from this Figure.
Tab. 2. Significant results of the linear mixed effect models in the clicker training task

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This Table shows the significant results (p <0.001) of the linear mixed effect models performed for latency to eye contact and latency to find food, with age, weight and clicker experience as fixed effects.

Using the Spearman’s rho we could also show that the latency to eye contact is positively correlated with the latency of finding food (Spearman’s rho = 0.589, p <0.001).

Finally, with the Wilcoxon Signed Ranks Test we could on the one hand demonstrate that the dog’s performance in establishing eye contact significantly improved during the task (Z = -4.928, p < 0.001), but on the other hand they did not improve their ability in finding food during the task (Z = -0.334, p = 0.739). Therefore we only could demonstrate learning across trials for establishing eye contact, but not for finding food, similar to the findings by Chapagain et al. (2017a) and Wallis et al. (2014).
3.4. Discussion

In the task for selective attention (clicker training task) we analyzed the effect of age, sex, weight, food, lifelong training score and clicker training experience on selective attention in elderly dogs (dogs elder than six years).

As mentioned above, aged dogs significantly exhibited a higher latency for eye contact and finding food than younger dogs, similar to other studies (e.g. Mongillo et al. 2010, Salvin et al. 2011, Snigdha et al. 2012). These findings may be caused by health problems (e.g. deteriorations of visual, audio or olfactory organs or movement disorders), motivation or alterations in cognitive processing of sensory information, as older dogs are more likely to suffer from such issues (e.g. shown by Salvin et al. 2011 for finding food, Wallis et al. 2014). Although all dogs underwent clinical examinations before attending our test, we cannot totally eliminate the fact that some impairments may not have been detected during the clinical examinations (e.g. limited ability to clinically investigate the olfactory sense).

Landsberg and Araujo (2005) revealed that aged dogs show changes in social attitudes, like a decrease in social interactions and a reduced responsiveness towards human commands suggesting a decline in motivation to interact with people. In line with this, it is often reported by owners that the motivation for working or playing decreases with ongoing age, therefore maybe older dogs may not be so motivated to search for food or establishing eye contact. As all dogs found the food and also established eye contact with the experimenter, we remain of the conviction that motivation was not the main factor, which led to these differences, but it may have influenced the outcome. Similar to Salvin et al. (2011b), we think that the poorer performance of elderly dogs might reflect age dependent alterations in the processing of sensory information. Or it may mirror age dependent deficiencies in attentional control or an increased distractibility of elderly dogs, which has been argued by Wallis et al. (2014).

Referring to the publications of Chapagain et al. (2017a) and Wallis et al. (2014), we expected that age is going to have significant effects on the dog’s performance in the clicker training task. Through our findings we could confirm this hypothesis. In contrast to the study by Chapagain et al. (2017a), we found a positive effect of age for the latency to eye contact as well as for the latency to find food (similar to the findings by Wallis et al. (2014)), whereas
they only observed a significant effect on the latency to find food. This difference might be due to an ongoing training experience of each dog as suggested by Milgram (2003). While Chapagain et al. (2017a) based their analyses on data collected before the food intervention, we analyzed the data of the repetition of the same task after the feeding period (one year later). Hence all dogs were already familiar with the proceeding of the clicker training task and had similar experiences through the test beforehand, we suppose that this circumstance might have led to a clearer age related effect (compared to the study by Chapagain et al. (2017a)), as the factor of different experiences in a similar situation had been removed.

On the other hand we have to keep in mind, similar to findings described by Nippak et al. (2006), that the age effects may be partly an effect of the previous training experience which, due to not being able to counterbalance the procedure, is an unavoidable confound. Mongillo et al. (2013) suggested that increasing age can lead to impairments in processing speed of novel information but not for already learned information.

The comparison of the study of Chapagain et al. (2017a) and our investigations is also limited to the fact that Chapagain et al. (2017a) were using three different age groups (late adulthood: 6-<8 years, senior: 8-<10 years and geriatric: ≥10 years), whereas we were only using two age groups (senior: <10 years, geriatric: ≥10 years), as our sample size was smaller (92 dogs participating in our study versus 185 dogs participating in the study of Chapagain et al. (2017a)).

Additionally, in previous studies it was shown, that the ability for inhibiting distracting stimuli is decreased in aged dogs, using selective attention tasks (Mongillo et al. 2010), size and reversal tasks (Tapp et al. 2003) or visual search tasks (Snigdha et al. 2012). As the owner was within the room, during testing, we cannot exclude that this was influencing the performance of some dogs as well. Therefore it would be interesting to conduct further studies, investigating the effect of the attendance of the owner on the performance of the dog, in this specific task.

Although we did not find any effect of lifelong training on selective attention, we found an effect of clicker training experience on selective attention, similar to the findings of Wallis et al. (2014).
The positive effect of the clicker experience may be a result of the clicker training procedure. Commonly the clicker is used to reward positive performances, in the beginning often along with giving treats after the click (as a positive reinforcement). Most types of dog training (Mongillo et al. 2016), and therefore also clicker training, commonly involve establishing focus (e.g. looking) at the handler. Accordingly, dogs that underwent clicker training are used to establish eye contact with the handler (experimenter) and are also used to getting rewarded after the click, e.g. with treats that are thrown somewhere (Marshall-Pescini et al. 2008). This may lead to the better performance of dogs with clicker training experience compared to dogs with no experience, e.g. shown by Wallis et al. (2014).

Additional also the sound of the clicker may have had an influence on the performance of the dog. Similar to findings proposed by Svartberg (2002), we noticed that some dogs, notable most of the time dogs with no clicker experience, seemed to be scared of the clicker. But we suppose that this fact did not significantly influence our results as we directly stopped using the clicker, when we saw that a dog was scared, and went on with a verbal confirmation.

In human studies (e.g. Bamidis et al. 2015, Shatil 2013) it could have been shown that training does not affect all domains of cognition in the same way. Shatil (2013) describes that most of the time cognitive functions, which are relying on the same processes as the training task itself, are enhanced. Therefore we may conclude that selective attention is not positively influenced by all types of training and thus, while the lifelong training score was not affecting the performance, clicker training improved the dogs selective attention abilities, as we detected a significant effect on the dogs performance.

Additional it is also possible that some owners tended (consciously or not) to train more with their dogs, to generate better results in the follow-up or maybe tried to train their dogs specifically for some tasks, e.g. clicker training. As participating owners, (participation was optional), tend to have a heightened interest in improving the aging process of their companion animal, and therefore may be considered to more likely change the conditions the aging dog is living in (e.g. behavioural, cognitive, physical or environmental enrichment). This may also be influencing the outcome, as recommended by some authors (e.g. see for behavioural enrichment: Milgram et al. 2004/2005, Opie et al. 2008, Pop et al. 2010, Snigdha et al. 2011/2012b; cognitive enrichment: Milgram 2003, Milgram et al. 2006; environmental enrichment: Kramer et al. 2004, Petrosini et al. 2009; physical enrichment: Kramer et al.

Interestingly we also found a significant effect of weight for the latency to eye contact and also for the latency to find food. Although these findings, as far as we know, were not described before, a possible explanation may be that smaller dogs are more agile, so they were faster in finding the food and returning to the experimenter.
For further arguments see the general discussion below.
4. Attention task (sustained attention)

4.1. Procedure
In this task we measured attentional capture and sustained attention of dogs towards two
different stimuli (a toy train and a moving human). The owner entered the room with the dog
on leash, tied the dog on the red leash attached to a holder on the floor, sat down on the chair
and pretended to read a test protocol. Two conditions were presented in front of the dog in a
counterbalanced order: moving toy train and human moving in a circle.
In the toy train condition, after the owner and the dog were in position and the dog looked
away from the toy, the owner pressed the start button of the toy’s remote control, which made
the toy train move in circles. After two minutes, which the experimenter signaled from
outside (using the word “okay”), the owner pressed the stop button of the remote control,
stopped the toy train and walked outside with the dog.
In the human condition, after the owner and the dog were in position and the dog looked
away from the door, the experimenter entered the testing room, closed the door, walked to a blue
circle on the floor marked at a distance of 3m from the dog and started to move in circles.
After two minutes, the experimenter went outside and then signaled the owner to leave the
room with the dog by saying “okay”.

4.1.1. Variables coded
In this task we used
1.) the latency to orientate to the stimuli (toy/human),
as measurements for attentional capture, and
2.) the duration of looking at the stimuli (toy/human) and
3.) the duration of looking at the owner in the toy and the human condition
as measurements for sustained attention.

In this task we used latency to orientate at the door (human condition) and latency to orientate
at the toy as measures for attentional capture. The coding for these two variables started when
the first movement of the toy or the noise of the door (as the movement of the door was not so
clear to see in the videos) was visible/hearable in the videos, and it ended when the dog (head and nose) was orientated at the toy/door.

As parameters for sustained attention, we used the duration of looking at the toy (dogs head and nose orientated at the toy train) and the duration of looking at the human (dogs head and nose are orientated at the experimenter), within two minutes of testing.

Additional we also examined the duration of looking at the owner in both conditions (toy and human), when the dogs´ head and nose were orientated at the owner. As some authors (e.g. Marshall-Pescini et al. 2017, Miklosi et al. 2003, Udell 2015, Topál et al. 1997) cite that dogs have a greater sensitivity to social inhibition when they are tested in a condition were a human is present (Udell 2015, Topál et al. 1997). And to the fact that the same authors revealed that dogs, participating in a problem solving task or a manipulation task, tended to look back at the owner faster (Marshall-Pescini et al. 2017, Miklosi et al. 2003) and for a longer duration (Marshall-Pescini et al. 2017, Udell 2015) compared to their relative the wolf, if a task got unsolvable for them. We on the one hand wanted to know if we might observe the same effect also in our test for sustained attention and on the other hand, we wanted to know if this is going to have an influence on the outcome of our results, as assumed by authors like Horn et al. (2013b). As this circumstance has to be kept in mind when using the here mentioned sustained attention task, as part of the Vienna Canine Cognitive Battery, for the detection of aging effects on dogs´ cognitive abilities.

**4.1.2. Hypothesis for the sustained attention task**

We hypothesized that older dogs are going to show a longer latency to orientate to the stimuli and a shorter duration of looking at the stimuli, in this task.

Additional we also predicted that there are going to be differences between the group fed with the enriched diet and the control food. Dogs fed with the enriched diet are going to show a shorter latency for orientation and a longer duration of looking at the two stimuli compared to dogs fed with the control diet.
4.2. Statistical analysis

In this task we excluded all dogs from the statistical analysis for attentional capture, which were already orientated at the toy/door when testing started. Therefore we excluded one dog in the non-social condition (toy), of the attending 91 dogs.

To exclude coder specific faults we checked for inter observer reliability, choosing randomly 20 dogs, which were coded by a second coder. Afterwards we calculated the inter observer reliability, using intra-class correlation coefficients for each variable. The reliability was excellent for the latency to look at the toy (ICC = 0.99) and very good for the latency to look at the door (ICC = 0.86). We also had an excellent reliability for the duration of looking at the toy (ICC = 0.98), for the duration of looking at the human (ICC = 0.98), for the duration of looking at the owner in the toy condition (ICC = 0.95) and for the duration of looking at the owner in the human condition (ICC = 0.93).

Running further analysis, we used linear mixed effect models, using age, weight, sex, lifelong training score, food (square vs round), test condition (socials vs. non-social) and testorder (toy or human condition in the beginning) as fixed effects in the analysis of the duration of looking at the stimuli and duration of looking at the owner (in the social and non-social condition), to check for any effect on sustained attention.

We also included the two-way interactions of age with food, food with lifelong training score and lifelong training score with stimulus, in our statistical analysis.

Using the Spearmann’s Rank Correlation Test, we calculated the correlation between duration of looking at the human and duration of looking at the toy. To check for differences of the dogs’ attentional performance between the two conditions.

4.3. Results

4.3.1. Latency to orientate at the stimulus

In the sustained attention tasks dogs needed on average 1.49 sec (range: 0.10-9.70 sec, SD: 1.73) to orientate to the door (in the social condition) and 2.05 sec (range: 0.10-18.70 sec, SD: 3.02) for orienting to the toy (in the non-social condition). We excluded dog number 13
from the analysis of the data for latency to orientate at the toy, as the dogs’ latency to orientate at the toy (18.70 sec) was more than three times higher, than the standard deviation (3.02 sec) and therefore the dogs’ data reached the criterion for exclusion of outliers. After removing this outlier, the average latency to orientate at the toy was 1.86 sec (range: 0.10-9.90 sec, SD: 2.45). The further analysis for latency to orientate at the toy were run using the data after removing the outlier.

We could not detect a significant difference between the latency to orientate at the door and the latency to orientate at the toy, using the Wilcoxon Signed Ranks Test (Z = -0.381, p = 0.703).

Using the Spearman Rank Correlation Test we found a significant correlation between the latency to orientate to the door and the latency to orientate to the toy (Spearman’s rho = 0.214, p = 0.044), suggesting that dogs which were faster in orientating to the social stimulus were also faster in orientating to the non-social stimulus and therefore we may conclude that the latency to orientate is not only depending on the sort of stimuli but also influenced by the dog itself.

4.3.2. Duration of looking at the stimulus (human/toy)

On average the duration of looking at the social stimulus (experimenter) was 41.08 sec (range: 3.20-60.00 sec, SD: 15.28) and 31.55 sec (range: 1.90-59.60 sec, SD: 17.57) for the non-social stimulus (toy). Therefore the duration of looking at the experimenter was significantly higher than the duration of looking at the toy (for statistical results see Tab. 3, for graphical illustration Fig. 7). Moreover weight had a significant effect on how long the animals watched the stimuli (see Tab. 3 for statistical results and Fig. 8 for graphical illustration). Especially in the non-social condition, heavier dogs tended to look longer at the stimulus as light-weighted dogs.

We did not find any significant effect for age, lifelong training score and food (Fig. 9-11) on the duration of looking at the stimulus (social vs non-social).

Using Spearmans Correlation Test, we found a significant correlation between the duration of looking at the experimenter and the duration of looking at the toy (Spearman’s rho = 0.267, p = 0.11).
Tab. 3. Results of the linear mixed effect models for the duration of looking at the stimulus

<table>
<thead>
<tr>
<th></th>
<th>estimate</th>
<th>standard error</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>food</td>
<td>1.295</td>
<td>3.445</td>
<td>0.376</td>
<td>0.708</td>
</tr>
<tr>
<td>lifelong training score</td>
<td>0.150</td>
<td>0.199</td>
<td>0.754</td>
<td>0.453</td>
</tr>
<tr>
<td>age</td>
<td>-0.077</td>
<td>0.072</td>
<td>-1.068</td>
<td>0.289</td>
</tr>
<tr>
<td>stimulus</td>
<td>-11.999</td>
<td>2.507</td>
<td>-4.787</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>weight</td>
<td>0.500</td>
<td>0.212</td>
<td>2.363</td>
<td>0.020</td>
</tr>
</tbody>
</table>

The Table shows the results of the linear mixed effect models for the duration of looking at the stimulus (toy/experimenter) during the first minute of testing using age, food, lifelong training score, stimulus and weight as fixed effects. Significant results (p < 0.05) are highlighted.

Fig. 7-8. The boxplot on the left side, shows the significant difference between the duration of looking at the social (human) and the non-social (toy) condition (Spearman: r = 0.267, p = 0.011). The scatter plot on the right side, illustrates the effect of weight on the duration of looking, in the social (Spearman: r = 0.106, p = 0.317; blue) and the non-social (Spearman: r = 0.272, p = 0.009; green) condition.
Fig. 9-10. These scatterplots show the not significant effects of age (p = 0.289, left) and lifelong training score (p = 0.453, right) on the duration of looking at the stimuli (blue = human, green = toy).

Fig. 11. In the boxplot above, the not significant effect (p = 0.708) of food on the duration of looking at the stimulus (blue = human, green = toy) is shown.

4.3.3. Duration of looking at the owner in the social and non-social task
Additional to the data for the duration of looking at the stimuli, we also analyzed how long the dogs looked at the owners instead of looking at the stimuli, to see if the presence of the owner during testing might have had an effect on our results.
For the social condition the average duration of looking at the owner was 3.61 sec (range: 0.00-45.10 sec, SD: 7.41) and 8.63 sec (range: 0.00-45.90 sec, SD: 10.92) for the non-social condition.

Further analysis was conducted using linear mixed effect models with age, weight, sex, food, lifelong training score, stimulus and testorder as fixed effects. We also included the two-way interactions age with food, food with lifelong training score and training with stimulus in our analysis.

The duration of looking at the owner was significantly higher in the non-social condition compared to the social condition (for statistical results see Tab. 4, for graphical illustration Fig. 12).

Furthermore the duration of looking at the owner was also significantly affected by the testorder with being higher if the human condition was tested first (see Tab. 4 and Fig. 13).

The other parameters used in the linear mixed effects models did not reach significance, e.g. shown in Tab. 4 for food and lifelong training score (for graphical illustration see Fig. 14-15).

Tab. 4. Results of the linear mixed effect models for the duration of looking at the owner

<table>
<thead>
<tr>
<th></th>
<th>estimate</th>
<th>standard error</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>food</td>
<td>0.099</td>
<td>0.096</td>
<td>1.031</td>
<td>0.305</td>
</tr>
<tr>
<td>lifelong training score</td>
<td>0.003</td>
<td>0.006</td>
<td>0.495</td>
<td>0.622</td>
</tr>
<tr>
<td>stimulus</td>
<td>0.375</td>
<td>0.077</td>
<td>4.847</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>testorder</td>
<td>-0.205</td>
<td>0.097</td>
<td>-2.110</td>
<td>0.038</td>
</tr>
</tbody>
</table>

This Table shows the results of the linear mixed effect models for the duration of looking at the owner in the first minute of testing, using food, lifelong training score, stimulus and testorder as fixed effects. Significant results (p < 0.05) are highlighted.
4.4. Discussion

In the task for sustained attention we analyzed the effect of age, weight, sex, food, lifelong training score, stimulus and testorder on the sustained attention of 91 dogs, aged over six years. And if the two-way interactions age with food, food with lifelong training score and lifelong training score with stimulus have any effects on the measurements for sustained attention.
attention (duration of looking at the stimulus and duration of looking at the owner in both conditions).

As mentioned above, dogs needed longer for orienting to the toy compared to orienting towards the door (human), which may be explained by the relevance of a given stimulus for the dogs and not the novelty of a stimulus. As dogs likely do know that when a door is opening something is going to happen (e.g. the owner is coming home) and therefore they may be more attentive to the noise of an opening door, as to the novel noise of the toy train. We also recognized during coding of the videos, that the noise of the door appeared to be louder than the noise of the train, although the train was closer to the dog. Therefore it could be possible, that hearing problems of some dogs, may have led to these findings. However, this is unlikely since every dog underwent a clinical examination before performing in our tests, conforming that there were no impairments of the dogs’ sense of hearing. As we found a positive correlation between the latency to orientate towards the door and the latency to orientate towards the toy (dogs fast in orientating at the door also tended to be faster in orientating at the toy), we suggest that there was not a difference in the loudness of the stimuli, but rather hearing impairments (which we tried to eliminate with the above mentioned clinical examination) or a decline in attention, which seems to be the more likely, that led to the higher latency to orientate at the non-social stimulus.

As a measurement for sustained attention we used the duration of looking at the stimulus (human or toy). We could demonstrate a difference between the duration of looking at the experimenter (human) and the duration of looking at the toy (train) (shown in Fig. 7). We suppose that owing to lifelong learning, as the participating dogs were quite old, dogs tend to be more attentive towards humans (which seem to be a more relevant stimulus) compared to the toy. Which is also suggested by different authors as Herberlein et al. (2016/2017), Marshall-Pescini et al. (2009/2017) and Wallis et al. (2014).

We should also keep in mind that a moving person may be more attractive to pay attention to for dogs than a toy. As dogs know that most of the time, when a person is moving something is going to happen (e.g. going for a walk, the dog is getting food), they might be more sensitive to the movement of humans compared to moving objects. Although we have to
assume that it is not very likely that such a difference was only induced by the movement of
the experimenter itself, as the toy train was also moving around, but it may have strengthened
the outcome.

Also the fact that the dogs were already familiar with the experimenter (as the dogs
participated in some other tasks conducted by the same experimenter), may have led to the
longer duration of looking at the human compared to the duration of looking at the toy. Hence
it would be interesting to compare our data with data where instead of the already familiar
experimenter, an unknown person is introduced to the dog (then the toy as well as the
experimenter would have been new to the dog). Wallis et al. (2014) also emphasize that
performing a sustained attention test with a human with whom the dogs already had positive
experiences may motivate the dogs to pay more attention towards the experimenter compared
to the toy, which is also mentioned by Horn et al. (2013a) and Mongillo et al. (2010).

It was also remarkable that within the duration of looking at the toy the variance was higher
as in the duration of looking at the human. It is possible that on the one hand the toy train was
a new and therefore attractive utensil to pay attention to for some dogs (demonstrated in a
longer duration of looking), but on the other hand the unfamiliar toy was not very interesting
for other dogs and therefore these dogs were looking at the owner or somewhere else instead
of looking at the toy (shown as minor duration of looking). Similar findings have been
reported by Moretti and colleagues (2015), as in their study 50 % of dogs were interested in a
novel object and interacted with it, whereas the other 50 % did not.

Although we hypothesized to find an effect of age at the duration of looking at the stimulus,
as it was shown by Chapagain et al. (2017a) (similar findings were also made in human
literature, e.g. Callaghan et al. 2017, Commodari and Guarnera 2008), we could not detect a
significant effect of age. We suppose that this is due to the fact that the dogs already have
been familiar with the task, as they performed a similar task one year earlier (for further
information see Chapagain et al. 2017a), and therefore overall paid less attention to the toy
compared to the human, independent of age. As it is known that dogs can remember specific
situations for a long period of time, this explanation seems to be suitable.
Additional we also have to bear in mind that our sample only consisted of elder dogs that probably overall had similar experiences due to their age, and therefore we suggest that this circumstance might also have led to no significant findings for the lifelong training score. We also could not confirm our hypothesis that a fortified diet significantly affects the performance of elderly dogs in the sustained attention task. For detailed discussion see general discussion, further below.

Next to the significant effect of the stimulus on the duration of looking at the stimulus, interestingly we also could examine a significant effect of weight, similar to our findings in the clicker training task. Which is going to be discussed in the general discussion below.

Additional to the already executed studies by Chapagain et al. (2017a) and Wallis et al. (2014) using the same test battery (the Vienna Canine Cognitive Battery), we examined the effect of age, weight, sex, food, lifelong training score, stimulus and testorder, not only for the duration of looking at the stimulus (human vs toy), but also for the duration of looking at the owner in both conditions, to see if there were any impacts on this variable.

We found a significant effect for the type of stimulus (human vs toy) on the duration of looking at the owner. The duration for looking at the owner was significantly higher in the toy condition compared to the human condition, this is in accordance to the findings of the duration of looking at the stimulus part (see above) and findings by Rehn et al. (2017). If dogs were mainly orientated at the stimulus, they could not spend so much time looking at the owner. Which confirms our expectations and findings by Marshall-Pescini et al. (2017) and Udell (2015), which have shown that animals (e.g. dogs) which are less persistent tended to look back at the owner sooner and for a longer duration.

As mentioned above it also seems that a moving human is more interesting than the owner itself and therefore the duration of looking at the owner in the human condition was really low. The higher duration of looking at the owner in the toy task can be described, as a consequence of the lower average of looking at the toy. Additional it could also represent the dogs searching for help, as some authors (e.g. Marshall-Pescini et al. 2008/2009/2017, Miklósi et al. 2000/2003, Udell 2015) have mentioned, that if dogs were confused or
overstrained with a given situation they tend to look at their owner, in order to „ask“ for help. A lack of motivation/interest (Mongillo et al. 2015/2016) or the fear of the unfamiliar toy train could also have led to the higher duration of looking at the owner. The performance of the dogs may also have been influenced by the dog-owner relationship, as argued by different authors (e.g. Rehn and Keeling 2016, Rehn et al. 2017, Topál et al. 1997). Topál et al. (1997) for instance showed that dogs which were considered as family members performed worse compared to working dogs, in a novel and a problem-solving task. The authors declare that this may be a result of a more socially dependent behaviour shown by dogs with a close dog-owner relationship.

Additional to the stimulus also the testorder had a significant effect on the duration of looking at the owner. As the testorder only had an effect in the duration of looking at the owner, but not on looking at the stimulus, we do not have an explanation for this finding right now and therefore recommend to ascertain if these findings are common or just a discovery by accident.
5. General Discussion

Although we hypothesized that there might be an effect of the fortified diet on the cognitive abilities of dogs, which has been suggested by various authors (e.g. Chapagain et al. 2017b, Cory 2013, Davis and Head 2014, Fast et al. 2013, Fragua et al. 2017, Hadley et al. 2017, Ikeda-Douglas et al. 2004, Larsen and Farcas 2014, Snigdha et al. 2016), we could not find any effect of food at all.

This might be due to the circumstance that not all domains of cognition are equally affected by a dietary intervention. Thus it is possible that attention is not or at least not as easy affected by a dietary treatment as for instance memory and learning, which have been shown to be affected by various authors like Cotman et al. (2002), Fragua et al. (2017) and Siwak et al. (2005). Similar findings have been provided by Head et al. (2005), as they examined a significant effect of a enriched diet on the dogs performance in a landmark discrimination task after a feeding period of two weeks, while there was a feeding period of two years necessary to gain any significant difference between the same two feeding groups in a test for spatial memory.

Milgram et al. (2002a) for instance mentions that the usefulness of an animal model relating to the effect of interventions on age-dependent cognition is depending on the ability of the model to reflect age-related cognitive dysfunction. As we could not detect any influence of age at the performance of the pet dogs in the sustained attention task, we have to keep in mind the possibility that our selected tasks are not suitable for establishing any food effects on age-dependent cognition in pet dogs. Although we hypothesize that the circumstance that we did not find any age effect, in the task for sustained attention, was due to the familiarity of the dogs with the task.

Which leads us to the consideration of the possibility, that the task we used was too easy for the dogs to provide any effect of food, as mentioned by authors like Cotman et al. (2002), Milgram et al. (2002a) and Snigdha et al. (2016). In a study of Cotman et al. (2002) they demonstrated that the effect of a fortified food was only apparent when the complexity of the oddity discrimination task was increased. Therefore we hypothesize that both task used in this study were too easy to create any significant effect, of an enriched diet on the cognitive abilities of pet dogs.
We also have to be aware that the short period of time feeding the fortified diet (one year), which has been described as problematic by some authors like Christie et al. (2009) and Milgram (2003/et al. 2005), might also have led to no significant findings in our study. Milgram (2003) for example only found a significant effect for a fortified diet, combined with behavioural enrichment, after a feeding period of two years. In the same study, he also describes a mild effect for the combination of a fortified food and behavioural enrichment after one year of examination, but these effects did not reach the criterion for significance. Hence we suppose that the feeding period of one year might be too short to generate an effect on selective and sustained attention and that a long-term feeding might have been more successful.

Additional we also have to keep in mind that the participating dogs were all older than six years. As it is known, depending on different literature, that the onset of the aging of some cognitive domains varies between the age of six years (e.g. Studzinski et al. 2006, Wallis et al. 2014), across seven (Golini et al. 2009) and eight years (Siwak et al. 2005), until 10-12 years of age (Salvin et al. 2011a), therefore it is recommended by some authors (e.g. Fissler et al. 2017, Fragua et al. 2017, Siwak et al. 2005) that it might be better to start feeding the fortified diet at an earlier stage of life (when there are not already age related changes present), as seen in humans (Cory 2013). Thus we recommend to also include younger dogs in studies investigating the effect of a diet on the cognitive abilities of dogs.

Last but not least we also have to be aware of the fact, that the participating dogs in this study were pet dogs, therefore all of them were raised in different environments and had different experiences when the study was conducted. Most of the literature, about the effects of a dietary intervention on cognitive decline, which are cited in this diploma thesis, have been run using dogs (often beagle dogs) that have been raised under the same conditions in a laboratory setting. These dogs had all similar experiences when testing was started. Which might be problematic, when comparing our study to this literature, as authors like Snigdha et al. (2016) state that the physiological state of an individual might be affecting the outcome of such studies. Additional many studies (Milgram et al. 2004/2005, Opii et al. 2008, Pop et al. 2010, Siwak et al. 2005, Snigdha et al. 2011/2012b/2016) have revealed that a combination of a fortified diet with cognitive, behavioral or environmental enrichment can lead to a strengthened outcome of the effects on cognitive abilities in dogs. Thus it is possible that, as
we did not check for the dogs ongoing experiences, that being raised and living under different conditions might have been more influencing than being fed with the fortified food. Hence it would be very interesting to compare laboratory raised dogs with pet dogs fed with the same enriched and control diets, to see if there might be some differences. 

It also would be interesting to examine the effect of food comparing the performance of the dogs before and after the diet. Maybe we would then be able to reveal an improvement or at least a consistent performance of dogs fed with the fortified diet compared to the dogs fed with the control diet.

As mentioned above we could detect an effect of weight in the task for selective attention as well as in the sustained attention task. On the one hand, in the clicker training task (selective attention) heavier dogs needed longer for establishing eye contact with the experimenter and to find food and, on the other hand, in the sustained attention task heavier dogs tended to look for a longer duration of time, especially in the toy condition, at the stimulus.

In human literature, e.g. Condello et al. (2017) mention that the BMI (body mass index) should be included as a covariate in studies where the effect of physical activity on cognitive abilities is going to be assessed, as there is evidence that the habitual physical activity is influenced by human weight status. And in turn, the habitual physical activity is thought to influence the cognitive performance of humans across lifespan and aging. Therefore we recommend that in further studies, researcher should be aware that the weight of their participating dogs, might have an impact at the outcome of a study. To our knowledge there are no other findings in literature about the effect of weight on cognitive abilities in dogs.

We suppose that our findings could also reflect an effect of the size of different breeds, since we used the absolute weight and not relative weight (depending on body size). Gácsi et al. (2009) mention that the performance in a cognitive test does not only depend on the dogs cognitive performance, but is also influenced by other factors such as temperament. Merkman and Wynne (2014) state that smaller breeds are more temperamental and therefore have a shorter span of attentiveness or rather tend to get distracted more easily as taller and calmer breeds. Additionally, smaller breeds also have been suspected to be more human-orientated, which could explain the shorter duration to establish eye contact in the clicker training tasks.

Although Chapagain et al. (2017a) could not find an effect of breed in their study, we cannot totally exclude that we may have examined a special breed effect (especially in the sustained attention task), as Border Collies were overrepresented compared to other breeds (see Tab. 1 for further information) participating in our study. Border Collies are included in the group of „working dogs“, these group is attributed to be more attentive/focused and less distractible as other breed groups (Rehn and Keeling 2016), as they have been bred with special regard to these characteristics during working. Gácsi et al. (2009) showed that within the group of working dogs, cooperatively working dogs (e.g. Border Collies) performed better in a task using a human pointing gesture compared to dog breeds selected for independent working. They suppose that the better performance of cooperatively working dogs was not affected by differences in cognitive abilities, but is an effect of selection, as these breeds have been selected to be more responsive to social stimuli, in the way of keeping continuous visual contact to their handler. Gácsi et al. (2009) also hypothesized that cooperatively working dogs were more disposed to observe human behaviours and therefore their performance was less affected by other conditions.

Another fact that also should be kept in mind when investigating the effect of weight or age on cognitive abilities in elderly dogs, is the biological age of dogs. As it is known that taller breeds (and therefore also heavier once) tend to age faster than smaller breeds (e.g. Egenvall et al. 2005, Greer et al. 2007, Salvin et al. 2012). As in our study most of the participating dogs were of mixed breed, unfortunately it was not possible to check for the biological age and its impact on our results.
6. Conclusion
In this diploma thesis we investigated the impacts of aging on attentiveness in pet dogs and we could prove that different kinds of attentiveness are differently influenced by the dog’s age.

We can say that the dogs’ performance in our conducted attention tasks was probably on the one hand depending on the level of distractibility (selective attention task), which seems to be higher with ongoing age and may be a result of deficits in attentional control, and on the other hand depending on the relevance of the given stimuli (sustained attention task), due to the dogs’ different lifelong experiences with the given stimulus.

For the investigation of the effect of a fortified diet on the dogs’ cognitive abilities, we recommend to start with the feeding of the diet, before the onset of the cognitive aging and to extend the feeding period, at least to a period of two years. Additional we also suggest to use tests with different degrees of difficulty, as there is the possibility that some tests are too easy or too difficult to gain any food effect (e.g. Cotman et al. 2002, Milgram et al. 2002a, Snigdha et al. 2016). When using pet dogs in such a study also the fact of different experiences should be tried to include in the executed models. At least we recommend to compare the dogs performance before and after the treatment with the dietary intervention, to see if there have been, at least at the individuals level, any improvements on the cognitive abilities of dogs. The comparison of laboratory dogs and pet dogs fed with the same diet might also reveal some differences between these two groups of dogs.

We also recommend that the dogs’ weight should receive more attention as a determinant in further studies, as we could detect a significant effect in both attention tasks. And, as to our acknowledgment, there is no current literature concerning the influence of weight on cognitive functions in dogs existing.

Additional the fact that our results in the selective attention task were only influenced by the clicker experiences instead of the lifelong training scores, strengthens the hypothesis that the effect of training on the dog’s cognitive abilities is depending on the kind of training experienced.
Furthermore we are aware that the fact that we could not find any effect of the lifelong training score, in comparison with the common literature, might be due to our small sample size and the fact that all our dogs were all of a similar age. Therefore we recommend to use a larger sample size and also the comparison of different age groups (juvenile, adult, senior) to investigate the influence of the training score on cognitive aging.

All in all we could confirm that pet dogs are a suitable model in the field of cognitive aging research. We are convinced that cognitive studies using pet dogs are the perfect link between studies conducted on laboratory animals and human studies. As pet dogs share the same environment, similar experiences, are exposed to the same harmful environmental conditions, are having similar nutritional needs (Araujo et al. 2005) and absorbing nutrients in a similar way to humans (Opif et al. 2008) and are developing similar pathologies during aging as humans do.

But on the other hand they have a moderate lifespan, are available in a huge number, easy to handle, highly motivated and therefore easy to test (Chapagain et al. 2017a/b, Cory 2013, Davis and Head 2014, Milgram 2003, Opif et al. 2008, Szabó et al. 2015, Wallis et al. 2014/2016, Youssef et al. 2016), which enables us to investigate the effects of different interventions on cognitive aging. And onwards these findings may also lead to the establishment of methods that may help to maintain or improve cognitive abilities during aging in dogs as well as in humans.

We believe that with the two above described tasks, as part of the Vienna Canine Cognitive Battery, we are providing a excellent and easy tool, that can be used to collect data about the cognitive abilities of dogs and furthermore can be used, additional to the already existing questionnaires, as a tool for testing dogs, if they are suffering from the canine cognitive dysfunction syndrome (CCDS).
7. Summary

7.1. Summary (English)
In this diploma thesis we investigated the effect of age and a fortified diet on the attentiveness of 92 pet dogs, of different breeds, which were six to fourteen years old. All of the testing was conducted at the Clever Dog Lab, at the University for Veterinary Medicine, Vienna. The participating dogs were divided into two groups balanced for age, sex, training level, weight and breed. Each dog was fed with a test or a control diet for the period of one year and underwent a clinical examination, before testing started. As this study was a double blind study, neither the owner, nor the experimenter were aware which food was the fortified diet and which was the control diet.
All participating dogs were tested in two tasks, one test for selective attention (clicker training task) and the other one for sustained attention (attention task), which are part of the Vienna Canine Cognitive Battery.
Regarding the effect of age on the attentiveness of pet dogs, we suppose that the dogs’ performance in our conducted attention tasks was probably on the one hand depending on the level of distractibility (selective attention task), which seems to be higher with ongoing age and may be a result of deficits in attentional control, and on the other hand depending on the relevance of the given stimuli (sustained attention task), due to the dogs’ different lifelong experiences with the given stimulus.
We assume that we could not detect an effect of the fortified diet may be due to the fact, that our sample size might have been to small and the feeding period of one year to short, to generate a significant effect. Therefore we recommend to extend the feeding period to at least two years.

7.2. Summary (German)
Grundlage dieser Diplomarbeit, war die Fragestellung welche Auswirkungen das Alter des Hundes und ein mit bestimmten Inhaltsstoffen angereichertes Futter auf die kognitiven Fähigkeiten von Hunden hat. Dazu wurden 92 Hunde, unterschiedlicher Rasse, die zwischen sechs und vierzehn Jahre alt waren und als Haustiere gehalten wurden, im Clever Dog Lab an der Veterinärmedizinischen Universität in Wien untersucht.
Die Hunde wurden nach Alter, Geschlecht, Gewicht, Rasse und Trainingslevel gleichsam in zwei Gruppen aufgeteilt. Die eine Gruppe wurde mit einem Spezialfutter (reich an Inhaltsstoffen von denen vermutet wird, dass sie einen positiven Effekt auf die kognitiven Fähigkeiten haben) und die andere Gruppe mit einem herkömmlichen Futter, für den Zeitraum von einem Jahr gefüttert. Da dies eine Doppelblindstudie war, wussten weder wir noch die Besitzer, an welche Hunde das Spezialfutter verfüttert wurde.

Alle Hunde wurden in zwei unterschiedlichen Test, die Teil der Vienna Canine Cognitive Battery sind, bezüglich ihrer selektiven Aufmerksamkeit (clicker training task) und anhaltenden Aufmerksamkeit (attention task) getestet.


Wir vermuten, der Umstand, dass wir in beiden Tests keinen signifikanten Unterschied zwischen den beiden getesteten Hundefuttern finden konnten, ist der Tatsache geschuldet, dass wir eine relative kleine Stichprobe von Hunden getestet haben und das Futter nur über den Zeitraum eines Jahres verfüttert wurde, der, laut führender Literatur, vermutlich zu kurz ist um eine deutlichen Effekt zu erzielen.
8. References


9. List of Tables and Figures

9.1. List of Tables
Tab. 1. List of the 92 participating dogs
Tab. 2. Significant results of the linear mixed effect models in the clicker training task
Tab. 3. Results of the linear mixed effect models for the duration of looking at the stimulus in the attention task
Tab. 4. Results of the linear mixed effect models for the duration of looking at the owner in the attention task

9.2. List of Figures
Fig. 1. Scatter plots about the correlations between latency to eye contact and age in the clicker training task
Fig. 2. Scatter plot showing the correlation between latency to eye contact and weight in a task for selective attention
Fig. 3. Boxplot illustrating that clicker experience positively influences the latency to eye contact in a selective attention task
Fig. 4. Scatter plots demonstrating the effect of age on the latency to find food, after removing one outlier in the clicker training task
Fig. 5. Scatter plot about the effect of the dogs weight on the latency to find food, after removing one outlier in the task for selective attention
Fig. 6. Boxplots showing the effect of clicker experience on the latency to find food, after removing three outliers in the selective attention task
Fig. 7. Boxplot demonstrating the significant difference between the duration of looking at the social and the non-social condition in the task for sustained attention.
Fig. 8. Scatter plot illustrating the effect of weight on the duration of looking in the social and the non-social condition of the attention task
Fig. 9. Scatterplot showing the not significant effects of age on the duration of looking at the stimuli in the task for sustained attention
Fig. 10. Scatterplot illustrates the not significant effect of lifelong training score on the duration of looking at the stimuli in the attention task
Fig. 11. Boxplot demonstrating the not significant effect of food on the duration of looking at the stimulus in the attention task.

Fig. 12. Boxplot showing the significant effect of the stimulus on the duration of looking at the owner, in the human condition and the toy condition of the sustained attention task.

Fig. 13. Boxplot illustrating the significant effect of the test order on the duration of looking at the owner in the attention task.

Fig. 14. Boxplot showing the not significant effect of the different food groups on the duration of looking at the owner in the task for sustained attention.

Fig. 15. Scatterplot representing the not significant effect of the lifelong training score on the duration of looking at the owner in the sustained attention task.
10. Statutory declaration

I declare that this thesis was entirely written by myself, the work is exclusively my own except where explicitly stated otherwise in the text and that this work has not been submitted, in whole or in part, in any other application for a degree.