Associations between owner-reported fear and aggression in dogs and experimentally determined flexibility, socio-emotional information processing and cognitive abilities

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Summary:

Fear-related behavior, including aggression in dogs is a worldwide problem. Dog biting incidences lead to physical and emotional damage but also to hidden costs for our society. Possibly, such unwanted behavior in dogs is related to the functioning of specific brain centers, which can be deduced indirectly by behavior, namely flexibility in reversed reward tasks, socio-emotional information processing in following exposure to social cues and general cognitive ability demonstrated during task solving. Experiments in humans that were evaluated also for brain functioning using PET scans underlie these hypotheses. Knowledge on the mechanisms underlying problem behavior in dogs could aid strategies to predict, prevent and remedy fear-related behavior and aggression. In this study, 58 dogs were used for testing if a relationship exists between fearfulness / aggressiveness and the traits flexibility (switching ability in a reversed reward task), sensitivity to social cues (measured by reactions towards different types of vocalization) and problem solving ability. Relative scores for fear, aggression and trainability were determined by use of a dog personality questionnaire called C-BARQ that dog owners filled in before the start of the tests. Aggression in dogs is known to be in part caused by fear and also in this study the owner reports revealed a (near significant in) relationship between fear and aggression. The dogs' performances in behavior tests will be influenced by many factors and here we tested for the influence of two, namely trainability and gender.

The dogs' task switching ability or flexibility was tested by use of a T-maze, in which dogs had to select one of two arms and received a food reward from their owner if they chose correctly. This test consisted of three different phases: the training phase, when for 6 trials dogs were forced to leave the T-maze by alternating arms and always received a reward the preference reinforcement phase, when a given arm was rewarding on 8 consecutive trials, and the reversed learning phase, when the opposing arm was rewarding for 8 consecutive trials. For all dogs, the number of errors made in the reversed learning phase, as well as the side preference was noted. By use of Chi-square analysis, it was determined if the variance between the factors fear, aggression, trainability and gender could explain the variance in the performance score for the T-maze. A binominal test was used to test if the normal walking side of dogs, i.e. when being walked outdoors by their owners, could explain the variance in the preference side of dogs in the T-maze. The results showed that many dogs were relatively inflexible, as 45% of the dogs did not change side in the reversed learning phase. High levels in the relative scores for fear and aggression did not explain variation in behavioral flexibility; neither did the gender of the dog or the relative score for trainability. The walking/ working side of the dogs significantly explained the variance in the preference side of the T-maze, with dogs being walked on the right side having a preference for the right arm in the T-maze. For future flexibility testing in dogs, it is advised to account for such influences. By making use of a certain learning criterion, instead of predetermined numbers of trials, all animals will have the same number of rewards which might reduce the influence of rewarding the dog too often.

The dogs' reaction towards different social cues was measured by exposing the dogs to two different bark types: a 'play' bark and a 'territorial' bark. The barks were played during 17 seconds, for every dog in a random order, with a 60 seconds interval between the barks. A principal component analysis (PCA) on behavior score resulted in three principal components, here labeled as 'submissiveness', 'vocal responsiveness' and 'vigilance'. To test which behaviors discriminated the dogs' reactions to

the ‘play’ bark and the ‘territorial’ bark, all component scores and remaining behaviors (i.e. which did not fit into one of the 3 components) were tested for bark type effects by use of a General Linear Model (GLM) analysis. The results show that aggressive and fearful labeled dogs do not have difficulty in discriminating ‘play’ barks from ‘territorial’ barks. Fearful dogs stood out by their strong submissive behavior in response to a territorial bark, with normal responses to a play bark. When considering their anxious nature and the fact that they did not show this in response to play barks indicates good social skills in discriminating between threats and friendly signals. No significant interaction effects of the relative aggression scores and bark type were found, in that way it cannot be stated that aggressive dogs were less sensitive to social cues than control dogs with low scores for aggression / fear. Fearless dogs showed higher vocal response during the expose of the ‘territorial’ bark than during the pose of the ‘play’ bark. Fearful dogs showed a higher vocal response during the expose of the ‘play’ bark. To obtain more insight in differences in vocal reaction towards the different bark types, it might be considered to determine the function of the vocalization. By means of the analyzing the amplitude and frequency of the vocalization, functional differences in reactive vocalization between the ‘play’ bark and ‘territorial’ bark might be determined.

Problem solving ability was tested by presenting the dogs a commercially available puzzle (‘dog brick’) with two movable lids, under which a reward could be hidden, and a wooden cone to prevent these puzzle pieces from moving. By moving the puzzle pieces with nose or paws the dogs could obtain food rewards. When the cone was placed in the puzzle this had to be removed first, by use of the mouth, to allow the lids to shift and access the food rewards. The test consisted of three different levels; each level consisted of three trials, each lasting for 30 seconds. When the dog obtained the reward, the dog earned points according to the complexity level of the test. Correlations between scores for fear, aggression or trainability, and the dogs’ cognition scores were tested. As stress may interfere with good learning and memory but also be a causal link between fear/ aggression and such effects are checked by testing if variation in stress behavior explains variation in cognition scores. The dogs’ performances in the cognition test were not associated with relative scores for fear, aggression, trainability or gender. However, stress behavior did explain variation in the cognition score. The lower the cognition score of the dog, the more stress the dog showed during the cognition test. This linear relationship was especially evident during the more complex stages in the test. This shows that the present test results were strongly influenced by levels of stress, which may have interfered with the assessment of a dogs potential to solve problems (e.g. under more optimal conditions). For future studies it is advised to set-up a test in such a way that stress has a minimal influence on the results so that the latter are more specific for cognitive ability. Ideally, the task is designed in a manner that the dog can perform the task without guidance of an owner or an experimenter, to exclude influence of having other people in the room.

This study could not demonstrate that a relationship exists between relative scores of fearfulness / aggressiveness and task switching ability and problem solving ability. By measuring the dogs’ reaction towards social cues (here different types of vocalization) insensitivity to social cues in aggressive dogs was not found, but increased sensitivity in fearful dogs. Fearful dogs discriminated between play barks and territorial barks, in that especially the latter triggered social insecurity. Fearful dogs may be relatively sensitive to (threatening) social cues and more so than aggressive dogs. Since there were no significant interaction effects of aggression scores x bark type (play, territorial) on behavioral response it cannot be stated that aggressive dogs were less sensitive to social cues than control dogs with low scores for aggression / fear. This study cannot demonstrate that unwanted behavior in dogs is related to the malfunctioning of specific brain centers, which have been demonstrated in human.

Possibly, this reflects that the study dogs did not suffer from pathological fear and / or aggression and were relatively normal. It cannot be excluded that in cases of severe (pathological) fear and / or aggression this is accompanied by impaired flexibility, sensitivity to social cues and cognitive abilities, but within a range of ‘normal’ scores such links seem to be lacking. More research on this topic is needed, as a higher number of pathological fearful and/ or aggressive dogs in combination with improvement in some of the behavior tests might lead to different and more valid outcomes.

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Introduction:

Aggression in our society:

They are often called: man's best friend and dogs number about 1.9 million in the Netherlands (Frissen et al., 2008). Despite the dogs' reputation of man's best friend, every year 150,000 people in the Netherlands are bitten by a dog, of which 50,000 people have to be treated by a doctor (Frissen et al., 2008). Annually, on average 1.2 people die as a result of a dog bite, most of them being young children (Frissen et al., 2008 p. 7; Brogan, 1995). Besides the physical and emotional damage, dog biting incidences also lead to hidden costs for the society. In the US, every year more than $1 billion is paid by the insurance industry to home owners' liability claims. Hospital expenses related to dog bites in the US are about $102.4 million per year (Beaver et al., 2001).

Biology of aggression:

Aggression in dogs is a normal innate behavioral response to a pain causing stimulus, an adaptive learned behavior to access resources, or a strategy to minimize damage in a physical confrontation (O'Heare, 2004, p. 7-12; Heath, 2002). Aggression is commonly defined as: "a behavior that causes (or leads to) harm, damage or destruction of another organism" (Moyer, 1986). This definition is not comprehensive; it does not include related affective responses like unfriendliness and threat. Furthermore, the definition is based on social psychology and gives no information about underlying neuronal mechanisms (Weinshenker and Siegel, 2002). Literature review of 49 articles on dog personality or dog traits showed that aggression is indexed by behaviors such as: biting, growling, and snapping at people or other dogs (Jones and Gosling, 2005). Aggression is the most common reported problem behavior of dogs. i.e. by owners who visit an animal behavior center or veterinary clinic. Seventy-five of 1644 studied dogs of the Animal Behaviour Clinic at Cornell University between 1991 and 2001 were diagnosed with aggression; most of these cases were prone to human directed aggression (Bamberger and Houpt, 2006).

From a biological point of view, fear is agitation or panic in the anticipation or presence of danger (Abrantes, 1997). Fear is, just like aggression, a normal innate behavior, vital to the survival of the individual. Fear is the motivational factor which elicits defense or flight (Abrantes, 1997). Fear is thought to have a high correlation with aggression. A study by Vage et al. (2008) on 52 aggressive English Cocker Spaniels and 65 control English Cocker Spaniels showed, firstly, that fear was common when dogs were handled and, secondly, that the dogs which were classified as aggressive showed a higher level of fear in such situations (Vage et al., 2008). A study done on dog bites in children in the Czech Republic showed that the resulting aggressive behavior was mostly of the possessive, territorial and fear type (Nahlik et al., 2010). Evaluation of the sociable acceptance behavior test to detect human directed aggression showed a relationship between aggressive behaviors and fear, indicating that fear played a role when the dog attacked in one of the subtests. This does not mean that there are no other triggers for showing aggressive behavior, but the present findings suggest that aggressive behavior in the absence of fear remains undetected in the SAB test (Van den Borg et al., 2010).

Canine aggression is a multi factorial issue involving genetics, environment and learning (Heath, 2002). There are various trigger stimuli of aggression and, assumingly, different motivational states that underlie aggressive behavior. Trigger stimuli can for example be: the context of a dog's territory, presence of other dogs, people, children, or the presence of food or toys, sometimes in combination with each other. The internal motivation for aggression can, for example, be related to competition, sexual, status (interdog aggression), territorial defense, predatory behavior, pain or fear, idiopathic causes and learned responses (Beaver, 1982; Blackshaw, 1991; Heath, 2002). As discussed earlier, probably fear is a high motivational factor for aggressive behavior. A common used approach for the classification of aggression is the classification based on the function, namely, affective and non-affective aggression. Affective aggression involves high emotional content and stimulates sympathetic autonomic arousal. All forms of aggression, except predatory aggression are included in this aggression class. Non-affective aggression involves the use of segments of the predatory sequence and only consists of predatory aggression (O'Heare, 2004).

Aggression is the result of a complex interaction of the limbic system and other brain areas, neurotransmitters and the endocrine system. Parts of the limbic system that play an important role in aggression are the hypothalamus and amygdala. Other brain areas important for aggressive responding are the medial preoptic area, lateral septum, periaqueductal gray and bed nucleus of the stria terminalis (Nelson and Trainer, 2007). Neurotransmitters like dopamine, adrenaline, norepinephrine and serotonin, glutamate and GABA establish signaling between nerve cells in case of (canine) aggression. The working of the endocrine system is intertwined with that of the nervous system, manifesting for example as the production of cortisol in periods of stress (Lindsey, 2000). As aggressive responses involve numerous brain structures and neurotransmitters, not all direct causal brain mechanisms are identified yet (Nelson and Trainer, 2007).

Coccaro and his colleagues reviewed literature for findings in relations to human and animal brain lesion, with a focus on three neural systems involved in impulsive/ reactive aggression: subcortical neural systems that support the production of aggressive impulses, decisions-making circuits and social-emotional information processing circuits that evaluate the consequences of aggressing or not aggressing, and the frontoparietal regions that are involved in regulation emotions and impulsive motivational cues urges (Coccaro et al., 2011). The study focused on human psychiatric disorders but was founded with animal studies as well. Three main characteristics, based on the neural system in relation to aggression, underlying decision making, the detection and assessing of social cues and the regulation of emotions could be deducted (Coccaro et al., 2011). Figure 1 shows the top down regulation from lateral prefrontal regions and dorsal and ventral medial prefrontal cortex, which serves to modulate key nodes in the response sequence. Also, the roles of decision making and socio-emotional information processing, experience of aggressive impulses and the emotional regulation in relation to various brain regions are shown.

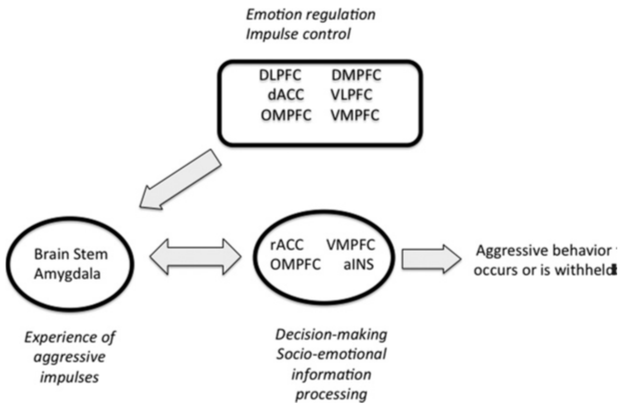


Figure 1: Sequence model of an unfolding aggressive response starting with the experience of an aggressive impulse and terminating in a behavioral response. Top-down regulation from lateral prefrontal regions and dorsal and ventral medial prefrontal cortex serves to modulate key nodes in the response sequence. aINS, anterior insular cortex; dACC, dorsal anterior cingulate cortex; DLPFC, dorsolateral prefrontal cortex; DMPFC, dorsomedial prefrontal cortex; OMPFC, orbital medial prefrontal cortex; rACC, rostral anterior cingulate cortex; VLPFC, ventrolateral prefrontal cortex; VMPFC, ventromedial prefrontal cortex (copied from Coccaro et al., 2011).

Role decision making processes in aggressive behavior:

Life involves decision making, as an outcome of related processes that allow selecting actions that promote accomplishment of one's goals or overall interest (Rangel et al., 2008). Persons with impaired decision making processes were found at increased risk of engaging in aggression. These people may have more difficulties in recognition of the possible consequences of aggressive behavior and anticipating its social consequences (Coccaro et al., 2011). The orbito medial prefrontal cortex (OMPFC) has repeatedly been associated with aggression. This prefrontal region is also associated with certain characteristic decision-making abnormalities, suggesting a link between the two (Coccaro et al., 2011). One hypothesis links OMPFC with reversal learning: persons with OMPFC damage may have difficulty altering representations of value associated with earlier conditioned stimuli (Rolls et al., 1994, Fellows and Farah, 2003). In reversal learning, a person or animal is presented with two stimuli simultaneously, with one being associated with a reward and the other not. After the person or animal has achieved a certain learning criterion, or after it has experienced a predetermined number of trials, the reinforcement value of the two stimuli is reversed. Often, several errors in the first reversal trials are made, which are known as negative transfer, but performances improve over successive reversals (Boogert et al., 2010). Reversal learning is often used in psychology and neuroscience to quantify behavioral flexibility (E.G. Fellows and Farah, 2003; Izquierdo et al., 2007; Haluk and Floresco, 2009). Behavioral flexibility is for many animal species an important adaptive response to a changing environment (Boogert et al., 2010). To determine behavioral flexibility by use of reversal learning different approaches can be used, for example a T-maze. The T-maze is often used in mice, as researchers have tried to establish the mouse as a useful animal model for assessing behavioral inflexibility found in human patients with frontal lobe damage, neurophysiological damage and aging-associated cognitive decline (Endo et al., 2011).

In 1985, Oortmerssen et al. revealed that aggressive wild mouse individuals functioned better under stable conditions, whereas non-aggressive individuals performed better under changing circumstances. In 1987, Benus et al. showed that a possible factor underlying this mechanism is the routine-like behavior the aggressive individuals showed. Both aggressive male rats and mice performed better in a maze task when only minor changes (to the maze) were made, whereas the non aggressive males performed better when the conformations of the maze were altered. During the repeated runs, aggressive males seemed to build up a routine and consequently their performance was stable. Non-aggressive males omitted the formation of routines, their performance was more variable and directed at details of the environment. In a further study, done in 1990, Benus et al. suggested that male mice of a line selected for short attack latency (SAL) are more routine-like in their behavior than individuals of a line selected for long attack latency (LAL). In this test, a Y-maze was used, with one of the arms leading to a food containing compartment. It was suggested that the SAL mice during the training period probably developed a strongly fixed locomotion pattern, which is difficult to oppress when a change is introduced (Benus et al., 1990). Something similar was found in a study by Bolhuis et al. that focused on individual coping characteristics, rearing conditions and behavioral flexibility in pigs (Bolhuis et al., 2004). In this study, the pigs were trained to obtain a food reward on one side of a T-maze until nine consecutive correct trails (which meant the pig obtained the food reward within 15 seconds) were obtained. In the reversal phase, first five normal training trials were performed with the reward on the same site as in the training phase. Subsequently, during 6 consecutive reversal trials the food reward was placed on the opposite arm of the T-maze. Pigs that did not reach the food within 300 s where gently directed to the food, so every trial the pig was eventually rewarded with the food. Starting from the third reversal trial, pigs (around 3% of the pigs, depending on the housing environment and resistance level) faithlessly started to enter the new rewarded arm. During the last trial, only 40% of the high resistant pigs, which compare to SAL individuals, were able to enter the new rewarded arm without errors contrary to 68% of the low resistant pigs, which compare to LAL individuals. In this task, the great majority of pigs with incorrect reversal performance did not randomly walk around in the maze, but repeatedly made the same perseverative set of errors. These pigs had more difficulty in inhibiting their previously reinforced response, suggesting that the types of pigs may have used different mechanism to solve the learning task. In this case 'high resisting' (SAL type) pigs may have developed more routine-like patterns that are relatively resistant to change, just as the more aggressive mice and rats studied by Benus et al. (1990).

T-maze tests are not used frequently in dogs, but were applied to detect cognitive deficits in Dachshunds. In this study, the preference site of 13 Dachshunds was detected by giving food rewards on both sides of the maze. After this phase, the preference site was reinforced by rewarding the dogs only on this side until the dogs reached a certain criterion. This criterion was 8/10 choices on two consecutive days or 9/10 correct choices on a single day. After passing this phase, the dogs progressed immediately to the reversed learning phases, which consisted of three reversals. For the first reversal, food rewards were switched to the dogs' non preferred side, and the dogs underwent daily testing of 10 trials per day until they reached criterion. In the second and third reversed learning phases, food rewards were placed on the dogs' preferred and non-preferred sides respectively. As for the first reversal, the dogs underwent daily testing of 10 trials per day until they reached criterion for each of the second and third RL phases. The number of errors the dogs made in the reversed learning phase was recorded and an average score over the three reversals was used for

analyses (Sanders et al., 2011). Normal 4 months old Dachshunds made on average, in the three reversal phases, 9 mistakes before reaching the criterion, normal 5 months old Dachshunds 5 mistakes, 6 month old Dachshunds 3 mistakes and 7 months old Dachshunds only 2 mistakes. The same dogs were used, the older the dog became, the more learning experience the dog had and less errors were made (Sanders et al., 2011).

In the current study it is investigated if the degree of aggression and fearfulness in dogs, as reported by their owners, is linked to the dogs' abilities to alter representations of value associated with earlier conditioned stimuli. Possibly, the propensity to act aggressively or fearful is related to the function of the OMPFC, which functioning can be demonstrated indirectly by behavioral responses in reversal learning, as demonstrated in human.

Role socio-emotional information processing in aggressive behavior:

Closely related to decision making and valuation is socio-emotional information processing. This means the detection and evaluation of social cues, like facial expression and vocal intonation. Patients with OMPFC lesions show a reduced capability to correctly identify social and emotional cues (Hornak et al., 2003; Hornak et al., 1996). In the study done by Hornak et al., performed in 1996, 11 patients with ventral frontal lobe damage and a control group of 18 people listened to a tape of emotional sounds corresponding to 7 emotions: sad, angry, frightened, disgusted, puzzled, contented and neutral. All people were asked to match sounds with emotions on a list. Nine of these 11 patients misinterpreted the emotional sounds. It was also shown that the greater the reported brain damage-related emotional change in emotional experience in the group of patients, the worse the performance in the vocal expression identification test (Hornak et al., 1996).

Domestic dogs have a rich vocal repertoire, which they use in a wide variety of social contexts (Tembrock, 1976). Research by Cohen and Fox (1976), Tembrock (1976) and Lehner (1978) concentrated on the functional contexts of the different kind of acoustic signals. Table 1 shows the comparative functional categorization of canid vocalizations based on study performed by Cohen and Fox in 1976 (copied from Pongracz et al., 2010). Pongracz et al. interpret dog barking functioning as a means of dog communication which conveys information about the dog's inner state. Related to the inner state of the dog, barking should be shown situation-specific and acoustic measures or testing the behavior of potential receivers must show consistency (Pongracz et al., 2010). Study by Pongracz et al. (2006) as well as Yin (2002) supported these claims. Acoustic parameters, like amplitude and frequency, showed consistency within (Pongracz et al., 2006) and between breeds (Yin, 2002) as a response to strangers, fight, walk, being alone, ball, and play (Pongracz et al., 2006) or disturbance, isolation and play (Yin, 2002). The behavior of potential receivers was tested on consistency by use of three groups of adult human listeners. Participants were asked in two separated experiments, to categorize the context of the barking or score the motivational state of the signaler (aggression, fear, despair, happiness and playfulness). The study showed that people categorized the barks correctly with a similar success rate above the chance level (Pongracz et al., 2005).It is surprising that few studies have examined the intraspecific role of barking, though recently, Cs Molnar et al. (unpublished) suggested that dogs can distinguish both between barks recorded in different situations and possibly between barks emitted by different individuals. These findings still need to be supported by observations by dogs in the field (Pongracz et al., 2010).

Table 1: The comparative functional categorization of canid vocalizations (based on Cohen and Fox, 1976)

	Meows	Grunts	Whines	Yelps	Screams	Whimpers	Howls	Bleats	Growls	Yaps	Barks	Clicks	Snapping the teeth	Pants
Greeting	F	WD	W C D	D	F	C	WD	F	WD	–	D	–	–	F
Play initiation	–	–	D	D	–	–	–	–	–	–	D	–	WD	FD
Submission	F	–	W C D	D	W C D	–	–	–	–	–	–	–	–	–
Defence	–	–	WCD	D	CF W	–	W	–	WCDF	WCDF	WD	F	WCD	–
Threaten.	–	–	–	–	–	–	–	–	WCDF	WCDF	WCDF	F	WCD	–
Contact seeking	nbF	DW	nb, D W C	nb-W nb-C D	F	C	–	F	–	–	D	–	–	–
Pain	nb	–	nb, D W C F	nb-W nb-C D	nb, D W C F	–	–	–	D	–	D	–	–	–
Loneliness	–	–	nb, D W C	D	–	–	W C D	F	–	–	D	–	–	–
Group	–	–	nb, D W C	–	–	C	W C D	–	W C D	–	–	–	–	–

Abbreviations: C = coyote, D = dog, F = red fox, W= grey wolf, and nb = newborn

In the current study, it is investigated if the degree of aggression and fearfulness in dogs, as reported by their owners, is linked to the dogs' capability to correctly identify social cues. Possibly, the propensity to act aggressively or fearful is related to the function of specific brain centers, like the OMPFC, which functioning can be demonstrated indirectly by behavioral responses to a social cue like vocal intonation, as suggested in human.

Role cognitive abilities in aggressive behavior:

Cognitive ability is thought to play a role in aggressive behavior, as high cognitive individuals better suppress primary urges to fear and aggression. Study by Emerson and colleagues showed that 42% of patients with intellectual disorders display aggression towards others (Emerson et al., 2001). The direct causal mechanisms of aggression in persons impaired in cognition have not been identified yet, but it is known that aggression is associated with a variety of environmental influences (Brame et al., 2001; Broidy et al., 2003; Nagin & Tremblay, 1999). For example chronic aggression during childhood is considered as a major predictor of aggressive behavior later in life (May, 2011). The relationship between cognitive ability and aggression in dogs has not been investigated so far.

Stress is thought to have a negative relationship with cognition and findings support that exposure to stress can block learning and memory (Cazakof et al., 2010; Diamond et al., 2005; Joels et al., 2006). These effects can be complex and a study by Li et al. (2012) showed the effects of acute moderate stress on various phases of memory, this within one single study. The results indicated that acute stress can disrupt the memory retrieval and interrupt the alliance of short-term memory into long-term (Li et al., 2012). Stress in dogs is typically manifested in behavioral parameters like increased restlessness, oral behaviors, yawning, open mouth and a moderate lowering of the posture (Beerda et al., 1997). In another study performed by Beerda et al. (1998), a group of Beagle dogs were socially and spatial restricted to induce chronic stress. This study considered that low postures, high frequencies of auto grooming, paw lifting and vocalizing are behavioral parameters indicating chronic stress. More popular literature also including freezing, shaking of the head and/ or body and turning the head and body away from the stimulus as behavioral indicators of stress (O'Heare, 2004; Rugaas, 2006).

In the current study it is investigated if the degree of aggression and fearfulness in dogs, as reported by their owners, is related to general cognition. Presence of stress can be a disturbing factor for testing the general cognition of the dog, but stress could also be the causal link between aggression /fear and impaired cognitive skills. Therefore, it is important to observe stress related behavior and test for relationship between cognition and possible stress level.

Main aims of the study:

Here, it is investigated if the degree of aggression and fearfulness in dogs, as reported by their owners, is linked to: the dogs' abilities to alter representations of value associated with earlier conditioned stimuli, the dogs' capability to correctly identify social cues and the dogs' general cognitive ability. Possibly, the propensity to act aggressively or fearful is related to the function of specific brain centers, which functioning can be demonstrated indirectly by behavioral responses to test-stimuli. Knowledge on this could aid strategies to predict, prevent and remedy fear-related behavior and aggression.

For testing if aggressive or fearful dogs have more difficulty in altering representations of value associated with earlier conditioned stimuli, a population of dogs will be tested by use of a T-maze, which is employed as a reversal learning test. It is expected that aggressive and fearful dogs will make more errors in the first reversal trials, compared to non aggressive/ non fearful dogs. As the trainability of the dog and the gender also might explain variation in the flexibility of the dog, these parameters are taken in account.

For testing differences in capability of correctly identification of social cues (here vocalizations) in aggressive or fearful dogs and non-aggressive/ non fearful dogs, test subjects are exposed to a 'play' bark and a 'territorial' bark. It is expected that aggressive and fearful dogs have more problems correctly reacting to the 'play' and the 'territorial' bark than 'normal' control dogs.

For testing differences in the cognition between aggressive or fearful dogs and 'normal' controls, test subjects are tested for their puzzle solving ability. The puzzle contains different levels and requires insight learning. It is expected that aggressive and fearful dogs have a lower rate of success in the dog puzzle in contrary to non aggressive/ non fearful dogs. As the trainability of the dog and the gender also might explain variation in the dogs' cognition, these parameters are again taken in account. Stress may interfere with good learning and memory but may also be a causal link between fear/ aggression and such effects are checked by testing if variation in stress behavior explains variation in cognition scores.

2. Material and Methods:

By use of a dog personality questionnaire in combination with three different behavior tests, associations between flexibility, socio-emotional information processing and cognitive abilities in dogs and owner-reported fear and aggression were experimentally determined. The next paragraphs describe the materials and methods used in this study.

2.1 Animals:

Dog-owner combinations were obtained by use of advertisements in local shops, a dog school, and newsletters, to acquire a population of dogs that is representative to the Dutch population. Owners were able to register themselves on the website: www.dierenwetenschap.com. On this website, owners were asked to fill in a simple questionnaire about the history and some personality traits of their dog. This information was used for the selection of suitable dogs for the behavior tests. Dogs with an age below 2 years were excluded as the total time of for the tests was too long and the personality of the dog is thought to be still under development. In total, 58 dog-owner combinations participated in the behavior tests. In total 39 different breeds and 9 crossbreeds were tested.

2.2 Personality questionnaire:

Next to the behavior tests, the dog owner was asked to fill in a detailed questionnaire on the personality of the dog and the personality of the owner itself. The questionnaire of the dog personality was mainly used to determine the relative fearfulness and aggressiveness of the dog. This questionnaire is based on the C-BARQ (Canine Behavioral Assessment and Research Questionnaire) developed by Hsu and Serpell (2003). The C-BARQ questionnaire contained 92 questions. The questionnaire concerned general information on the owner and the dog with questions about the background of the dog and whether or not the dog participated in obedience training for example. Other parts of the questionnaire focused on: (1) training and obedience (2) aggression (3) anxiety (4) separation anxiety (5) excitement (6) attachment and drawing attention (7) remaining. All these different parts were scored and the degree of the score gives more information of the dog personality regarding the part scored for. For detailed description see Hsu, Y. and Serpell, J.A. (2003). In this study, the relative score for fear and aggression was used to get more insight in the relationship between flexibility, cognition and socio-emotional information processing between fearful and aggressive dogs, and dogs with relative low scores for fear and aggression. Also, the relative score for trainability was taken in account and the gender of the dogs, as these parameters also might explain variation in the dogs' behavior regarding flexibility, cognition and socio-emotional information processing.

2.3 Behavior tests:

In total, the dog and its owner could participate in 6 different tests of which two of them where performed outdoor and 4 of them indoor. All tests used for this study were performed indoor. The tests were planned from 14-11-2011 until 17-12-2011. The order of the tests was kept the same, but the tests were not dependent from each other. The behavior tests were designed such that they could be performed in different order, on different locations including training fields. The total duration of all test never exceeded two hours, which included pause and play time between tests to facilitate relaxation between tasks and prevented carry-over effects. All tests were performed in the

presence of the owner. Owners had a say in which behavior tests they wanted to participate in. The tests were not expected to cause significant stress in the dogs. The flexibility test and the cognition test involved task performance for food rewards and these were expected to be pleasurable to the dogs. The socio-information processing test could evoke reactions to the barking sound which could cause a degree of surprise/ startle in some sensitive dogs leading to some short lasting stress. This test was always performed last, so stress could not influence the other tests.

2.3.1 Flexibility test:

Test apparatus:

To determine if fearful or aggressive labeled dogs have more difficulty altering representations of value associated with earlier conditioned stimuli; 58 dogs were tested by use of a T-maze (Fig. 2). The T-maze consisted of a start box, where the dog was placed before every trial and two arms, one to the left and one to the right, and a screen in the length of the arms where behind the owner was positioned. A door in the middle of the screen helped the owner to walk in a straight line to the startbox and back, preventing the walking route to become the walking route of the dog. The owner was positioned right in front of the start box, with his face directed towards the wall in front of him. The position of the owner was marked on the floor with a black cross. The instructor opened the startbox manually after 5 seconds after the owner stood on his place. When the door of the startbox was opened, the owner was allowed to call the dog. During the trials, the owner was not allowed to give the dogs directions with neither his face, arms or body direction.

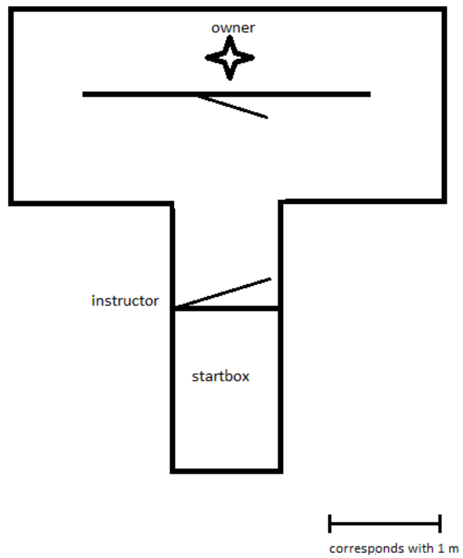


Figure 2: Position T-maze. For each trial, the dog was placed in the start box. The owner was positioned behind a screen with his face directed towards the wall. The dog had to choose left or right, to arrive to the owner where it obtained a reward.

Testing protocol:

The test was based on reversal learning and consisted of three different phases: the training phase, the preference reinforcement phase and the reversed learning phase. In the training phase consisted of 6 trials whereby the dog learned to go to the owner and receive a reward. The reward consisted of 1/3 of a frolic and vocal praises by the owner. A screen blocked one of the two arms (either the left or right arm) and each trial the side of the blockade was reversed. In this way, the dog learned that it could use both sides to go to the owner. The second phase was the preference reinforcement phase which consisted of 8 trials. The first side the dog chose became the preference side and every time the dog chose this side in this phase, it was rewarded by use of a reward (1/3 of a frolic) and vocal praises by the owner. When the dog chose the other the side, the dog was not rewarded and it was brought back to the startbox without any praise. After four trials, there was a small break of 1 minute, whereby the owner was asked to stay on his position but he was allowed to give the dog any kind of attention the owner whished. The last phase consisted of the reversed learning phase and also contained 8 trials. In this phase the rewarded side was reversed to the non preferred side. Again, when the dog chose the right side, which was in this case the reversed side, the dog was rewarded with 1/3 of a frolic and vocal praise by the owner. When the dog chose his preference side, the dog was not rewarded and was brought back to the start box without any praise of the owner. After four trials, there was a small break of 1 minute, whereby the owner was asked to stay on his position but he was allowed to interact with the dog. For the preference reinforcement phase and the reversal phase, the chosen side was noted.

Statistical analysis:

To determine the flexibility of the dog, the number of errors the dog made in the reversed learning phase was noted. This score was coupled to the relative score of fear and aggression, the relative score of trainability and the gender of the dog, obtained by use of the C-BARQ questionnaire. The relative scores for fear, aggression and trainability were continue, and varied between 0 and 100. The 'gender' of the dog consisted of 4 groups: female and male dogs which could be intact or neutered. By use of Chi-square analysis in SPSS, it was tested if the variance between the factors fear, aggression, trainability and gender, which were the independent variables, could explain the variance in the performance score for the T-maze, which was the dependent variable. In a Chi-square analysis, the variables: fear, aggression, trainability score, performance score and gender were assembled in 2 discrete groups. The groups considered were mutually exclusive and had a total probability of 1 (Ott and Longnecker, 2001). Chi-square analyses counted data per combination of 2 discrete factors and tested which combinations deviated from expectations. Asymptotic significances with p-values <0.05 were considered to be significant.

Furthermore, the influence of the independent variable: the walking side of the dog was coupled to the preference side of the dog. A binominal test was used to test if the walking side of the dog could explain the variance in the preference side of the dog in the T-maze. Binomial probability values of <0.05 were considered to be significant.

2.3.2 Socio-emotional information processing test:

Testing devices:

To determine differences in capability of correctly identification of vocalization, in aggressive and fearful dogs and non-aggressive/ non fearful dogs, 58 dogs were exposed to a 'play' bark and a

'territorial' bark. The bark recordings which were used for this test can be found on the cd: 'Barking behavior of dogs (in this test the Dutch version was used named: 'blafgedrag van honden') which was included in the similar named book by Turid Rugaas (ISBN 978-90-807584-5-2).

Testing protocol:

During the test the dog was fixed on a safety line which was secured to a hook in the wall. The owner was asked to bring the dog towards the middle marked black square on the floor, where the dog was put in a sit position (Fig. 3). Preferably, the dog faced the wall in front of him, but some dogs just moved with the direction of the owner. The owner was asked to step back from the dog (approximately 1 m) towards the chair where the owner had to sit down. The owner had to keep looking at the wall in front of him without paying attention to the dog and thereby having their arms neutral next to the body. Behind a screen a 'play' bark was played for 17 seconds. The behavior of the dog was recorded by use of video. The owner was not allowed to correct the behavior the dog showed. After these 17 seconds, the owner was asked to do nothing at all, for an extra 13 seconds, to record the recovery behavior of the dog. After these 13 seconds, there was a period of 60 seconds where the owner could walk towards the dog and comforted him if necessary. In this period, the dog was allowed to walk around and do whatever the owner or the dog wanted to do. After this 60 seconds break, the owner was asked to rehearse the same procedure. Instead of the 'play' bark, the second time the 'territorial' bark was played off. During all tests, the 'play' and 'territorial' barks were played in a random order. During the test, the reaction of the dog was verified by use of video recording.

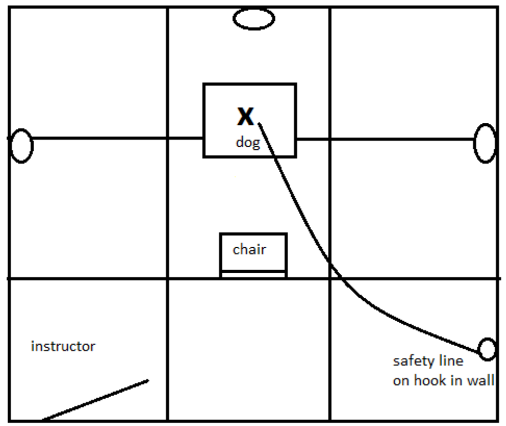


Figure 3: Position of owner, dog and instructor during the socio-emotional information processing test. The behavior of the dog was recorded by use of three video recorders which were placed in the middle and the sides of the room. During the test the dog was fixed with a safety line on a hook in the wall.

Behavioral observations of the recordings by means of focal sampling continuous recording were computer aided using the Observer® 10.0 software (Noldus Information Technology, 6709 PA Wageningen, The Netherlands). The ethogram included the behavioral classes: general posture, tail wagging, tail posture, panting, and remaining events (Table 2). Posture, tail wagging, tail posture and panting ('states') were expressed as a percentage of the observation time. The other class with remaining behaviours ('events') was expressed as frequency of occurrence.

Table 2: Ethogram for the behaviors scored in the socio-information processing test

Behavioural Class	**Behaviour**	**Description**	**Properties**
General posture	High	Head and body in upright position	State
	Neutral	Head and body natural, according to the breed standard	State
	Low	Head and body lower than the breed standard	State
	Out of sight	Dog is out of sight	State
Tail wagging	Wagging off	No tail wagging and/or tail between legs	State
	Normal tail wag	Normal tail wagging	State
	Stiff tail wag	Wagging tail with a stiff tail	State
	Tail not visible	The tail is not visible	State
Tail posture	High	Upright position of the tail	State
	Neutral	Natural position of the tail, according to breed standards	State
	Low	Tail between the legs	State
	Tail not visible	The tail is not visible	State
Ear posture	High	The ears are directed towards the front, in and/or in upward position	State
	Neutral	The ears are between low and high posture	State
	Low	The ears are directed towards the back, and/or in a low position	State
	Not visible	The ears are not visible	State
Panting	Panting off	No visible panting	State
	Panting on	Visible panting	State

	Mouth not visible	The mouth is not visible	State
Locomotion	Walking	Walking at least one step with all four paws	State
	Sitting	Hind quarters on ground and forelegs supporting the body	State
	Standing	All four paws on ground with legs upright and extended supporting the body	State
	Laying down	In ventral or lateral position, all four legs make contact with the ground	State
Events	Yawning	Involuntary intake of breath through a wide open mouth	Event
	Oral behaviours	Licking around lips and nose, tongue flicking	Event
	Turning away	Turning head and/or body away from the stimulus	Event
	Freezing	General rigidity of the body	Event
	Paw lifting	Lifting one of the front paws	Event
	Growling	Growling	Event
	Barking	Single bark	Event
	Repetitive bark	Hard bark that is often repeated in quickly	Event
	Nose wrinkling	Wrinkling of the nose	Event
	Show front teeth	Only showing front teeth	Event
	Show all teeth	Mouth corners are visible	Event
	Snapping	Fast movement towards the stimulus, attempt to bite but does not make physical contact	Event
	Biting	Teeth make physical contact with the stimulus	Event
	Escape	Running/jumping away from the stimulus	Event
	Crouching	Rapid and pronounced lowering of the body without large	Event

	movement	
Trembling	Shaking of legs and/or whole body	Event
Staring	Fixated look at the stimulus with eyes wide open	Event
Support seeking	Seeking support at the owner, looking at the owner, jumping at the owner	Event
Elimination	Urination or defecation	Event
Shaking	Fast sideward movement of the whole body, possibly head also	Event
Pilo-erection	Erection of hears on the back, behind the head or near the tail	Event

Behavioral classes (column 1), behaviors (column 2) and their properties (column 4).

Statistical analysis:

For the purpose of data reduction and to investigate relationships between behaviors a principal component analysis was performed (Lattin et al., 2003; Tabachnick and Fidell, 2001). Also, the PCA results in component scores that have a normal distribution, which facilitates subsequent REML analyses. Briefly, in PCA, underlying correlation matrices in sets of parameters were represented by principal components as linear combinations of parameter scores. Principal components identify parameters that co-vary (in the same or opposite direction) as indicated by relatively high absolute loadings, which like correlations range from −1 to +1, for the same component. The relative importance of a component was indicated by the percentage of variation in the data set that it explains (i.e., can be attributed to interrelationships between parameters). The first principal component explains the maximum variance and each successive component explains a smaller proportion of the variance. Here, only the behaviors with an absolute loading > 0.4 were retained in a dimension and a maximum of 5 components were defined (Tabachnick and Fidell, 2001). The PCA had been performed two times, the first time to assess which behaviors fitted the components, and a second time to precisely calculate component scores of the fitted behaviors.

To test which behaviors differed in expression, percentage of time and/or frequency, between the reaction according to the playing off the 'play' bark and the 'territorial' bark, all component scores and remaining behaviors were tested for bark type effects by use of a mixed model analysis (restricted maximum likelihood: REML). The REML approach is a particular form of maximum likelihood estimation. This estimation uses a likelihood function calculated from a transformed set of data, so that nuisance parameters have no effect (Dodge, 2003). The data were checked for deviation from the normal distribution by plotting fitted values against residuals, i.e. screening for changing variance, and log transformed if the variance clearly depended on the level of measurement. REML estimates components of variation and treatment effects where the sources of variation are at different strata (Pryce et al., 1999). REML generates Wald test statistics, which approximate a Chi-

square distribution with degrees of freedom equal to the fixed model term. In univariate analyses, the Wald test statistics were used to test for significance. The Wald statistics that were calculated in this way ignored terms that were fitted later in the model and, therefore, the fixed effect that was of most interest was fitted last to the model. Dogs were fitted as random effect to account for covariance between multiple measurements in the same individual. Variance components for the random dog effect and the fixed effects were estimated simultaneously in the model. They were assumed to be normally distributed with a mean of zero. Means for the different levels of fixed effects were estimated whilst adjusting for the effects of other fixed effects (Pryce et al., 1999).

To test the difference in behaviour shown by the playing off off the 'play' bark and the 'territorial' bark, the bark type was tested for the effect on the behaviour of the dog by use of REML analysis. To test if behaviours differed between aggressive and fearful dogs, the score for fear and aggression was tested for the effect on the behaviour of the dog. Also the interaction between bark type and score for fear and aggression could explain variation in the behaviour of the dog; this was tested by the two-way interaction of bark type and the relative score for fear/ aggression for the effect of the relative score for aggression/ fear in combination with a specific bark type on the behaviour of the dog. The behavior scores were included as co-variates.

The analysis was performed with the elaborated statistical model: $Y_{ijkl} = \mu + \alpha_i + Barkingtype_j + Scorefear_k + Scoreaggression_l + Barkingtype.Scorefear_{jk} + Barkingtype.Scoreaggression_{jl} + e_{ijkl}$

Y_{ijkl} being the record (i.e. outcome of the measurement), μ: overall mean, α_i: random term of the dog (as multiple analyses on the same animal were done), tested for the following levels of fixed effects: $Barkingtype_j$, $Scorefear_k$, $Scoreaggression_l$ and the interaction between Scorefear orScoreaggression and the barkingtype and e_{ijkl} describing the random error term.

The procedure test for fixed effects was used to calculate F values. All statistical analyses were performed in Genstat®. Differences with p-values <0.05 were considered to be significant.

2.3.3 Cognition test:

Testing devices:

To determine differences in the cognition between aggressive and fearful dogs and non aggressive/ non fearful dogs, 58 dogs were tested for their general intellectual ability by use of a dog puzzle. The puzzle was manufactured by Nina Ottosson, under the name: dog brick. Three of the four rows were covered by a wooden plate, to keep the dogs focused on one row only. The puzzle contained two movable lids, under which a reward could be hidden, and a wooden cone to prevent the puzzle pieces from moving. By moving the puzzle pieces by the dogs' nose or paws, to allow the lids to shift and access the food rewards. When the wooden stick was placed in the puzzle, the stick had to be removed by use of the mouth first.

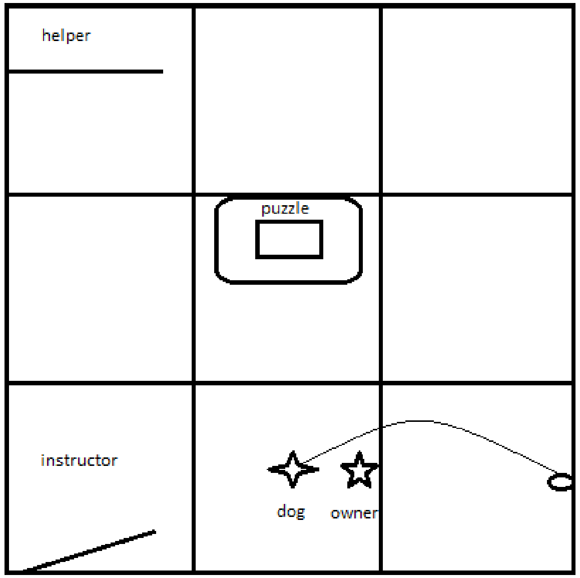


Figure 4: Position of owner, dog, instructor and helper during the cognition test. The puzzle was placed in the middle of the room. During the test the dog was fixed with a safety line on a hook in the wall.

During the test, the dog was fixed with a safety line which was secured on a hook in the wall (Fig. 4). The puzzle was placed in the middle of the room. The owner was asked to stand on the white cross with the dog on the left, sitting on a black cross which was directly in front of the dog puzzle (Fig. 5).

Figure 5: Position of owner and dog during the puzzle test. The puzzle was manufactured by Nina Ottosson, under the name: dog brick. Three of the four rows were covered by a wooden plate. The puzzle contained two movable puzzle pieces and one wooden stick, which was introduced in the most difficult level. During the test the dog was fixed with a safety line on a hook in the wall.

Testing protocol:

Before the test started, the owner was asked to make the dog familiar with the puzzle by showing it the puzzle and putting rewards under the puzzle lids. The owner had to show the dog that the puzzle lids were movable and the dog was stimulated to interact with the puzzle by help of the owner for the duration of one minute.

The test consisted of three phases, whereby the reward was hidden in a more difficult place each phase. Every single phase consisted of three trials, all trials lasted 30 seconds. A reward which consisted of a piece of Frolic was shown by a helper. In the easy phase, the reward was hidden under only one puzzle piece, near the helper's side (Fig. 6). In the average phase, the reward was hidden under the same puzzle piece but now both puzzle pieces covered the reward (Fig. 6). The difficult phase was the same as the average phase, but now a wooden cone was placed in the hole near the dog's side (Fig. 6). After the helper had placed the reward in the right spot, the helper stepped back to the left corner behind a small wall.

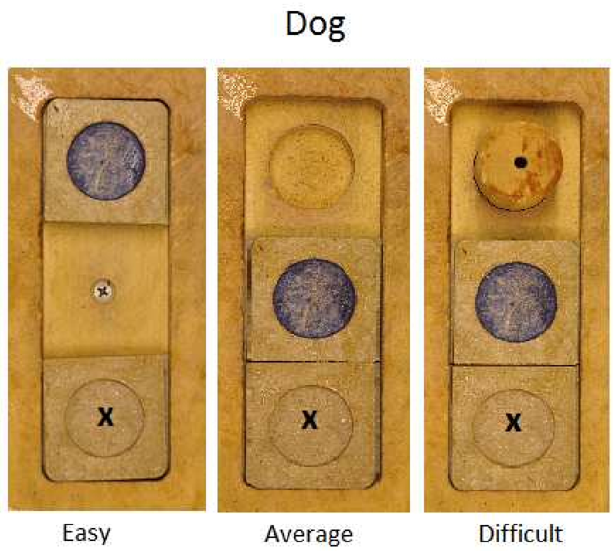


Figure 6: Position of the reward (X) and the puzzle pieces in the three different phases: easy, average and difficult. The reward was always hidden under the puzzle piece the most distant from the dog. The cone prevented the puzzle pieces to be moved and needed to be taken away by use of the mouth of the dog.

The dog was shown were the reward was hidden, but was not helped during the task. The owner was allowed to draw the attention of the dog by pointing at the puzzle and giving the dog a command like: 'search for it'. The number of success per phase was noted. When the dog as not able to obtain the reward within 30 seconds, this was noted as a failure. The dogs' behavior during the test was documented by use of video recording. After the dog had played the game, the owner was asked if they had a similar game at home and if the dog was already familiar to it, as this might influence the results

Statistical analysis:

To determine the general cognition of the dog, the numbers of success were noted. Dogs could obtain different points, according to the complexity level of the test (Table 3).

Table 3: Distribution of the points according to the complexity level of the cognition test

Level	Easy	Average	Difficult
Points per successful trial	1	2	3
Maximum total points per level	3	6	9

Different points can be obtained according the complexity of the test. In the easy phase, only one point is obtained per successful trial. In the difficult phase, three points are earned per successful trial. Every phase consists of three trials, each trial with duration of 30 seconds.

The total score obtained in the cognition test was coupled with the relative scores of fear, aggression and trainability, and the gender which were obtained by use of the C-BARQ questionnaire. The scores for fear, aggression and trainability were as explained. By use of bivariate analysis in SPSS, the correlation between the independent variables: relative scores for fear, aggression and trainability and dependent variable: the cognition score was tested. The correlations between the four variables and the cognition score were obtained by use of the Pearson's r. The Pearson product moment coefficient of correlation, r, is a measure of the strength of the linear relationship between the two variables, X and Y. A value of 0 implies little or no relationship between the two variables. The closer r comes to -1 or 1, the stronger the linear relationship between the relative score for fear, aggression or trainability and the cognition score (McClave and Sincich, 2002).

The gender of the dog consisted of 2 groups: female and male dogs. By use of Chi-square analysis in SPSS, it was tested if the variance between the genders, which was the independent variable, could explain the variance in the cognition score, which was the dependent variable. In a Chi-square analysis, the variables gender and cognition score were both assembled in 2 discrete groups. The groups considered were mutually exclusive and had a total probability of 1 (Ott and Longnecker, 2001). Chi-square analyses counted data per combination of 2 discrete factors and tested which combinations deviated from expectations. Asymptotic significances with p-values <0.05 were considered to be significant.

Stress may interfere with good learning and memory but also be a causal link between fear/ aggression and such effects are checked by testing if variation in stress behavior explains variation in cognition scores. By use of the videotapes, stress signals were scored (Table 4). Behavioural observations of the recordings were computer aided using the Observer® 5.0 software (Noldus Information Technology, 6709 PA Wageningen, The Netherlands). The ethogram included the behavioural classes: attention, tail posture, ear posture, locomotion, vocalization, miscellaneous, and puzzle level. For this study, only behaviours related to stress were used.

Table 4: Ethogram of stress related behavior in dogs

Behavior	Description	Properties
Panting	Breathing in a high frequency which is often accompanied by the protrusion of the tongue	State
Yawning	An involuntary intake of breath through a wide open mouth	Event
Oral behaviours	Licking around lips and nose, tongue flicking	Event
Turning head/ body away	Turning head and/ or body away from the stimulus	Event
Freezing	General rigidity of the body	Event
Paw lifting	Lifting one of the front paws	Event
Shaking	Fast sideward movement of the whole body, possibly head also	Event

Scored behaviours (column 2) description of this behaviour (column 2) and properties (column 4).

All behaviors were expressed as frequency of occurrence, panting was also expressed as a percentage of the total observation time. By use of bivariate analysis in SPSS, it was tested if the variance in stress behavior had a correlation with the variance in the performance score of the cognition test. Also for this test, the correlation between the number of stress signals and the cognition score were obtained by use of the Pearson's r. Whereby the Pearson product moment coefficient of correlation, r, a measure is of the strength of the linear relationship between the two variables (McClave and Sincich, 2002).

3. Results:

During a period of 5 weeks, 58 dogs were tested for behavioral flexibility, general cognition, and socio-emotional information processing. In total, 39 different breeds and 9 crossbreeds participated in the behavior tests. Nine of the dogs that participated were castrated male dogs, 20 were intact male dogs, 23 were neutered female dogs, and 5 dogs were intact female dogs. The gender of one dog was unknown. Owners were asked to fill in a dog personality questionnaire, to determine the relative level of fear, aggression and trainability of their dog. Nine owners did not fill in this questionnaire, resulting in 49 useful records to determine possible relationship between fear, aggression, gender and trainability and behavioral flexibility, general cognition, and socio-emotional information processing.

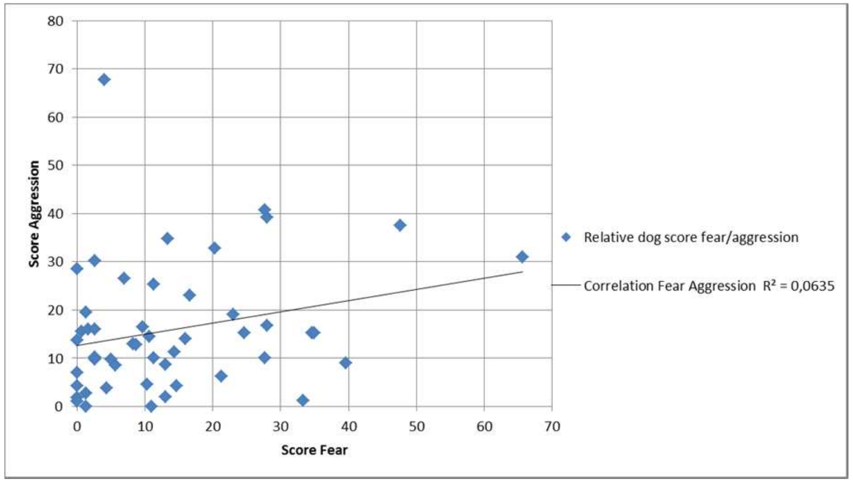
Table 5: Correlations between the relative score of fear, score aggression and score trainability

		Score fear	Score aggression	Score trainability
Score fear	Pearson Correlation	1		
	Sig. (2-tailed)			
Score aggression	Pearson Correlation	.25	1	
	Sig. (2-tailed)	.081		
Score trainability	Pearson Correlation	.038	-.11	1
	Sig. (2-tailed)	.80	.48	

Presented are the Pearson correlation coefficients between owner reported scores for fear, aggression and trainability of their dogs. No significant correlations between the three parameters were determined. A trend between the correlation of the owner reported score for fear and aggression was found (P=0.081). The correlations were based on 49 records for fear and aggression and 46 records for trainability.

As fear is thought to have a high correlation with aggression, the correlation between these two parameters was determined. The correlation with trainability was also taken in account, to obtain an overview of the correlation between the three parameters: fear, aggression and trainability. The mean scores for fear, aggression and trainability were 13.9, 15.8 and 60.7 respectively, with scores representing percentages of maximum scores according to the behavior the dog shows in some pre-defined situations and adjusted to the number of answered questions. The correlations were based on 49 records for fear and aggression and 46 records for trainability. No significant correlations between the three parameters could be observed (Table 5). The correlation between the scores for fear and aggression show a trend with a P value of 0.081. The Pearson correlation coefficient for the score of fear and aggression is 0.25, implying a small positive linear relationship between the two parameters (Fig.7).

Figure 7: Trend (P= 0.081) in correlation between relative scores for fear and aggression, as reported by the owner of the dog. The y-axis indicates the relative score for aggression of a dog in combination with the relative score for fear (x-axis). The black line indicates the correlation between fear and aggression, with a correlation coefficient of 0.25.



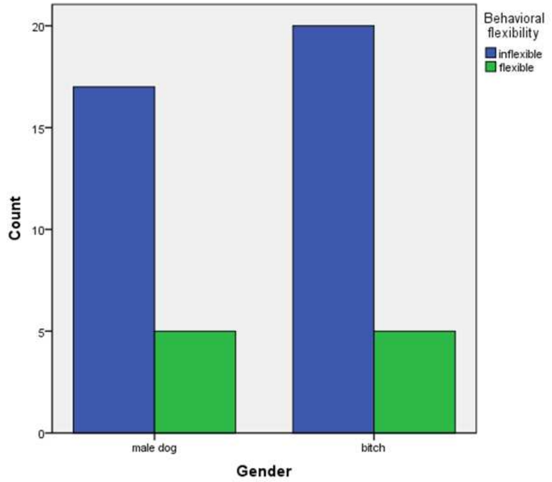
3.1 Flexibility test:

For testing if the degree of aggression and fearfulness in dogs, as reported by their owners, is linked to the dogs' abilities to alter representations of value associated with earlier conditioned stimuli , a population of dogs was tested for behavioral flexibility by use of a T-maze. In total, 58 dogs were tested. One dog jumped over the T-maze and the test was stopped for this dog, resulting in 57 records. Twenty-nine percent of the dogs preferred choosing the left side during the test; the remaining 71% of the dogs preferred the right side during the test. Forty-five percent of the dogs kept choosing the same side during the reversal learning phase and thereby did not show any flexibility during the test. The remaining dogs did choose, at least once, the non-preference side during the revered learning phase. On average, 6.3 errors per dog were made during the 8-trial reversed learning phase. Nine owners did not fill in this questionnaire, resulting in 48 useful records to determine a possible relationship between the owners determined score of fear, aggression, trainability, gender and experimentally determined behavioral flexibility.

3.1.1 Relationship gender and behavioral flexibility:

The relationship between the gender of the dog and behavioral flexibility was determined by use of Chi-square analysis. The gender of the dogs consisted of two groups: male and female dogs. The dogs' behavioral flexibility, determined by use of the number of errors made in the reversed learning phase of the flexibility test, consisted of two groups: inflexible animals and flexible animals. Inflexible animals made 5 errors or more in the reversed learning phase, flexible animals made 4 errors or less in the reversed learning phase of the flexibility test. Seventeen out of 22 male dogs showed inflexible behavior in the flexibility test, whereas 20 out of 25 bitches showed inflexible behavior. The gender of 1 dog was unknown, resulting in 47 records taken in account in this analysis (Fig. 8). Chi-square analysis showed that variation in the gender of the dogs did not explain variation in behavioral flexibility and no significant differences between scores for combinations of gender and flexibility were found (P = 0.82).

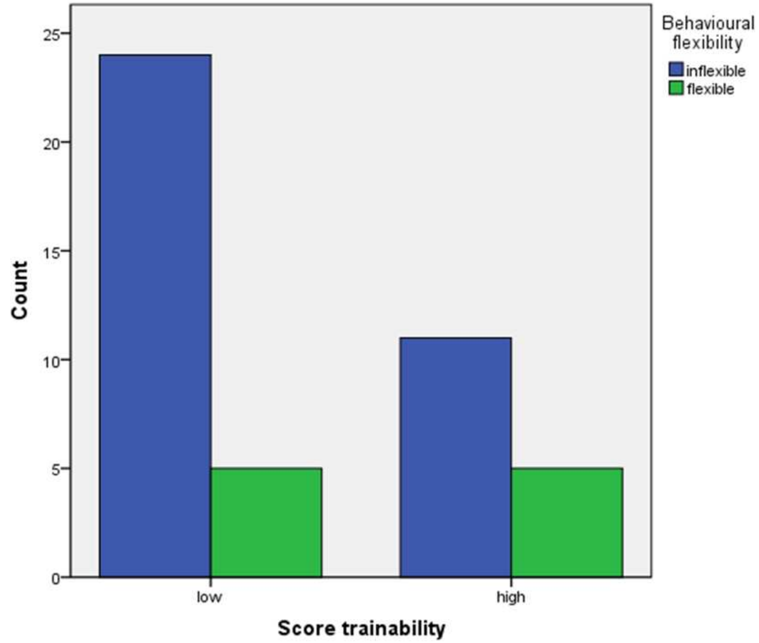
Figure 8: Bar chart of the gender of the dog and the behavioral flexibility. The y-axis indicates the number of dogs of a given gender (x-axis) that acted flexible (green bars) or inflexible (blue bars) in a reversal reward test. The gender of the dogs did not explain variation in behavioral flexibility (P = 0.486).



3.1.2 Relationship trainability score and behavioral flexibility:

The relationship between the relative trainability score of the dog and behavioral flexibility was determined by use of Chi-square analysis. The relative trainability scores of the dogs were assembled in two groups: relative low trainability score and relative high trainability score. The low trainability score was determined by the having a lower score than 65 % for trainability. The high trainability score was determined by having a higher score than 65% for trainability. The dogs' behavioral flexibility, determined by use of the number of errors made in the reversed learning phase of the T-maze test, was classified as inflexible or flexible following procedures described in section 3.1.1. Twenty-four out of 29 dogs with a low score for trainability showed inflexible behavior in the T-maze test. Eleven out of 16 dogs with a high score for trainability showed inflexible behavior in the T-maze test. The trainability score of 3 dogs was unknown, resulting in 45 records taken in account in this analysis (Fig. 9). Chi-square analysis showed that variation in the trainability score of the dogs did not explain variation in behavioral flexibility. No significant differences between the groups of the trainability score and flexibility were found (P = 0.279).

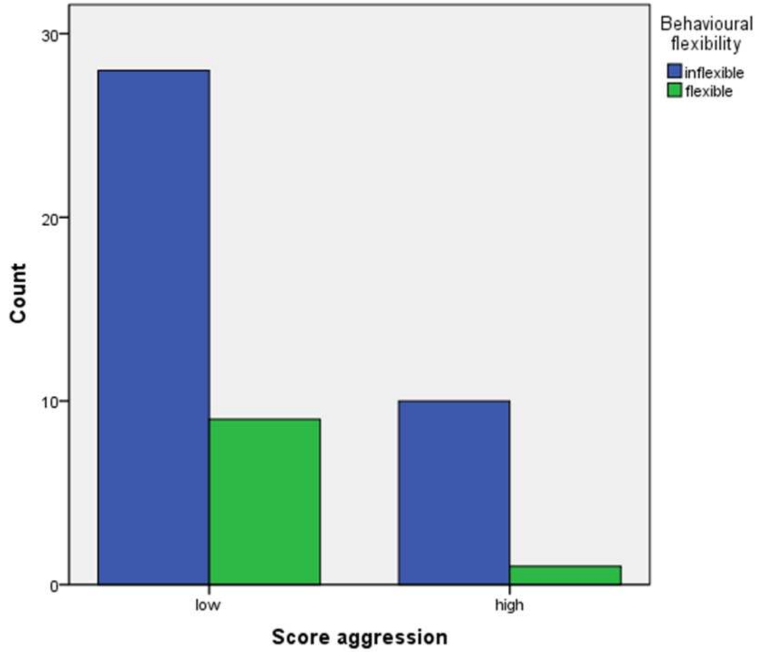
Figure 9: Bar chart of the trainability score of the dog and the behavioral flexibility. The y-axis indicates the number of dogs of a given trainability score (x-axis) that acted flexible (green bars) or inflexible (blue bars) in a reversal reward test. The trainability score of the dog did not explain variation in behavioral flexibility (P=0.279).



3.1.3 Relationship score aggression and behavioral flexibility:

The relationship between the aggression score of the dog and behavioral flexibility was determined by use of Chi-square analysis. The relative aggression scores of the dogs were assembled in two groups: relative low aggression score and relative high aggression score. The low aggression score was determined by the having a lower score than 20 % of the possible maximum for aggression. The high aggression score was determined by having a higher score than 20% for aggression. The dogs' behavioral flexibility, determined by use of the number of errors made in the reversed learning phase of the T-maze test, was classified as inflexible or flexible following procedures described in section 3.1.1. Twenty-eight out of 37 dogs with a low score for aggression showed inflexible behavior in the flexibility test. Ten out of 11 dogs with a high score for aggression showed inflexible behavior in the flexibility test. Forty-eight records were taken in account in this analysis (Fig. 10). Chi-square analysis showed that the aggression score of the dogs did not explain variation in behavioral flexibility. No significant differences between the groups of the aggression score and flexibility were found (P = 0.275).

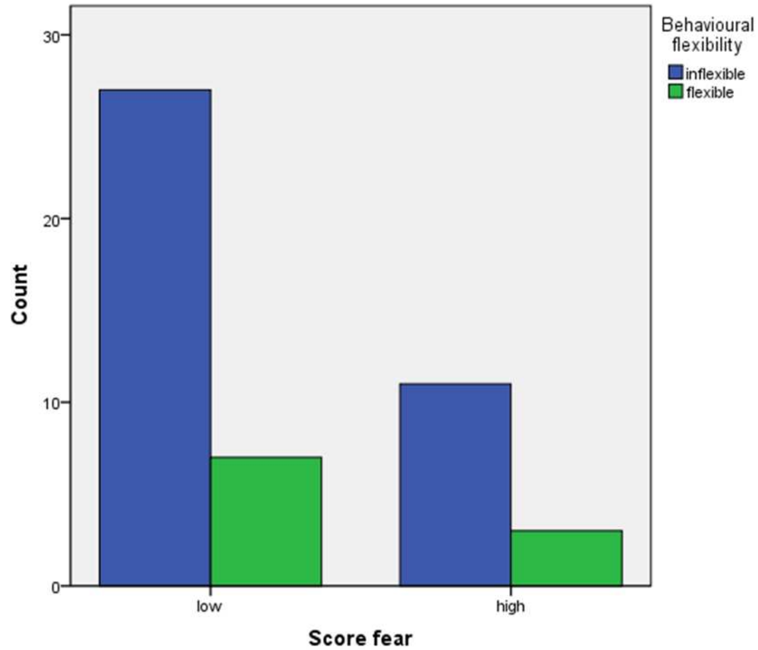
Figure 10: Bar chart of the aggression score of the dog and the behavioral flexibility. The y-axis indicates the number of dogs of a given aggression score (x-axis) that acted flexible (green bars) or inflexible (blue bars) in a reward reversal test. The aggression score of the dog did not explain variation in behavioral flexibility (P = 0.275).



3.1.4 Relationship score fear and behavioral flexibility:

The relationship between the fear score of the dog and behavioral flexibility was determined by use of Chi-square analysis. The relative fear score of the dogs were assembled in two groups: low fear score and high fear score. The low fear score was determined by the having a lower score than 20% for fear. The high fear score was determined by having a higher score than 20% for fear. The dogs' behavioral flexibility, determined by use of the number of errors made in the reversed learning phase of the T-maze test, was classified as inflexible or flexible following procedures described in section 3.1.1.Twenty-seven out of 34 dogs with a low score for fear showed inflexible behavior in the flexibility test. Eleven out of 14 dogs with a high score for fear showed inflexible behavior in the flexibility test. Forty-eight records were taken in account in this analysis (Fig. 11). Chi-square analysis showed that the fear score of the dogs did not explain variation in behavioral flexibility. No significant differences between the groups of the fear score and flexibility were found (P = 0.948).

Figure 11: Bar chart of the fear score of the dog and the behavioral flexibility. The y-axis indicates the number of dogs of a given fear score (x-axis) that acted flexible (green bars) or inflexible (blue bars) in a reward reversal test. The fear score of the dog did not explain variation in behavioral flexibility (P = 0.948).



3.1.5 Relationship preference side T-maze and preference walking side:

To determine a possible relationship between the preference side of the dog in the T-maze and the side the dog normal walks or works, dog owners were asked which side their dog normally walks or works. Twenty dog owners answered this question. The most chosen side in the T-maze determined the preference side for this test. For 19 dogs the preference side was the first chosen side, for one dog this was the opposite side (Table 6). Eighty percent of the dogs choose the left side of the T-maze as the preference side. Three out of 16 dogs which had the left side as the preference side did not normally walk on the left. One of the four dogs which had the right side as a preference side did not had this side as the normal walk side. A binominal test showed that there is a strong relationship between the preference side in the T-maze and the normal walking side of the dog, with a binominal probability of P = 0.0046.

Table 6: Preference side and working side of 20 dogs that participated in the T-maze test

Preference side T-maze	Preference working/ walking side	
	Left	Right
Left	13	3
Right	1	3

Presented are the numbers of dogs having the left or the right side as preference side of the T-maze or working/ walking side.

3.2 Socio-emotional information processing test:

To determine if the degree of aggression and fearfulness in dogs, as reported by their owners, is linked to the dogs' capability to correct identify social cues, 58 dogs were exposed to a 'play' bark and a 'territorial' bark. In general, the calculated mean percentages and the average total frequencies of the observed behaviors, between the 'play' bark and the 'territorial' bark, only show small differences (Table 7). A difference of 10% of the observed time was seen for having the ears in an upward or down position, whereby the dogs have a more upward position of the ears during expose of the 'play' bark and a more down position of the ears during the expose of the 'territorial' bark.

Table 7: An overview of the mean scores for all observed behaviors (see first column) during the 17s expose of a 'play' bark and a 'territorial' bark.

Behavior	'play' bark	'territorial' bark
neutral posture %	69.82	68.87
high posture %	24.19	27.66
low posture %	5.54	6.44
normal tail wagging %	4.01	4.42
wagging off %	95.86	96.03
neurtal tail posture %	67.59	70.00
high tail posture %	4.74	6.91
low tail posture%	25.70	24.81
panting %	20.52	17.94
panting off %	79.30	82.45
oral behaviour #	0.19	0.24
turning head/ body #	3.07	3.10
barking #	0.76	0.74
support seeking #	0.66	0.62
ears neutral position %	12.67	14.26
ears up position %	54.53	45.04
ears down position %	31.07	42.42
sitting position%	50.24	51.74
standing still %	20.48	19.40
walking %	9.88	8.15
lay down %	19.40	22.44

Calculated means of the behaviors are presented as average percentage of the observed time (%) or as an average frequency (#) of the observed time. The mean values are based on 98 records on 58 dogs exposed to a 'play' bark and / or 'territorial' bark.

3.2.1 Data reduction:

Principal component analysis was used to determine the relationships between all observed behaviors and to convert these behaviors of possible correlated variables into a set of values of uncorrelated variables called principal components. In this way, the large dataset of all behaviors was reduced to a dataset of behaviors clustering together (the principal components), with some remainder of behaviors that could not be clustered.

Only behaviors that occurred in more than 10% of the recordings were used for the PCA, resulting in 21 behaviors: neutral posture, low posture, normal tail wagging, not tail wagging, neutral tail, high tail, low tail, panting, not panting, oral behavior, turning head/ body, barking, ears neutral, ears up, ears down, standing still, walking and laying down. In order to calculate component scores in a

precise way the PCA was repeated with only the behaviors that fitted in the first, second and third component (Table 8). The behaviors: low posture, oral behavior, not panting, normal tail wagging, not tail wagging, and turning head/ body did not fit in one of the four components and were excluded.

Table 8: An overview of the refined results from the PCA on behaviors shown by the dogs during the socio-emotional information processing test.

Behavior	CS 1: Submissiveness	CS 2: Vocal responsiveness	CS 3: Vigilance
Neutral posture	0.36	-0.18	-0.17
Low posture	**0.54**	0.12	0.08
Neurtal tail posture	0.12	**0.83**	0.08
High tail posture	-0.01	**-0.49**	-0.16
Low tail posture	**0.85**	0.05	0.17
Panting	-0.23	0.18	**0.47**
Barking	0.16	**-0.50**	-0.14
Supportseeking	**0.49**	-0.09	0.14
Ears neutral position	0.09	0.19	**-0.42**
Ears upwards	-0.27	-0.34	**0.65**
Ears down	**0.39**	0.19	**-0.48**
Sitting	0.29	**0.53**	**0.40**
Standing	**0.81**	-0.32	0.13
Walking	**0.78**	-0.11	0.22
Lay down	0.12	0.17	**-0.54**

Presented are the loadings, with absolute values > 0.4 indicating significant fit in one of the 3 components (columns 2, 3 and 4). The percentages of variation explained by the 3 components 'submissiveness', 'vocal responsiveness' and 'vigilance' were 20.51, 12.55, and 11.31 respectively.

The first component explained 20.5% of the variation and was labeled 'submissiveness' as high percentages of a low posture were accompanied by having a low tail posture, ears down while standing and walking (Table 8). The second component score explained 12.6% of the variation and was labeled 'vocal responsiveness' as high frequencies of barking were accompanied by high percentages of having a high tail posture. There was a strong negative correlation between the vocal responsiveness behaviors and sitting and having a neutral tail posture. The third component score explained 11.3% of the variation and was labeled 'vigilance' as high percentages of panting were accompanied by sitting and having the ears upwards. There was a strong negative correlation with the behaviors: lay down and having the ears down and neutral. These behaviors represent a relaxed state.

Component scores for the three dimensions were calculated per record individually. These scores were analysed with REML. A strong positive score for 'submissiveness' meant that the dog showed high levels of submissive behavior. A strong positive score for 'vocal responsiveness' meant that the dog showed very low levels of 'vocal responsiveness', a strong negative score represented high levels of 'vocal responsiveness'. A strong positive score for 'vigilance' meant that the dog showed high levels of vigilance representing behaviour.

3.2.2 Behavioral differences in identification of vocalization in aggressive and fearful dogs:

For testing differences in capability of correct identification of vocalization, in aggressive and fearful dogs and non-aggressive/ non fearful dogs, REML analyses were performed with the fixed effects: barking type, score fear, score aggression and the interaction between the score of fear or the score of aggression and the barking type. A more precise description of the model can be found in the material and method section. Nine dog owners did not fill in the C-BARQ questionnaire, for these dogs the score for fear, aggression and trainability could not be determined. In total, for each REML analysis, 98 records on 49 different dogs exposed to a 'play' bark and a 'territorial' bark were used (Table 9).

Table 9: fixed effects of the tested behaviours (see column 1) on the fixed terms (see column 2)

Tested behaviour	Fixed term	F pr
CS 1: Submissiveness	bark_type	0.668
	Score fear	0.328
	Score aggression	0.276
	bark_type.scorefear	**0.020**
	bark_type.scoreaggression	0.956
CS 2: Vocal responsiveness	bark_type	0.985
	Score fear	0.758
	Score aggression	**0.006**
	bark_type.scorefear	*0.066*
	bark_type.scoreaggression	0.677
CS 3: Vigilance	bark_type	**0.046**
	Score fear	0.656
	Score aggression	0.186
	bark_type.scorefear	0.877
	bark_type.scoreaggression	0.188
Low posture	bark_type	0.887
	Score fear	0.406
	Score aggression	0.371
	bark_type.scorefear	0.997
	bark_type.scoreaggression	0.419
Oral behavior	bark_type	0.654
	Score fear	0.265
	Score aggression	0.108
	bark_type.scorefear	0.116
	bark_type.scoreaggression	0.215
Non panting	bark_type	0.322
	Score fear	0.560
	Score aggression	0.244
	bark_type.scorefear	0.292
	bark_type.scoreaggression	0.250
Normal tail wagging	bark_type	0.567
	Score fear	*0.079*
	Score aggression	0.159
	bark_type.scorefear	0.853
	bark_type.scoreaggression	0.754
No tail wagging	bark_type	0.553
	Score fear	0.465
	Score aggression	0.292
	bark_type.scorefear	0.119
	bark_type.scoreaggression	0.903
Turning head/ body	bark_type	1.000
	Score fear	0.776
	Score aggression	0.867
	bark_type.scorefear	0.192
	bark_type.scoreaggression	0.863

For all tested behaviours, 98 records consisting 49 dogs exposed to a 'territorial' and 'play' bark were used. P values marked in italic showed a trend (P<0.10), values showed in bold differ significantly (P<0.05).

Two way interaction between the fear score and bark type:

A significant effect of the two-way interaction between the score for fear of the dog and the bark type was found for 'submissiveness' (P=0.02). Contrasts between the 'play' and 'territorial' bark for the 0, 30, 40, 50, 60 and 70% score for fear were considered to be significant (Table 10, P=0.02, SED=0.22). The dogs that scored 0% for fear showed more submissiveness during the 'play' bark than during the 'territorial' bark. Dogs that scored 30% or more for fear showed higher levels of submissiveness during the expose of the 'territorial' bark than for the 'play' bark. Contrasts within the 'play' bark were not considered to be significant. Contrasts within the 'territorial' bark were considered to be significant, the higher the score for fear, the more submissiveness the dog showed during the expose of the 'territorial' bark.

Table 10: predicted means of component score 1: submissiveness, for the interaction of score for fear and bark type

Bark type	Score for fear %							
	0	10	20	30	40	50	60	70
'play'	$0.23^{b,1}$	$0.24^{a,1}$	$0.26^{a,1}$	$0.27^{a,1}$	$0.29^{a,1}$	$0.30^{a,1}$	$0.32^{a,1}$	$0.33^{a,1}$
'territorial'	$-0.38^{a,1}$	$0.00^{a,1,2}$	$0.39^{a,2,3}$	$0.78^{b,3,4}$	$1.16^{b,4,5}$	$1.55^{b,5,6}$	$1.93^{b,6,7}$	$2.32^{b,7,8}$

Submissiveness is presented as an average component score. Mean values are based on 2 records per dog (n = 49). Positive values indicate high levels of submissiveness and vice versa. Mean values that do not share a letter in the superscript differ significantly within a column, mean values that do not share a number in the superscript differ significantly within a row (P<0.05).

A trend in the effect of the two-way interaction between the score for fear and the bark type was found for 'vocal responsiveness' (P=0.066). Contrasts between the 'play' and 'territorial' bark within the 0, 30, 40, 50, 60 and 70% score for fear were considered to show a trend (Table 11, P=0.066, SED=0.16). The dogs that scored 0% for fear showed more vocal responsiveness during the 'territorial' bark than during the 'play' bark. Dogs that scored 30% or more for fear showed higher levels of vocal responsiveness during the expose of the 'play' bark than for the 'territorial' bark. Dogs that scored 10 and 20% for fear showed the same levels of vocal responsiveness as well for the 'play' bark, as well as for the 'territorial' bark. Contrasts between the scores for fear within the barks showed a trend. The higher the score for fear, when exposed to the 'territorial' bark, the lower the level of vocal responsiveness. The higher the score for fear, when exposed to the 'play' bark, the higher the level of vocal responsiveness (Table 11, P=0.066, SED=0.16).

Table 11: predicted means of component score 2: vocal responsiveness, for the interaction of score for fear and bark type

Bark type	Score for fear %							
	0	10	20	30	40	50	60	70
'play'	$0.14^{b,6,7}$	$0.02^{a,5,6,7}$	$-0.10^{a,4,5,6}$	$-0.22^{a,3,4,5}$	$-0.34^{a,2,3,4}$	$-0.46^{a,1,2,3}$	$-0.58^{a,1,2}$	$-0.70^{a,1}$
'territorial'	$-0.34^{a,1}$	$-0.12^{a,1,2}$	$0.10^{a,2,3}$	$0.32^{b,3,4}$	$0.53^{b,4,5}$	$0.75^{b,5,6}$	$0.97^{b,6,7}$	$1.19^{b,7}$

Vocal responsiveness is presented as an average component score. Mean values are based on 2 records per dog (n = 49). Positive values indicate low levels of vocal responsiveness and vice versa. Mean values that do not share a letter in the superscript show a trend within a column, mean values that do not share a number in the superscript show a trend within a row (P<0.10).

Main effects for tail wagging, 'vocal responsiveness' and 'vigilance':

A trend in the effect of the score for fear for normal tail wagging was found (P=0.079). The higher the score for fear, the lower the predicted mean for normal tail wagging during the expose of the 'play' bark and the 'territorial' bark. This means, the higher the score for fear, the less tail wagging the dog shows, regardless the bark type.

Table 12: predicted means of normal tail wagging, for the main effect of the fear score

Score for fear %	0	10	20	30	40	50	60	70
Predicted means tail wagging	6.65^{h}	5.09^{g}	3.53^{f}	1.97^{e}	0.41^{d}	-1.15^{c}	-2.71^{b}	-4.27^{a}

Tail wagging is presented as an average frequency. Mean values are based on 2 records per dog (n = 49). Mean values that do not share a letter in the superscript show a trend (P<0.10).

A significant effect of the score of aggression for 'vocal responsiveness' was found (P=0.006). Mean component scores for 'vocal responsiveness' were significantly higher for the low scores for aggression, representing low scores of 'vocal responsiveness'. The level of 'vocal responsiveness' significantly increased every step the score for aggression increased (Table 13, P=0.006, SED=0.16). Dogs with high scores for aggression showed higher levels of 'vocal responsiveness' in the socio-emotional information processing test compared to dogs with low scores for aggression. No significant effect was found for the interaction between the score of aggression and barking type, showing no difference in 'vocal responsiveness' between the two barking types.

Table 13: predicted means of the component score vocal responsiveness for the aggression score

Score for Aggression %	0	10	20	30	40	50	60	70
Predicted means vocal responsiveness	0.54^{h}	0.18^{g}	-0.18^{f}	-0.54^{e}	-0.90^{d}	-1.26^{c}	-1.62^{b}	-1.98^{a}

Vocal responsiveness behaviour is presented as an average component score. Mean values are based on 2 records per dog (n = 49). Positive values indicate low levels of vocal responsiveness behaviour and vice versa. Mean values that do not share a letter in the superscript show a significant difference (p<0.05).

A significant effect of the bark type for 'vigilance' was found. The mean component score for the 'play' bark was 0.25 and for the 'territorial' bark -0.10 respectively (Table 14, P=0.046, SED=0.16). This means that the dogs showed a higher level of vigilance behaviour when exposed to the 'play' bark in comparison to the expose of the 'territorial' bark.

Table 14: predicted means of the component score vigilance for the bark type

Bark type	'play' bark	'territorial' bark
Predicted mean	0.2552^{b}	-0.1037^{a}

Vigilance is presented as an average component score. Mean values are based on 2 records per dog (n = 49). Positive values indicate high levels of vigilance and vice versa. Mean values that do not share a letter in the superscript show a significant difference (P<0.05).

3.3 Cognition test:

To determine if the degree of aggression and fearfulness in dogs, as reported by their owners, is linked to the dogs' cognitive ability, 58 dogs were tested by use of a dog puzzle. Three tests were stopped because of severe anxiety or aggression towards the experimenters. Seven records were deleted because these dogs already had experience with the dog puzzle and the results of those dogs would not be representative for their cognition. Dogs could earn a score varying between 0 and 18 depending on the number of food rewards the dog obtained within 30 s. A more precise description of the test can be found in the material and method section. On average, the dogs earned 7.76 points in the cognition test. Six owners did not fill in the C-BARQ questionnaire, resulting in 42 useful records to determine a possible relationship between fear, aggression, trainability, gender and the cognition level of the dog. As Stress may interfere with good learning and memory but may also be a causal link between fear/ aggression, the correlation between the total number of stress related behavior, shown by each dog during the first level of the puzzle test as well as the total number of stress related behavior shown during the total puzzle test, was tested for correlation with the cognition score. For these analyses 46 records were used.

3.3.1 Correlation between trainability, fear, aggression and cognition:

Pearson correlation coefficients between trainability, aggression, fear and the cognition score were determined by use of SPSS. The mean scores for fear, aggression and trainability were 14.6, 16.0 and 56.52 respectively, with scores representing percentages of maximum scores according to the behavior the dog shows in some pre-defined situations and adjusted to the number of answered questions. The correlations were based on 42 records for fear, aggression, gender and trainability. No significant correlations between the four parameters and the cognition score could be observed (Table 15). Variance in the score of trainability, aggression and fear did not explain variation in the score for cognition obtained by a dog puzzle.

Table 15: Correlations between the score of gender, trainability, aggression, and fear and the cognition score.

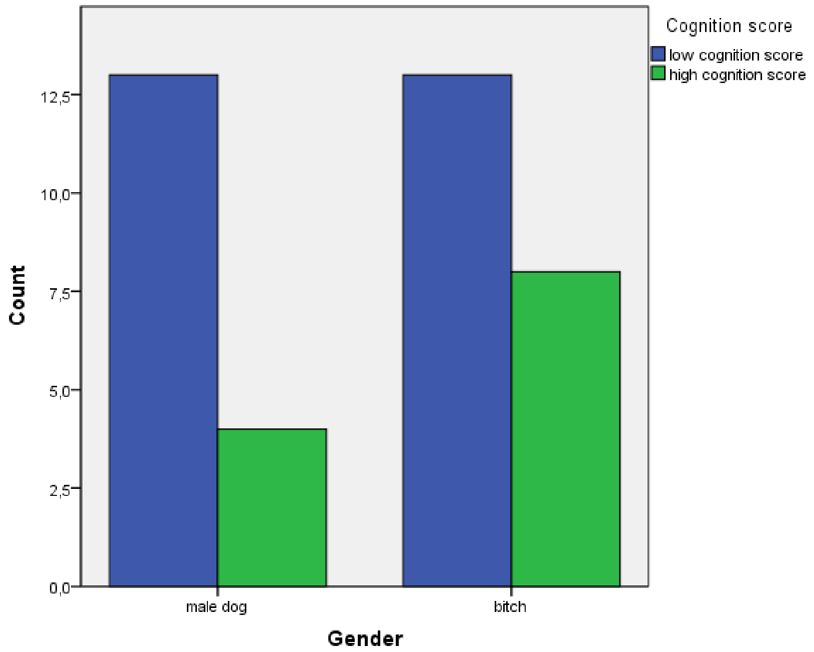
	Pearson correlation coefficient of the cognition score	Sig. (2-tailed)
Score trainability	-0.025	0.88
Score aggression	0.21	0.19
Score fear	-0.005	0.98

Presented are the Pearson correlation coefficients of the behaviors shown in column 1 and the cognition score. The higher this value comes to 1 or -1, the stronger the linear relationship between the two parameters. Significant values were not found.

3.3.2. Relationship between gender and cognition:

The relationship between the gender of the dog and the experimentally determined cognition was determined by use of Chi-square analysis. The genders of the dogs were assembled in two groups: male dogs and bitches. The dogs' cognitive ability, determined by use of the total points obtained in the cognition test, were classified as low cognitive scores (0-9 points) and high cognitive scores (10-18 points). Thirteen out of 17 male dogs scored low in the cognition test. Thirteen out of 21 bitches scored low in the cognition test (Fig.12). Chi-square analysis showed that the gender of the dogs did not explain variation in the cognition. No significant differences between the groups of the gender and cognition score were found (P = 0.34).

Figure 12: Bar chart of the dogs' gender and the cognition score. The y-axis indicates the number of dogs of a given gender (x-axis) that obtained low scores (blue bars) or high scores (green bars) in a cognition test. The gender of the dog did not explain variation in cognition (P = 0.34).



3.3.2 Correlation stress and cognition:

Presence of stress can be a disturbing factor for testing the general cognition of the dog, but stress could also be the causal link between aggression /fear and impaired cognitive skills. Therefore, stress related behavior showed during the cognition test, was scored by use of the Observer software. As stress could occur as a consequence of not being able to fulfill the puzzle task, stress related behavior shown in the first level, as well as stress related behavior shown during the whole cognition test was tested on correlation with the cognition score. Per dog, panting was shown the most with an average occurrence of 2.1 times in the first level and an average occurrence of 4.2 during the whole test. Freezing was shown the least, with no freezing behavior shown in the first level of the puzzle task and an average occurrence of 0.1 times per dog during the total test (Table 16). For determining the mean frequencies, 55 records were used.

Table 16: General stress related behavior during the first level of the cognition test and the total test

Behavior	**Average occurrence level 1**	**Average occurrence total test**
Panting	2.1	4.2
Paw lifting	0.0	0.0
Looking away	0.2	0.4
Yawning	0.0	0.0
Shaking head/ body	0.1	0.1
Oral behaviour	0.2	0.3
Freezing	0.0	0.1

Presented are the mean occurrences for stress related behaviors (see column 1) expressed as times per dog for the first level of a problem solving task and for the total test, which included 3 levels of increasing complexity.

As some stress behaviors were only shown incidentally, the total frequencies of the seven stress behaviors were summed. The sum of stress related behavior of the first level and of the total test were tested for correlation with the cognition score in SPSS. On average, three stress related behaviors were shown in the first level of the puzzle task; on average 6.05 stress related behaviors were shown during the total test.

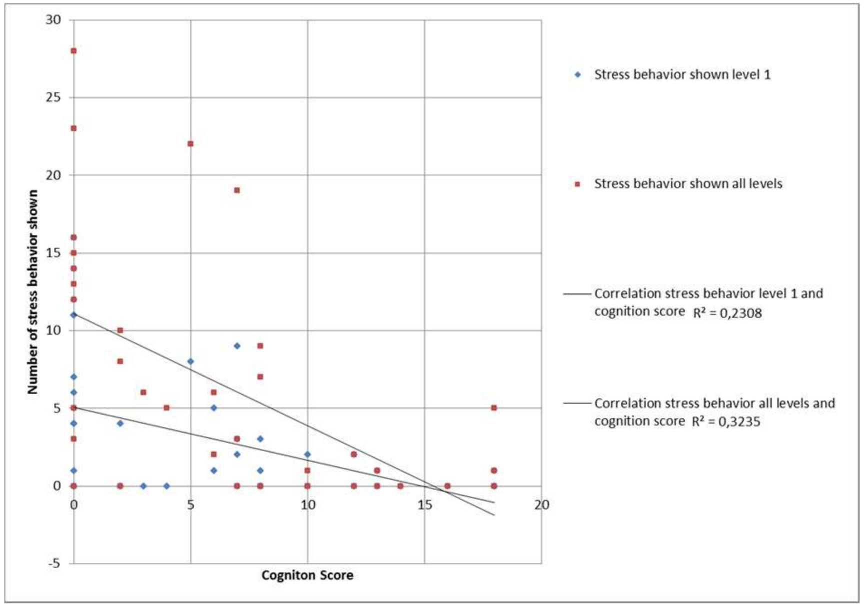
Table 17: Correlations between the score of gender, trainability, aggression, and fear and the cognition score.

	Pearson correlation coefficient cognition score	**Sig. (2-tailed)**
Stress related behavior level 1	-0.48	**0.001**
Stress related behavior total test	-0.57	**0.000**

Presented are the Pearson correlation coefficients of the behaviors shown in column 1 and the cognition score. The higher this value comes to 1 or -1, the stronger the linear relationship between the two parameters. Significant values are shown in bold.

The correlation coefficients were based on 46 records for the first level of the puzzle test and on 39 records for the total puzzle test, as 7 dogs completed the first level of the puzzle test only. Significant correlations for stress related behavior in the first level and the cognition score as well as significant correlation for stress related behavior in the total test and the cognition score were found (Table 17). The correlation of stress related behavior of the first level and the cognition score had a Pearson correlation coefficient of -0.48. The correlation of stress related behavior of the total test and the cognition score had a Pearson correlation coefficient of -0.57. This means, the lower the cognition score of the dog, the more stress the dog showed during the puzzle test. This linear relationship is even higher when dog comes further in the test. During the puzzle test, low scores for cognition were explained by high levels of stress (Fig. 13).

Figure 13: Correlations between stress and cognition. The y-axis indicates the cognition score of a given dog in combination with the number of stress behavior shown (x-axis). The black lines indicate the correlation between stress behavior shown in level 1 and the cognition score and the correlation between stress behavior shown in the whole test, with correlation coefficients of -0.48 and -0.57 respectively.



4. Discussion and conclusion:

The aim of this study is to determine if the degree of aggression and fearfulness in dogs, as reported by their owners, is linked to: the dogs' abilities to alter representations of value associated with earlier conditioned stimuli, the dogs' capability to correctly identify social cues and the dogs' general cognitive ability. This study was founded on studies in human, which showed that persons which are more prone to aggression have difficulty altering representations of value associated with earlier conditioned stimuli, have reduced capability to correctly identify social cues, like vocalization and that they have a lower level of general cognitive ability. It is expected on forehand that this will be the same for aggressive dogs. As aggression is thought to have a high correlation with fear, fear was also taken in account. Trainability and gender could explain variation in the results of the three different tests; these two parameters were taken in account as well. Knowledge on this subject could aid strategies to predict, prevent and remedy fear-related behavior and aggression.

4.1 Correlation fear and aggression

Aggression and fear are both part of the normal behavioral innate responses of dogs, vital for the survival of the individual (O'Heare, 2004; Abrantes, 1997, Heath, 2002). Fear is thought to have a high correlation with aggression (O'Heare, 2004; Vage et al., 2008; Nahlik et al., 2010). In the current study, dog-owners were asked to fill in a so called 'C-BARQ' questionnaire concerning the personality of their dog including fear and aggression. A trend in the correlation between the relative scores of fear and aggression was found (P= 0.081). The Pearson correlation coefficient for the relative score of fear and aggression in this study is 0.25, implying a small positive linear relationship between the two parameters. This result is in line with other studies. Study by Vage et al. (2008) on 52 aggressive English Cocker Spaniels and 65 control English Cocker Spaniels showed that dogs which were classified as aggressive showed a higher level of fear in several situations. Also, when handling these dogs fear was observed commonly. Study by Nahlik et al. (2010) showed that aggression was mostly of the possessive, territorial and fear type. This study focused on dog bites in children in the Czech Republic. Furthermore, evaluation of the SAB test indicated that fear played a role when the dog attacked in one of the subtests (Van den Borg et al., 2010). In a study similar to the current study, volunteer puppy trainers scored the behavior of 1097 future guide dogs by means of a five point score questionnaire. The results showed that fear and aggressive responses toward unfamiliar people were highly correlated (Serpell and Hsu, 2001). Though fear and aggressive responses towards unfamiliar people emerged in one factor in the performed factor analysis, they were associated with different reasons for relinquishing dogs. The author shares the notion with Serpell and Hsu that although aggression is a common response to fear evoking stimuli in dogs, both traits may also appear in the absence of the other and should therefore be treated and measured as two separate temperament aspects.

4.2 High levels of fear and aggression do not explain variation in behavioral flexibility

For testing if aggressive or fear dogs have more difficulty altering representations of value associated with earlier conditioned stimuli, 57 dogs were tested for behavioral flexibility by use of a T-maze, which is an example of a reversal learning test (Boogert et al., 2010). In reversal learning, an animal is presented with two stimuli at the same time. One stimulus is associated with a reward while the other one is not. In this case, the dog could chose left or right to come to the owner, whereby the first chosen side became the rewarding side. After the dog had experienced a predetermined number of trials, in this case 8 trials, whereby we presume the dog had achieved a certain learning criterion, the reinforcement value of the two stimuli was reversed. On forehand, it was expected that aggressive and fear labeled dogs would make more errors in the first reversed trials, compared to non aggressive/ non fearful labeled dogs.

Forty-five percent of the dogs did not change side during the reversed learning phase, showing no behavioral flexibility at all. On average, a dog made 6.3 errors during the reversed learning phase. It is difficult to compare these values with the performance of other animals in a T-maze, as no general method is used between studies. In the present study, there was only one reversal phase and there was no learning criterion. In other studies, animals often had to obtain a certain learning criterion during the different phases. In study done on pigs (Bolhuis et al., 2004), 60% and 32% respectively of the animals kept making errors, depending on the resistance level. This is comparable with the present study, whereby 45% of the dogs kept making errors in the last trial. In the study of Bolhuis et al., pigs that did not reach the food within 300 s where gently directed to the food, so every trial the pig was eventually rewarded with the food. In the present study, when the dogs chose the non-rewarding side, the dogs were not directed to the other arm. When compared to the only other study performed on behavioral flexibility in dogs, by use of a T-maze, the dogs also had to obtain a certain learning criterion. Normal 4 months old Dachshunds made on average (in the three reversal phases) 9 mistakes before reaching the criterion, normal 5 months old Dachshunds 5 mistakes, 6 month old Dachshunds 3 mistakes and 7 months old Dachshunds only 2 mistakes. It is difficult to compare these values with the present study, as they are average errors over three different reversal phases whereby a learning criterion had to be obtained. Unfortunately, there were no results published over how many errors the dogs made in the first reversal phase only. When we presume that in the study of Sanders et al. (2011) in the first reversal phase before obtaining the learning criterion nine errors were made, the 8 reversal trials of the reversal learning phase in the present study are too less to achieve a switch to the newly rewarding side. It is also possible that the learning phase, which also consisted of 8 trials, was too long. The dogs got rewarded very often for not showing behavioral flexibility in that phase and trying to turn this behavior around in 8 trials could be too difficult.

As 55% of the dogs did change side at some time during the reversed learning phase, the procedure of the T-maze seems valid and the reality may be that many dogs were relatively inflexible. An influence, which was not present in other studies, is the presence of the owner. It might be that being with the owner was already a reward for the dog itself, making them less motivated to 'solve' the problem of re-obtaining rewards after rewarding-arm reversals. However, a drop in the speed of walking was observed when the dog was not rewarded for several times, indicating that the reward was of importance for the dog. To obtain more insight why most dogs behave inflexible in the T-maze test it is advised to test the behavioral flexibility without the presence of the owner, to exclude possible influence of the owner. By making use of a certain learning criterion instead of a predetermined number of trials, the influence of rewarding the dog too often might be reduced. In this way, all animals entering the reversed learning phase are more or less on the same level. For the reversed learning phase, more trials might be needed, so differences between animals can be observed more easily. Also for this phase, a criterion might be useful. Implying this advice will result in more time needed to perform this test.

During the flexibility test, 80% of the dogs chose the left side as the preference side. First it was thought that the door, used in the startbox of the T-maze, might induce this outcome. After changing the door, still the majority of the dogs kept choosing the left side as the preference side. For twenty dogs it was tested if the preference side of the T-maze had a relation with the normal walking or working side of the dog. A binominal test showed that there is a strong relationship between the preference side in the T-maze and the normal walking side of the dog, with a binominal probability of P = 0.0046. In literature, there are no studies which give insight in side preference of animals. Study on behavioral flexibility performed on 13 Dachshunds also did no give insight in this phenomenon (Sanders et al., 2011).

The relationships between the gender of the dog, relative trainability score, relative scores for fear and aggression and behavioral flexibility were determined by use of Chi-square analysis. Variation in the gender of the dog and the relative scores for trainability, fear and aggression did not explain variation in behavioral flexibility. For the statistical analysis, 11 dogs were classified as high fearful and 10 dogs as high aggressive, 37 dogs were classified as low aggressive and 34 dogs were low in fear. The numbers of dogs seem sufficient for being able to detect significant relationships between flexibility and aggression, fear or trainability. Prior to the study it was expected that fearful and aggressive dogs would make more errors, in the reversal trials but this was not the case. In human, people that are prone to aggressive behavior show a lower level of behavioral flexibility. For example, persons with orbital medial prefrontal cortex damage may have difficulty altering representations of value associated with earlier conditioned stimuli (Rolls et al., 1994, Fellows and Farah, 2003). In mice, study by Benus et al. (1990), suggested that male mice of a line selected for short attack latency are more routine-like in their behavior and thereby show less behavioral flexibility than individuals of a line selected for long attack latency. It was suggested that the mice during the training period probably developed a strongly fixed locomotion pattern, which is difficult to oppress when a change is introduced (Benus et al., 1990). The two dogs with the highest relative scores of aggression that were pathologically classified as aggressive, were more flexible in their behavior than the average dog, by making only 1 error in the T-maze (aggression score of 41%) and making 6 errors (aggression score of 66%). The three most fearful dogs were, or very flexible in their behavior, by making only 1 error in the T-maze (dog with fear score of 65%), or very inflexible in behavior by making only errors (dogs with relative fear scores of 48% and 40%). The results indicate that many dogs were relatively inflexible and there is no relationship between flexibility and owner determined scores for fear and aggression. Also trainability and gender did not explain variation in behavioral flexibility. This part of the study indicates that task switching ability is not linked to aggression and fear traits reported by owners. Thereby, this part of the study can not demonstrate that such behavior in dogs is related to the function of specific brain centers, which have been suggested in human.

4.3 Aggressive and fearful dogs discriminate between 'play' and the 'territorial' barks

For testing differences in capability of correctly identification of social cues, like vocalizations, in aggressive and fearful dogs and non-aggressive/ non fearful dogs, 58 dogs were exposed to a 'play' bark and a 'territorial' bark. The exposures to the barks lasted for 17 seconds and were played back in a random order. By means of principal component analysis the 22 different behaviors the dogs showed during the test were converted in three principal components called: 'submissiveness', 'vocal responsiveness', and 'vigilance' and remaining behaviors. The principal components and remaining behaviors were tested for effects of the bark type, the dogs' scores for fear and aggression and possible interactions. It was expected on forehand that aggressive and fearful labeled dogs have more difficulty correctly reacting to the 'play' and the 'territorial' bark than non aggressive/ non fearful labeled dogs.

In human, socio-emotional information processing has found to be related to the function of specific brain centers. For example, patients with lesion in the orbital medial prefrontal cortex region showed reduced capability to correctly identify social and emotional cues (Hornak et al., 2003; Hornak et al., 1996). Patients with ventral lobe damage were not able to interpret emotional sounds. The greater the reported emotional change in emotional experience in the group of patients found, the worse the performance in the vocal expression identification test (Hornak et al., 1996). In dogs, no study between reaction towards barks in relation to fearful and aggressive dogs have been performed yet, though unpublished results of Cs Molnar et al. indicated that dogs can distinguish both between barks recorded in different situations and possibly between barks emitted by different individuals (in Pongracz et al., 2010). In this study, a significant effect of the two-way interaction between the fear score and the bark type was found for 'submissiveness' (P=0.02). The higher the score for fear, the higher the level of submissiveness shown during the exposure to the barks, this effect being significant for the 'territorial' bark only. The dogs that scored 0% for fear did differentiate between the barks, but reacted more submissive to the 'play' bark perhaps as they anticipated a friendly greet and meet with another dog. The fact that fearful dogs behaved strongly submissive in response to territorial barks makes sense when considering their anxious nature and the fact that they did not show this in response to play barks indicates good social skills in discriminating between threats and friendly signals. This finding opposes the idea that fearful dogs have reduced capability to interpret social and emotional cues. Submissive behaviour was not affected by the interaction between bark type and a dog's aggression score, meaning that aggressive dogs and friendly dogs could not be proven to behave differently to the different barks. Thus, there was no evidence that aggressive dogs had impaired social skills, though the fact that submissive behaviour was rare in control and aggressive dogs will have played a role in this.

A trend for a two-way interaction between the score for fear of the dog and the bark type was found for 'vocal responsiveness' (P=0.066). Dogs that scored low for fear reacted more vocally during the expose of the 'territorial' bark, than during the expose of the 'play' bark. Dogs that scored high in fear responded more vocally towards the 'play' bark than towards the 'territorial' bark. The higher the score for fear, when exposed to the 'territorial' bark, the lower the level of vocal responsiveness. The higher the score for fear, the higher the level of vocal responsiveness, when exposed to the 'play' bark. This seems consistent with the observation that high scores of aggression were associated with much barking with a high tail position. Such behaviour seems to indicate arousal and a willingness to establish contact, which fearful dogs show in response to a play bark but not in response to a territorial bark (in contrast to aggressive dogs). Whether or not reacting more vocally during the expose of the 'play' bark, observed in the more fearful dogs, is a correct reaction towards the type of bark is hard to say. To determine the vocal context, the vocal responsiveness should be analysed on frequency and amplitude to get more insight in the function of the vocalisation. This has been performed for example by Yin (2001), which showed that barks emitted by different individuals in

different contexts (disturbance, isolation and play) were distinguishable on the basis of acoustic parameters like amplitude and frequency.

A trend for effects of fear score on normal tail wagging was found (P=0.079). Fearful dogs did not differentiate according to the type of bark by means of normal tail wagging behaviour. The higher the scores for fear, the less tail wagging the dog displayed during both exposure to the 'play' bark and the 'territorial' bark. It might be that fearful dogs suffer from behavioural inhibition following danger signals and stop tail wagging. It was hypothesised that fearful labeled dogs have more difficulty correctly reacting to the 'play' and the 'territorial' bark than non fearful labeled dogs, when looked at tail wagging, no differentiation according to the expose of the different barks can be made.

A significant effect of the aggression score on 'vocal responsiveness' was found (P=0.006). Differentiation in 'vocal responsiveness' according to the barks was expected on forehand, according to studies performed in human, however in this study differentiation between the types of bark in accordance to the score for aggression could not be demonstrated. The higher the score in aggression, the more vocally the dogs reacted. The dogs with 0 and 10% aggression scores showed very low levels of 'vocal responsiveness', dogs with a 60 and 70% score of aggressive behaviour showed high levels of 'vocal responsiveness' during the socio-emotional information processing test. No significant effect was found for the interaction between the score of aggression and barking type, showing no difference in 'vocal responsiveness' according to the two barking types..

A significant effect of the bark type for 'vigilance' was found. The mean component scores for 'vigilance' according to the 'play' bark and the 'territorial' bark showed that the dogs displayed a higher level of 'vigilance' when exposed to the 'play' bark in comparison to the expose of the 'territorial' bark. It seemed that dogs were keener to inspect (as deduced from ears upwards) dogs producing 'play' barks in comparison to dogs producing 'territorial' barks. This makes sense as territorial dogs should best be avoided to prevent harmful fights and playful dogs approached for rewarding social interactions. The absence of interaction effects with bark type suggests that both aggressive and fearful dogs were capable of discriminating between territorial barks and play barks.

Summarizing, in the socio-emotional information processing test, the principal components and remaining behaviors were tested by use of REML analyses to see if the bark type, the relative scores of fear and aggression and the two way interaction between the bark type and scores of fear and aggression could explain variation in the dogs' behavior. In accordance to studies performed in humans it was expected on forehand that aggressive and fearful labeled dogs have more difficulty correctly reacting to the 'play' and the 'territorial' bark than non aggressive/ non fearful dogs, but the results do not support this. All dogs seemed to have good social skills, with fearful dogs being especially sensitive to danger signals shown by the fact that fearful dogs behaved strongly submissive in response to territorial barks. Fearful dogs did not show strong submissiveness in response to play barks indicating good social skills in discriminating between threats and friendly signals. There was no evidence that aggressive dogs had impaired social skills, though the fact that submissive behaviour was rare in control and aggressive dogs will have played a role in this. The higher the score for fear, the higher the level of vocal responsiveness, when exposed to the 'play' bark. This seems consistent with the observation that high scores of aggression were associated with much barking in combination with a high tail position. Such behaviour seems to indicate arousal and a willingness to establish contact, which fearful dogs show in response to a play bark but not in response to a territorial bark, in contrast to aggressive dogs. For tail wagging and 'vocal responsiveness' a differentiation according to type of bark or score for fear or aggression could simply not be made. Dogs in general may be good in interpreting intraspecific vocalizations, but dog-owners are known to re-teach dog language to dogs, suggesting some dog signal reading difficulties. For future research it might be interesting to obtain some more detailed information on the dogs' reaction in terms of vocalization. Analysis of the vocalization by means of the frequency and amplitude of the sounds might give beneficial insight in terms of the context of the vocalization.

4.4 Low cognition scores can be explained by high levels of stress:

For testing differences in cognition between aggressive and fearful dogs and non aggressive/ non fearful dog, 55 dogs were tested for their general intellectual ability by use of a dog puzzle. It was expected that aggressive and fearful labeled dogs had relatively lower rates of success in the dog puzzle as in human cognitive ability is thought to play a role in aggressive behavior, as high cognitive individuals better suppress primary urges of fear and aggression. On the one hand, 42% of patients with intellectual disorders display aggression towards others (Emerson et al., 2001), but on the other hand intellectual disabilities are not more common in prison populations. Fazel et al. (2008) studied the prevalence of intellectual disabilities among 12000 prisoners by means of 10 surveys from four different countries. When considered the results, variation in definition as well as survey and diagnostic methodologies need to be taken in account. The findings suggest that typically between 0.5 and 1.5% of the prisoners are diagnosed with intellectual disabilities. This value is comparable with a general population of similar age (Kavanagh& Opit, 1999). However, in death row a higher prevalence in intellectual disabilities, ranging from 2–20%, might be found, though some notice that 'there are no definitive statistics on this' (Hall, 2002). In the present study, correlations with the scores for fear or aggression and the cognitive ability were not found. Also the gender of the dog and the trainability of the dog could not explain variation in the cognitive ability of the dog. The results seem to indicate that cognition score in dogs is not linked to traits as fear and aggression as reported by dog owners.

Stress may interfere with good learning and memory but also be a causal link between fear/ aggression and such effects are checked by testing if variation in stress behavior explains variation in cognition scores. Stress related behavior (panting, yawning, oral behaviors, paw lifting, shaking head/ body, freezing and looking away) shown during the cognition test, was scored and as stress could also occur as a consequence of not being able to fulfill the puzzle task, stress related behavior shown in the first level, as well as stress related behavior shown during the whole cognition test was tested on correlation with the cognition score. Significant correlations for stress related behavior in the first level and the cognition score as well as significant correlation for stress related behavior in the total test and the cognition score were found (Pearson correlation coefficients of -0.48 and -0.57 respectively). This means that the lower the cognition scores of the dog, the more stressed it was during the cognition test, with this linear relationship strengthening during the test. Thus, relatively low cognition scores may have resulted from some degree of stress. This result reveals imperfections of the present test in testing cognitive ability of dogs, as cognition scores in part mirror stress susceptibility.

For future studies focused on the relationship between aggression and fear and general cognition of dogs, it is advised to set-up a test in such a way that stress has a minimal influence on the results, by letting the dogs feel as comfortable as possible. It might be that for this the dogs need to become

habituated to the task in familiar environments. Also, the task must be designed in such a way that the dog can perform the task without guidance of an owner or an experimenter, to exclude the influence of having other people in the room. During the puzzle test it seemed that the dog-owner interactions, by means of encouragement, or the lack of encouragement, might influence the results. It would be interesting to get more insight in the relationship between dog and owner behavior. At this point, the results oppose the idea that aggressive and fearful dogs have reduced cognition than non fearful and non aggressive dogs. Contrary to what is suggested in human, it might be that cognitive ability has no effect on fear and aggression in dogs.

4.5 Conclusion:

Fear-related behavior, including aggression in dogs is a worldwide problem. Dog biting incidences lead to physical and emotional damage but also to hidden costs for our society. Possibly, such behavior is related to the function of specific brain centers, which functioning can be demonstrated indirectly by behavior, namely reversed reward (flexibility) and socio-emotional information processing, which have been demonstrated in human. Alternatively, general cognitive ability, assessed by task solving, could be of influence. Knowledge on this could aid strategies to predict, prevent and remedy fear-related behavior and aggression. In this study, it is tested if a relationship exists between fearfulness / aggressiveness and the traits task switching ability, reaction towards different types of vocalization and problem solving ability.

In accord with literature, in the current study a relationship (trend) between fear and aggression was found. Behavioral flexibility tests showed that high levels in the relative scores for fear and aggression did not explain variation in behavioral flexibility. Also, no relationship between the gender of the dog, the relative score for trainability and the behavioral flexibility was found. Forty-five percent of the dogs did not change side during the T-maze and on average 6.3 errors per dog were made. This indicates that many dogs were relatively inflexible. Fifty-five percent of the dogs did change side at some time during the reversed learning phase, suggesting the procedure of the T-maze to be useful. To obtain more insight why most dogs behave inflexible in the T-maze test it is advised to test the behavioral flexibility without the presence of the owner, to exclude possible influence of the owner. By making use of a certain learning criterion instead of a predetermined number of trials, the influence of rewarding the dog too often might be reduced. The cognition test did not show that variation in the cognition score of the dog could be explained by the relative scores for fear, aggression, trainability or the gender. However, stress behavior did explain variation in the cognition score. The lower the cognition score of the dog, the more stress the dog showed during the cognition test. This result reveals imperfections of the present test, as cognition scores in part mirror stress susceptibility. For future studies it is advised to set-up a test in such a way that stress cannot have an influence on the results. Also, the task must be designed in such a way that the dog can perform the task without guidance of an owner or an experimenter, to exclude the influence of having other people in the room. The socio-emotional information processing test gave more insight in the relationship between relative scores of fearfulness / aggressiveness and the reaction towards different types of vocalization. Fearful dogs discriminated between play barks and territorial barks in that especially the latter triggered social insecurity. Fearful dogs may be more sensitive to (threatening) social cues than aggressive dogs. Since there were no significant interaction effects of aggression scores and bark type (play, territorial) on behavioral response it cannot be stated that aggressive dogs were less sensitive to social cues than control dogs with low scores for aggression /

fear. Interestingly, instead of the prior expected insensitivity to social cues in aggressive dogs, increased sensitivity in fearful dogs was found.

This study could not demonstrate that a relationship exists between relative scores of fearfulness / aggressiveness and task switching ability and problem solving ability. Thereby, this study can not demonstrate that such behavior in dogs is related to the function of specific brain centers, which have been demonstrated in human. Also, insensitivity to social cues (vocalizations) in aggressive dogs was not found, but rather increased sensitivity to such cues in fearful dogs. Possibly, the results reflect that the study dogs did not suffer from pathological fear and / or aggression and were relatively normal. It cannot be excluded that in cases of severe (pathological) fear and / or aggression this is accompanied by impaired flexibility, sensitivity to social cues and cognitive abilities, but within a range of 'normal' scores such links seem to be lacking. More research on this topic is needed, as a higher number of pathological fearful and/ or aggressive dogs in combination with improvement in some of the behavior tests might lead to different outcomes. Overall, obtaining more insight in this topic will provide important knowledge which could aid strategies to predict, prevent and remedy fear-related behavior and aggression.

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