



Norwegian University of Life Sciences  
Faculty of  
Department of Animal and Aquacultural  
Sciences

Master Thesis 2014  
30 credits

# Laterality in Dogs in Response to Odour of Human Stress

Maren Helene Burdahl Teien

## **Abstract**

Can dogs smell that humans are stressed? Lateralization of behaviour and neural functions is found among humans and non-human animals like mammals, amphibians, birds, fishes and reptiles. The structures in the right hemisphere tend to be more active in response to novel stimuli, and intense emotions like fear, escape behaviour and aggression, while those in the left hemisphere tend to be more active in response to familiar stimuli and learning of systematic rules. Lateralization studies on dogs have been based on visual and vocal tests, while only a few studies have been based on dogs' primary sense – olfaction. Sniffing behaviour is measured in this study to discover laterality asymmetry in dogs when they were presented odours of human stress. Odour samples were collected from 8 women at 3 different stress levels: when odour giver was calm, when odour giver was immediately stressed by riding a roller coaster, and 15 minutes after the odour giver rode the roller coaster. Twenty-nine dogs completed 8 trials in which they were sequentially presented with four odour samples per trial in a counter-balanced sequence across trials: control sample with no human odour, and odours from calm, immediate stressed and later stressed odour giver. The dogs' use of left and right nostril was video recorded and analysed. There was no effect of the four treatment groups control, calm, immediate stressed or later stressed on total duration of sniffing or laterality of sniffing. However, a laterality effect of the odour givers' stress score was found, which indicate that dogs can sense how stressed a human is by sniffing at an odour sample. Dogs had a higher investigation time for the first trial when all stimuli were novel to the dog. Males showed a clear left bias on sniffing behaviour, while females show lower left bias. Nose length affected the dogs' investigation time, where dogs with longer nose had a lower investigation time than dogs with shorter nose. Long nosed dogs did also show less left bias than dogs with shorter nose. Laterality effects were also found in relation to daily physical training, previous experience and how obedient the dog reported to be. This study will attribute to a better understanding of the communication between dogs and humans and could lead to a new method to study dogs' emotions and brain activity.

## Sammendrag

Kan hunder lukte at mennesker er stresset? Lateralisering av atferd og neurale funksjoner finnes hos mennesker og en rekke ikke-menneskelige dyr som pattedyr, amfibier, fugler, fisker og reptiler. Strukturer i høyre hjernehalvdel tenderer til å være mer aktive i respons til ukjente stimuli og intense følelser som frykt, fluktatferd and aggresjon, mens strukturer i venstre hjernehalvdel er mer aktive i respons til kjente stimuli og læring av system og regler. Lateralitetsstudier på hunder har vært basert på visuelle og vokale tester, mens bare et fåtall av studiene har vært basert på hundens primærsans – luktesansen. Sniffatferd måles i dette studiet for å utforske lateral asymmetri hos hunder som ble presentert lukter av menneskelig stress. Luktprøvene er hentet fra 8 kvinner ved 3 ulike stressnivåer: når luktgiver var avslappet, når luktgiver var umiddelbart stresset mens hun tok en berg og dalbane, og 15 minutter etter turen med berg og dalbanen. Tjueni hunder utførte 8 testserier der hundene fikk lukte på fire luktprøver i hver testserie: luktprøve uten lukt fra menneske, luktprøve fra kvinne som var avslappet, luktprøve fra umiddelbart stresset kvinne og luktprøve fra kvinne 15 minutter etter hun ble utsatt for en sterk stressor. Hundens bruk av venstre og høyre nesebor ble filmet og senere analysert. Det var ingen effekt av de fire behandlingsgruppene kontroll, avslappet, umiddelbart stresset eller senere stresset. Derimot ble det funnet effekt av luktprøvegivers stress-score, hvilket indikerer at hunder er i stand til å sanse hvor stresset et menneske er ved å lukte på luktprøver fra dette mennesket. Hunder hadde lengre utforskingstid på den første testserien der alle luktprøvene var ukjente. Hanner viste en klar preferanse for å bruke venstre nesebor, mens hunner viste en mindre klar preferanse for venstre nesebor. Lengden på hundens snute påvirket utforskingstiden, der hunder med lenger nese brukte kortere utforskingstid enn hunder med kortere snute. Hunder med lang snute brukte også venstre nesebor mindre enn hunder med kortere snute. Lateralitetseffekter ble også funnet i relasjon til daglig fysisk trening, tidligere erfaring med nesearbeid og hvor lydig hunden var. Dette studiet bidrar til forståelsen av kommunikasjon mellom hunder og mennesker og kan være utgangspunkt for en ny metode som kan brukes for å studere hunders følelser og hjerneaktivitet.

## **Acknowledgement**

This thesis is submitted to obtain a Master degree (30sp) in Animal Science – Ethology at the Norwegian University of Life Sciences (NMBU). This is the final assignment after 5 years of study at the UMB/NMBU.

Twelve years old, I decided to become an “animal psychologist”, specialized in dogs. I was told that we could not know what a dog was thinking and that I instead could study the *behaviour* of animals to get *indications* of what was going on their mind. Thanks to Geir Marring and Morten Bakken for talking me into this field of study.

I train and compete with dogs and have noticed how sensitive dogs are to human emotional states. I wanted to figure out how.

Animal physiology and brain studies have always attracted me and it was clear to me that I wanted to include that perspective in my thesis. I also wanted to increase my understanding of how dogs are affected by human stress and nervousness. I ended up with studying brain laterality in dogs when they are presented odours of humans at three different stress levels.

I am really thankful to my supervisor Professor Ruth Newberry for helping me with choice of topic and analysing the data. Thank you for working day and night when it was needed most! Thanks to all volunteering dog owners (and dogs) that came to me and let their dog sniff at human sweat for 30 minutes, and thanks to the 8 volunteers that took the roller coaster and sweated on cotton pads for me. Thanks to Camilla Haugen for doing the blinded analyses. A huge thanks should also be given to Jørgen Rørvik, my motivator, supporter and love, who gave me perspective and pushed me forward every time it felt hopeless.

**Department of Animal and Aquacultural Sciences, NMBU**  
Ås, November 2014

**Maren Teien**

# Table of Contents

<b>1. INTRODUCTION</b>	<b>6</b>
1.1. CEREBRAL LATERALITY	6
1.2. EVOLUTION OF LATERALIZATION	7
1.3. LATERALIZATION IN DOGS	9
1.3.1. LATERALITY RELATED TO VISUAL STIMULATION	9
1.3.2. LATERALIZATION RELATED TO ACOUSTIC STIMULI	10
1.3.3. PAW PREFERENCE	10
1.4. LATERALITY OF THE OLFACTORY SENSE	12
1.4.1. LATERALIZATION OF OLFACTORY SENSE IN DOGS	13
1.5. AIM OF THE RESEARCH	13
1.6. QUESTIONS OF INTEREST	14
1.7. PRELIMINARY STUDY	15
<b>2. METHODS</b>	<b>17</b>
2.1. SUBJECTS AND HOUSING	17
2.2. ODOUR COLLECTION AND STORAGE	17
2.3 TEST APPARATUS	18
2.4 TEST PROCEDURE	19
2.5 VIDEO ANALYSES	22
2.6. STATISTICAL ANALYSES	24
<b>3. RESULTS</b>	<b>26</b>
3.1. TREATMENT EFFECTS	26
3.2 TRIAL EFFECTS	26
3.2 STRESS SCORE EFFECTS	27
3.3 SEX EFFECTS	29
3.4. NOSE LENGTH EFFECTS	30
3.5 EFFECTS OF OBEDIENCE	31
3.6. EFFECTS OF DAILY PHYSICAL TRAINING	33
3.7 EFFECTS OF PREVIOUS EXPERIENCE WITH NOSE WORK	35
<b>4. DISCUSSION</b>	<b>37</b>
4.1. DISCUSSION OF RESULTS	37
4.2. CHALLENGES OF STUDYING DOGS	40
4.3. CONSEQUENCES OF REWARDING SNIFFING BEHAVIOUR	41
4.4. DISCUSSION OF PREVIOUS STUDIES ON OLFACTORY LATERALITY IN DOGS IN RELATION TO CURRENT FINDINGS	41
<b>5. CONCLUSIONS</b>	<b>43</b>
<b>6. LITERATURE</b>	<b>44</b>
<b>APPENDIX 1</b>	<b>48</b>
<b>APPENDIX 2</b>	<b>49</b>
<b>APPENDIX 3</b>	<b>50</b>
<b>APPENDIX 4</b>	<b>53</b>

# 1. Introduction

## 1.1. Cerebral laterality

The mammalian brain is divided into two cerebral hemispheres. There are left and right halves of structures in the brain, like thalamus, amygdala, hippocampus, caudate and other important structures for higher mental processes (Davidson & Haugdal, 1998). The orbitofrontal cortex and amygdala are essential parts of the brain when processing olfactory stimuli. These regions are distributed over the two halves of the brain, the left- and right cerebral hemisphere (Royet & Plailly, 2004). Also the peripheral nervous system has a left and right branch (Davidson & Haugdal, 1998). Intense emotions, such as aggression, escape behaviour and fear (summarized by Rogers & Andrew, 2002) and response to novel stimuli, or more precisely, the familiarity judgment task, is associated with activity in the *right hemisphere* of the brain (Royet & Plailly, 2004). It is also reported that there are higher levels of noradrenalin in the right side of the brain, compared to the left side of the brain (Oke et al., 1978) The *left hemisphere* responds to familiar stimuli and is related to learning of systematic rules (Vallortigara, 2006), and is dominated by dopaminergic activity (Willing, 1995). Brain asymmetry is found in control of both cerebral cognitive functions, emotional states and cerebral regulation of autonomic-physiologic processes (Witting, 1995). Specialization of the two hemispheres of the brain controls functional systems where one side of the brain is more critically involved in some particular functions, while the other side of the brain has higher importance in other functions. Most processes are controlled as an interaction between the two hemispheres of the brain (Witting, 1995).

Lateralization in behaviour and neural functions is found in humans and non-human animals like amphibians, birds, fishes, mammals and reptiles (Bradshaw & Rogers, 1993; Rogers & Andrew, 2002; Siniscalchi et al., 2008) as well as among invertebrates (Rogers & Vallortigara, 2008). It is suggested that cerebral asymmetry is a functional feature among vertebrate brains (Siniscalchi et al., 2008).

Humans and Macaque monkeys (*Macaca mulatta*) process species-typical vocalizations in the left hemisphere of the brain (Hauser & Anderson, 1994). Male lemurs (*Microcebus murinus*) show the same tendencies, as male lemurs show significant right-ear (left hemisphere) bias in response to conspecific communication

sounds (Scheumann & Zimmermann, 2008). Both humans and rhesus monkeys exhibit right hemisphere dominance for facial expression of negative emotions like fear (Hauser, 1993). The same asymmetric behaviour in use of senses related to negative emotions is found in dogs as a response to both visual stimuli and to acoustic stimuli (Siniscalchi et al., 2008; Siniscalchi et al., 2010).

## 1.2. Evolution of lateralization

Ethological evidence shows that asymmetrical sensory systems are not rare for animals in wild or domestic environments. At first glance, this could look like a disadvantage or weakness. So is there any reason why most animals have splitting of brain functions? And why is it always the left hemisphere that is specialized to find targets in rapid search, while the right hemisphere is specialized in approaching to selected targets and seizure (Vallortigara, 2006)?

Brain asymmetry, or lateralization, in dogs can be investigated by studying how dogs use their senses. The visual sense and the sense of hearing are contralateral senses, which means that the sense organ and the sensory system in the brain are located on opposite sides of the body. The olfactory sense is ipsilateral which mean that right nostril sends sensory information to the right half of the brain, while the left nostril sends sensory information to the left side of the brain (Broman et al., 2001).

In a lateralized brain, each hemisphere carries different functions and can process different stimuli. Lateralization is ubiquitous among vertebrates, but less attention has been paid to *why* lateralization is ubiquitous. Vallortigara (2006) discuss the effect of visual lateralization in biological fitness in a study looking into prey catching and agonistic behaviour. A prey was placed mechanically in a horizontal plane around the toad (*Bufo bufo*) so it entered either the right- or the left monocular visual field of the toad (Robins et al., 1998; Vallortigara et al., 1998). When the prey entered first the left and then the binocular field of vision, almost all of the tongue-strikes occurred in the right half of the binocular field. A more symmetrical distribution of strike in left and right halves of the binocular fields was observed when the prey first came in to the right binocular field of vision. It seemed like the prey had to enter the right half of the binocular field to evoke predatory behaviour in the toad. In the same way, the

toads were more likely to attack conspecifics to their left side and ignore them if they appeared on the right. (Robins et al., 1998; Vallortigara et al., 1998). Similar results have been obtained in birds (i.e. domestic chicks (*Gallus gallus*) and pigeons (*Columba livia*)) (Diekamp, 2005) and lizards (*Anolis sp.*) (Deckel, 1995).

Diekamp et al. (2005) tested behaviour laterality in the domestic chick and the pigeon. The birds were given food when they could only move their head and showed a significant leftward bias. The left eye seemed to be the most useful eye when tracking down grains and food. This could be disadvantageous because grains and food are unlikely to be more commonly located on the left side of bird's midline in a natural environment. The right eye, however, seems to be specialized for selecting targets and their seizure. The result is that these birds can spot grains and foods with their left eye, and at the same time monitor the environment for threats.

When one hemisphere is busy with those tasks it is specialized for, the other hemisphere is free to perform additional functions that are not taken care of by the first hemisphere (Denenberg, 1981). In this way, neural tissue can be spared, as there would be no or little useless duplication of brain activity. But why is it not a 50:50 distribution of left and right cerebral hemisphere activity in a population? Collins (1985) showed with mice that it was possible to select for the strength of lateralization. If left- and right lateralization in a population is equal, behaviour will be predictable to other organisms such as predators and competitors. The predictability would have been lower if the left- and right lateralization had a 50:50 distribution in a population. The most common situation among vertebrates is that populations are consisting of left-type and right-type individuals in unequal numbers (Vallortigara, 2006). This has been shown to be evolutionary stable if the costs and benefits depend on the *frequency* of being lateralized to left or right (Vallortigara, 2006). The evolutionary history of lateralization could be that lateralization appeared in the brain on an individual level, which lead to lateral biases in behaviour. Lateralized individuals started to interact with each other and if the lateralization had an effect on their success, selection pressure on genes that favoured lateralization would appear.



## 1.3. Lateralization in dogs

### 1.3.1. Laterality related to visual stimulation

Dogs show visual lateralization and asymmetry in use of eyes when looking at different stimuli (Guo et al., 2009; Siniscalchi et al., 2010). When humans and chimpanzees look at a human face, they show bias of using left eye and move both eyes so they are looking most at the right side of the face they are watching (Guo et al., 2008). Similar results have been obtained in dogs when they are watching a human face (Guo et al., 2008). This is called “left gaze bias” and only happens when we study faces. The hypothesis is that left gaze bias is functional because emotions are expressed most clearly on the right side of the human face (Guo et al., 2008). This ability to read human faces could have evolved after thousands of years of breeding and interactions between dogs and humans.

Siniscalchi et al. (2010) did a visual study on dogs where the dogs were eating while silhouettes of a dog, a cat and a snake were shown on a panel on both sides of the dog, left and right visual hemifields of the dog, to see what side the dog would turn its head towards. The silhouettes were presented repeatedly to see if the response changed over time. The results showed that dogs preferentially turned their head to the left side (right hemisphere) in response to the silhouettes of the cat and the snake, while there was no bias to the dog silhouette. This indicates a specialization of right hemisphere. The right hemisphere of the brain is known as specialized for expression of intense emotions like aggression, escape behaviour and fear (Mac Neilage et al., 2009; Rogers & Vallortigara, 2008; Vallortigara, 2000). Silhouettes of snakes are known as bringing up fear response in most mammals (Lobue et al., 2008) and the cat silhouette had a defensive threat posture, which has been shown to be more likely to activate the right hemisphere in other species (Rogers & Andrew, 2002; Siniscalchi et al., 2010; Sovrano, 2004). The dog silhouette was showing a dog with tail down and a neutral posture, which do not contribute to any lateralized brain activation (Parr & Hopkins, 2000; Siniscalchi et al., 2010). Even more interestingly, there was a trend for shifting from turning head to the left (right hemisphere) to turning head to the right (left hemisphere) with repeated presentations of stimuli. Such shift from activation of right hemisphere to left hemisphere as the stimuli gets more familiar and categorized has been reported across many species and conditions (Andrew, 2002;

Vallortigara et al., 1998). The experiments by Siniscalchi et al. (2010) suggest a higher right hemisphere activity can be expected for stimuli of higher emotional valence. This supports that right hemispheric sympathetic outflow is more effective, while parasympathetic outflow is under left hemisphere control (Wittling, 1995).

### **1.3.2. Lateralization related to acoustic stimuli**

Behaviour asymmetry is also found in relation to acoustic stimuli in dogs. When Siniscalchi et al. (2008) simultaneously played back the same sound from the left and the right, dogs were more likely to turn their head to the left when hearing the sound of a thunderstorm whereas they were more likely to turn their head to the right when hearing barks recorded from an isolated dog, a dog disturbed by a stranger knocking on the door, or a playing dog. Siniscalchi et al. (2012) observed that dogs were also more likely to turn their head to the right when hearing playbacks of their own barks. This suggests that the left hemisphere of the dog brain responds to familiar stimuli, while the right hemisphere of the dog brain is more active when the stimuli is related to intense emotions. This was found by using a head-orienting procedure and needs to be further investigated with studies of the brain to get more information about brain activity.

#### **1.3.2.1. Tail wagging**

Asymmetric tail wagging in dogs can be associated with different types of emotional stimuli (Quaranta et al., 2007). Dogs showed higher amplitude of tail wagging movements to their right side to stimuli eliciting approach responses, while tail movements to the left side predominated when stimuli elicited withdrawal responses. Dogs responded with right tail wagging (left brain activation is dominating) to familiar stimuli and “positive” stimuli such as seeing a dog’s owner. Tail wagging to the left (right brain activation is dominating) when the dogs were exposed to novel and “scary” stimuli, such as seeing a dominant unfamiliar dog.

#### **1.3.3. Paw preference**

The existence of handedness in humans is well known and accepted, as it exists in all modern human populations and 90% of our species prefer to use their right hand for complex behaviours (McManus, 2002). Lately, the same type of handedness has been discovered also among animal species (summarized in Poyser et al., 2006); like rats

(e.g. Collins, 1975), cats (e.g. Elalmis et al., 2003), horses (Murphy et al., 2005) and dogs e.g. Quaranta et al., 2004, Wells, 2003). Even though many studies on animals support the theory of handedness, the results have been inconsistent. In the same species, some studies can show left bias, while other studies can show right bias in the same species. This could be due to other variables, like for example sex, age or chemical/hormonal balance in the prenatal phase that could affect brain development (Poyser et al., 2006).

Both Wells (2003) and Quaranta et al. (2004) have reported that male dogs show a higher bias of left-handedness than females. Rogers & Andrew (2002) showed that hormonal effects on brain development absolutely affect laterality effects. Sex and its related hormones can therefore have a directly effect on laterality. On the other hand, later studies on handedness among dogs and its relation to gender have varying results. Wells (2003) showed that male dogs showed a significant tendency of using left paw when they responded to the command “give paw” (or other commando with the same learned behaviour) while the same males did not show any significant tendency of paw preference in a test where they where supposed to remove a blanket from their head. Quaranta et al. (2004) did a quite similar test, which gave more or less the same result – males showed a tendency of using left paw. Right paw preference among females was shown, though with a low significance level. Poyser et al. (2006) tested paw preference in dogs for three different tasks: removing a piece of tape from its nose, use of paw when chewing on a bone and rolling a ball that contained food. They found left paw preference for males in only one of the tests, which was to roll the ball. They could also see that tendency of left paw preference declined with repeated presentations. The decline with repeated sessions could indicate that laterality is affected by novelty, which is supported by a range of studies (Rashid & Andrew, 1989; Regolin & Vallortigara, 1996; Rochers et al., 2008; Siniscalchi et al., 2008; Sovrano, 2004; Vollortigara & Andrew, 1994; Vollortigara, 1992). The left paw is controlled by the right cerebral hemisphere, which is, as earlier mentioned, involved when experiencing some kind of novelty (Rogers & Andrew, 2002). Summarized, gender differences in laterality are shown as long as the stimulus is novel to the dog. The tendency of laterality and gender differences in laterality decreases if the stimulus is no longer novel to the dog.

Paw preference in dogs is documented to be associated with the immune system. Quaranta et al. (2004) measured immune parameters like total number of white blood cells including lymphocytes, granulocytes and monocytes; leukocyte formula; total proteins and  $\gamma$ -globulins) and showed that this could be related to paw preference. There were higher levels of lymphocytes among left-pawed than right-pawed and ambidextrous dogs. The level of granulocytes and  $\gamma$ -globin was higher among right-pawed than left-pawed dogs and ambidextrous dogs. These findings indicate that brain asymmetry also can be an effect of the immune system.

The immune system is controlled hormones in the brain by the dopaminergic and the hypothalamic-pituitary-adrenal axis (Quaranta et al., 2004). We can assume that right-paw preference is related to higher activity in the left cerebral hemisphere. In what order elevated hormones or paw preference are working is hard to say, but it could be that left hemispheric activation increases the secretion of stress hormones in right-pawed dogs, or that right pawed dogs have higher reactivity to stress and therefore will show a higher immune response (Quaranta et al., 2004).

#### **1.4. Laterality of the olfactory sense**

In contrast to other senses, the sensory receptors of olfaction and its centre in the brain are ipsilateral, which mean that they are placed at the same side of the body. The ipsilateral olfaction sense has a short and direct linkage between peripheral neurons and higher brain structures (Broman et al., 2001). Receptor information goes directly from each nostril to the olfaction bulb and continues to the primary olfactory cortex in the same hemisphere (Broman et al., 2001). Laterality of olfaction exists even though it is ipsilateral. Odours sniffed in with the right nostril are in human experiments rated as more familiar than odours sniffed with the left nostril (Broman et al., 2001). Humans seem to feel more emotionally positive when they sniff with their right nostril than when they sniff with their left nostril.

Human studies of olfaction asymmetry give divergent results about its relation to handedness. It seems like it can be a connection between these two features, where right-handed humans tend to be more sensitive in the right nostril (Broman et al., 2001; Youngentob et al., 1982), while some larger studies found conflicting results

(Hummel et al., 1998; Martinez et al., 1993). Broman et al (2001) reported that there is a right-nostril advantage for perceived odour familiarity that could reflect differential functioning of the cerebral hemispheres and a right-side advantage for tasks that are related to odour perception.

#### **1.4.1. Lateralization of olfactory sense in dogs**

The olfactory sense is believed to be the most sensitive sense in dogs (Berns et al., 2014). Behavioural lateralization in dogs is found in asymmetric tail wagging (Quaranta et al., 2007), paw preferences (Quaranta et al., 2004; 2006), visual gaze (Siniscalchi et al., 2010) and head orientation to acoustic stimuli (Siniscalchi, et al., 2008), but little about asymmetry in relation to olfaction have been published. This seems strange, as the olfactory sense is known as being the primary sense for dogs. Dogs have more than 220 million olfactory receptors in their nose, while humans have only about 5 million olfactory receptors (Bear et al., 2007).

Siniscalchi et al. (2011) investigated the left and right nostril use in dogs when sniffing at different emotive stimuli. Dogs used their right nostril for non aversive stimuli (food, vaginal secretion, cotton and lemon) but shifted over to left nostril bias with repeated stimuli presentations. Aversive stimuli (adrenaline and sweat from veterinarian) gave consistent right nostril bias over all stimuli presentations. This suggests the existence of right cerebral hemisphere bias for novel stimuli that shift to left cerebral hemisphere bias as the stimuli gets familiar and develops to more routine behaviour. Right cerebral hemisphere bias is sustained for aversive stimuli, maybe because stress is controlled by the sympathetic hypothalamic-pituitary-adrenal axis that is mainly under control of the right hemisphere (Siniscalchi et al., 2011).

#### **1.5. Aim of the research**

Cerebral laterality is found in a wide range of species, dogs included (e.g. Bradshaw & Rogers, 1993; Rogers & Andrew, 2002; Siniscalchi et al., 2008; Vallortigara, 2006). However, few studies on dogs are based on the olfactory sense (Berns et al., 2014; Siniscalchi et al., 2011). It would be interesting to study cerebral laterality in dogs based on the olfactory sense, since the olfactory sense is the primary sense for dogs (Siniscalchi et al., 2011). It is commonly thought that dogs' behaviour is affected by human stress (e.g. when we compete in sports with our dogs and the

handlers are nervous). It would therefore be interesting to get information about how dogs are affected by the smell of human stress through an odour sample collected on cotton pads, where only a smell of stress is presented to the dog. Furthermore, if the methodology in this research allows us to infer cerebral laterality, this could lead to a new method to study dogs' emotions and brain activity.

## 1.6. Questions of interest

This thesis is a study on how lateralization behaviour among dogs is affected by odours from stressed humans.

1. Do dogs show difference in lateral asymmetry by differential use of left nostril (left cerebral hemisphere) and right nostril (right cerebral hemisphere) when sniffing at odours from humans in the following stress levels: calm, immediate stressed and after a stressed situation (later stressed)?
2. How will these different odours (human stress levels) affect duration of sniffing with left and right nostril and laterality of nostril use (left or right) for the first sniff and last sniff each odour presentation?
3. Can laterality of olfaction be related to sex, age, nose length, distance between nostrils or daily activity/training?

It was hypothesized that odours from more stressed people would produce greater emotional arousal in dogs than those from calm people, resulting in less sniffing and greater right nostril bias when sniffing. It is predicted that odour from more and less stressed human would be aversive for the dog, while odour from calm human would be non-aversive. How stressed the odour giver was (odour givers stress score), is predicted to affect how aversive the odour is. A higher proportion of right sniff is expected for the aversive odours than for the non aversive odours. It is predicted to find a shift from right to left laterality for the non-aversive odours with repeated presentations (trials) and decrease in novelty.

### **1.7. Preliminary study**

The methodology of odour-studies can be challenging, as the dog can have other motivations than sniffing at the presented odour. The dogs can be trained to sniff on the presented odours, but this could lead to errors as the dog may have positive expectation of the reward and thereby not display their natural response to the odour stimuli. The dog can also be more interested in other odours than the odour sample we are presenting, such as the odour of food in the room or the odour of the testing equipment.

Preliminary studies were used to test different test apparatus to find one what favoured sniffing and excluded most other options. The dogs needed to be relaxed and be comfortable in the test apparatus to favour concentration, attention and sniffing behaviour. In addition, the odours and the dog's nose needed to be visible to the camera.

The dogs used in the preliminary study were the authors' own dogs; a 10 year old border collie bitch and a 4 year old border collie male. Odour samples used were control odours and odours collected from the author at calm stress level. The same odours samples were used repeatedly.

First try was constructed as a copy of Siniscalchi et al. (2011). I tried to get in contact with the authors to work out more details, but got no response. The test apparatus was simple: two plastic panels of 50x30 cm created a corner to favour a centred position of the dog in front of a video camera on a tripod. A stick with odours samples at the tip was attached to the camera, so the dogs' nose was visible right in front of the camera. When the dogs were presented to the test apparatus singly, they showed interest to the camera and tripod. The experimenter told the dog to sit while she touched the plastic tube, which had the odour at the tip, and motivated the dog by using voice. Next, the dog was given a "free signal". Both dogs responded by paying more attention to the plastic tube, but they started to bite the stick from the side instead of sniffing at the tip of it. They also tried to freeze with their nose on the tube, as they are trained to do this with other objects in obedience and tricks training. Out of 10 repetitions for each dog, where each repetition lasted for 3 minutes, only one of them showed sniffing behaviour once.

The next test apparatus was therefore designed to avoid the misunderstanding of biting the stick. The testing area consisted of two sides of lattice, 136x88 cm, attached to a screen of Plexiglas ® with the dimension of 80x100 cm. Odour samples were inserted in cytotrine vials which could be inserted into a suitable drilled hole in the Plexiglas, at a height suitable for the dogs' size – about neck height. A digital video camera was located on a tripod behind the Plexiglas. This test apparatus reduced biting behaviour. To make the dog attend to the plastic tube/odour, motivation was still necessary. The motivation was to show the dog that the tube was inserted into the hole in the plastic panel and use talk to the dog in a positive voice to keep the dog's attention. The dog was rewarded for attention after some of the odour presentations. Out of 40 repetitions for each dog, sniffing behaviour was shown in all repetitions within 20 seconds. The same odour sample was used in all 40 repetitions.

With this background, the test apparatus with Plexiglas was chosen for the apparatus design in this thesis. It was expected, based on the preliminary observation, that each dog would sniff at 32 presentations to complete the whole test.



## **2. Methods**

### **2.1. Subjects and housing**

The subjects were 32 pure breed dogs of different breeds (see Appendix 1) aged from two to seven years. Dog owners applied to participate in the experiment by filling out an online form with a short screening questionnaire (see Appendix 3). Dogs that were healthy, were in the age range of 2-7 years and where owner could bring the dog to testing at a convenient time were included in the studies. The age criteria were set to only include mature dogs that were still young and healthy. Bitches that were in a 3 months period around heat or that were pregnant or had litters younger than 3 months were not allowed to attend the experiment because varying gonadal steroid levels could affect behaviour and mask effects of the experimental treatment. The questionnaire consisted of questions that could give additional information about factors that could interact with lateral asymmetry. All dogs were housed in their home together with their family. They got stimulation every day both physically (e.g. walks, running, play) and mentally (e.g. obedience training based on positive reinforcement, searching tasks). These were included as criteria to be included in the study. Nutritionally complete commercial age-appropriate food was given once or twice a day as the dogs were used to before the experiment period. Water was available ad libitum.

### **2.2. Odour collection and storage**

Four different odour conditions were presented to each dog:

- Cotton pad containing sweat of a person, who was a stranger to the dog, at three different stress-levels:
  - Calm: Sample was collected when the person had been sitting down and relaxed for at least 30 minutes when the odour was collected. The duration of collection was 5 minutes.
  - Immediate stressed: Sample was collected while the person took a ride on a wooden roller coaster, “Thunder coaster”, built in 2001, 39m at highest point and producing a speed up to 100 km/h at “Tusenfryd”, Vinterbro, Norway, with cotton pads under armpits. The duration of collection time was 5 minutes.

- Later stressed: Sample was collected 15 minutes after the person had completed a ride on the Thunder coaster. The duration of collection time was 5 minutes.
- Control: Clean cotton pad (no sweat), treated the same way as the samples with odour from a human.

The day of odour collection was 29<sup>th</sup> of September 2014. Odour samples were collected directly on cotton pads (Softstar cotton pads, ICA Norge AS). All odours were collected from 8 healthy women volunteers aged between 20 and 40 years. They were randomly given a number between 1 and 8 to keep their identity anonymous. They had two cotton pads in each its armpit, held in place by clean clothing, for 5 minutes per stress-level.

Immediately after the collection the odours samples were quickly cooled down in a mobile cooler filled with ice. The four cotton pads collected per person per sampling treatment were later cut into 8 pieces to give 32 pieces per person per stress level. The pieces of cotton pads from the odour givers' armpits were placed singly in 2 mL plastic tubes (NALGENE ® cryogenic vials, Thermo Fisher Scientific Inc, Waltham, Massachusetts, USA) marked with number and stress-level, and secondly stored in plastic bags with zip-lock. All handling was done with medical examination gloves (ABENA A/S, 6200 Aabenraa, Denmark). The odour samples were stored in a freezer with -80°C until they were used in the tests, maximum 4 weeks after they were collected.

The dog owners that gave odour samples were instructed not to use deodorant/antiperspirant/perfume for 2 days before the experiment, to prevent samples from containing odorants such as perfume or alcohol, until after the collection of the cotton pads was over. They were also restricted from having any form of tobacco or caffeine on the day of odour collections.

### **2.3 Test apparatus**

The tests were performed in a living room, cleared of all furniture and personal belongings.

The test apparatus consisted of two sides of wire fence with the dimensions of 136x88 cm, attached to a screen of Plexiglas® with the dimensions of 80x100 cm. Each odour sample inside its Nalgene tube was inserted in a hole in the Plexiglas, at a height suitable to the dog's size (about neck height). The Plexiglas contained multiple holes at different heights to accommodate different dog sizes. A digital video camera (Canon EOS 700d, Canon U.S.A., Inc., Melville, New York, set on video function) was located on a tripod behind the Plexiglas (Figure 1). A circle with a radius of 5 cm around the hole for the plastic tube, scratched into the Plexiglas with a needle indicated the sniffing-area.

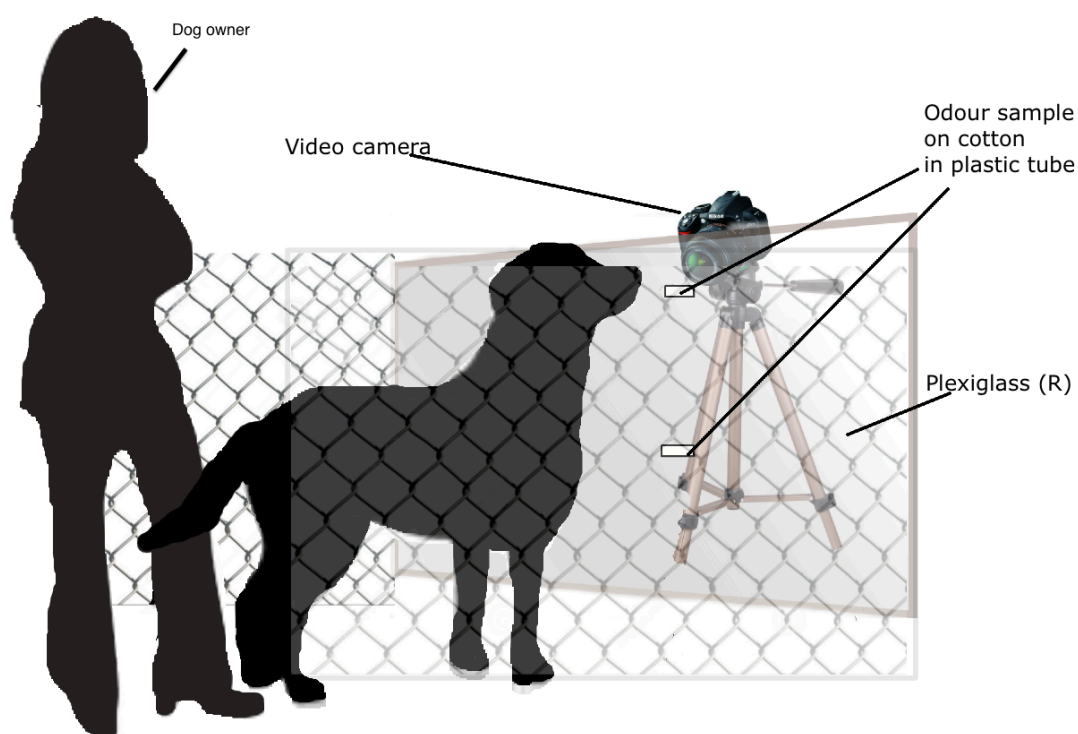


Figure 1: Schematic presentation of test apparatus

Pictures of the test apparatus in use are attached in Appendix 4.

## 2.4 Test procedure

All tests were performed in the period 11<sup>th</sup> of October to 20<sup>th</sup> of October 2014.

The dog was presented to the odours separately (one odour at a time). Effect of order was avoided by counterbalancing the 4 samples (SC, SIS, SLS, Control) as shown in Table 1. Each dog was given samples from one person, randomly assigned to each dog as (see Appendix 1). Each dog did 8 trials consisting of four odour

presentations/sniffing bouts (calm, immediate stressed, later stressed and control). The order of the odours was different in each trial (Table 1 and Appendix 2). This mean that at first trial, dog nr 1 (as an example) started with order code a (C - IS - LS - Control). On trial 2, the same dog got the odours presented in order b (IS - LS - Control - C), and so on, until 8 trials were completed. Dog 2 started with the order code b before continuing with c, d, e..., h, a. The odour plastic tubes were unused for each odour presentation – none of the odour samples were presented repeatedly.

**Table 1 Odour presentations were counterbalanced. Each order code represents a unique order of the four odours; calm (C), immediate stressed (IS), later stressed (LS) and Control.**

Order code	Odour order
a	C - IS - LS - Control
b	IS - LS - Control - C
c	LS - Control - C - IS
d	Control - C - IS - LS
e	IS - C - LS - Control
f	C - LS - Control - IS
g	LS - Control - IS - C
h	Control - IS - C - LS

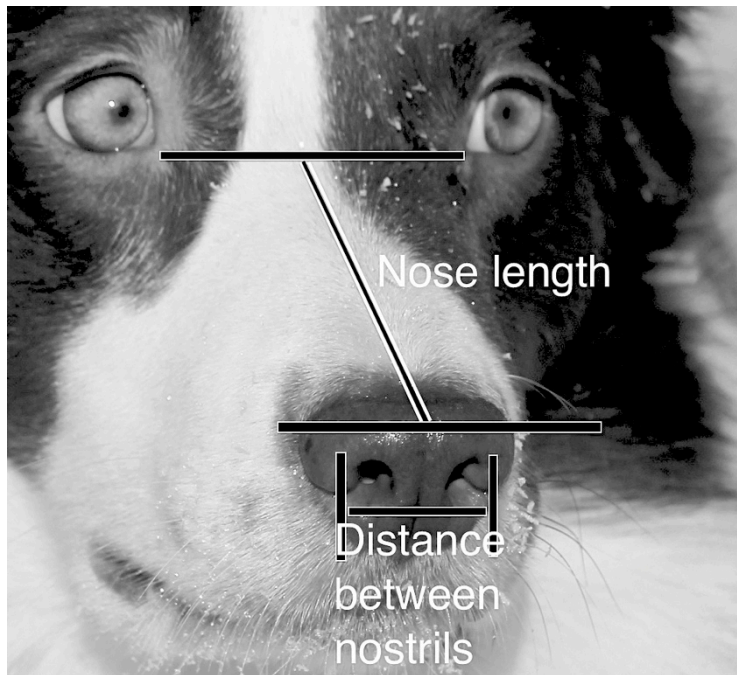
Stimuli were presented to each dog while their sniffing behaviour was video recorded. The dog was introduced to the room and was allowed to examine the room while the experimenter prepared the odour sample according to the predetermined order.

Every dog owner was instructed to act naturally, be quiet and have a relaxed body language. They could choose if they wanted to have their dog on a leash, or without, depending on what the dog was the most comfortable with. The dog was faced towards the camera at a 50 cm distance to the camera, and the owner was positioned beside the dog during the whole experiment (Figure 1). The owner was blind to the order of the odour treatments.

The video camera was started at once the dog was inside the test apparatus. Continuous recording was done until half of the trials were completed (trial 1-4).

Then, the dog was given 3 minutes break before the procedure was repeated with the last four trials (trial 5-8).

The odour samples were moved from the  $-80^{\circ}\text{C}$  freezer to a normal  $-20^{\circ}\text{C}$  freezer one day before the experiment. They were moved to room temperature 2 hours before they were presented to the dog and the cap was kept on the plastic tube until the odour was inserted in the hole in the Plexiglas. The experimenter got the dogs' attention and attached the odour (in the plastic tube) into the hole in the Plexiglas. The dog was allowed to start sniffing whenever it wanted to. The experimenter motivated the dog to sniff at the odour by pointing at the plastic tube from behind the Plexiglas, and talking in a friendly manner to the dog. The dog was rewarded with treats brought by its owner after sniffing at the odour in some of the trials. Not every sniffing was rewarded, but the reward frequency was increased if the dog lost interest in the odour samples. The reward was dropped over the top of the Plexiglas. The timing for the reward was varying because the goal of the reward was to keep the dog's attention, not to teach the dog a particular behaviour. A reward was given for instance when the dog was moving away from the odour, sniffing at the hole without plastic tube /odour in, looking at the experimenter or responding to its name.



**Figure 2** Measurements of the nose length and distance between nostrils was taken respectively on the top of the nose tip to between the eyes, and distance between each wing of the nostril at the widest.

All 8 trials consisting of 4 odour presentations, were performed the same day and took about 30 minutes including preparation and breaks.

The dog's length of nose and distance between nostrils were measured after the trials (Figure 2).

Two windows were kept wide open to ventilate the room during the whole process. The room was cleaned after each dog.

## 2.5 Video analyses

The following data were analysed from the videos, based on the dog's first sniffing bout at each odour stimulus presented, which was considered to start when the dog first placed a nostril within 5cm of the odour tube. The bout ended when it moved at least 10 cm away from the tube or turned its head to the side and did not start sniffing again within two seconds:

- Total duration of sniffing with one or both nostrils within 5 cm of the odour tube, including sniffing with the left, right or both nostrils, during the dog's first sniffing bout at the tube.
- Proportion of sniffing with left nostril and right nostril. Proportion of sniffing with left nostril was the total time spent sniffing with left nostril within 5 cm of the odour tube divided by the total time spent sniffing with the left nostril and the right nostril within 5 cm of the odour tube, during the dog's first sniffing bout at the odour tube. Other sniffing was not included in calculation since it did not provide laterality information.
- Nostril used (left or right) for the first sniff and the last sniff within 5 cm of the odour tube for each sniffing bout.

Video recordings were analysed in the program Solomon Coder Version beta 14.10.04 (Copyright © 2014 by András Péter, Budapest, Hungary, <http://solomoncoder.com>). Recordings were analysed and scored after a detailed behaviour description, the ethogram (Table 2). The observations registered in Solomon Coder were transferred to a Microsoft Excel file. Other measurements like size of the nose and answers from the questionnaire were included in the same file.

Duration of sniffing with left and right nostril was registered. Also an “other” score was used to register sniffing where it was unclear that left or right nostril was used. The duration for each nostril was summed so each sniffing bout gave duration (number of seconds) for each nostril (and “other”) Duration was scored in 0,2 second intervals. The following observations for each sniffing bout was analysed: duration sniffing with left nostril (L), duration sniffing with right nostril (R), duration other sniffing (O), total duration sniffing (L + R + O), first nostril used at the odour (left or right) and last nostril used at the odour (left or right).

**Table 2: Ethogram with description of the analysed behaviour**

Category	Behaviour	Description of behaviour	Code	Measurement
Sniffing	First sniff	First sniff at odour. Nostril wing vibrating.	L (left) or R (right)	Event
	Sniffing with left nostril at odour sample	Left nostril in contact with the plastic tube with odour inside. Wing of left nostril vibrating.	L (left)	Duration
	Sniffing with right nostril at odour sample	Right nostril in contact with the plastic tube with odour inside. Wing of right nostril vibrating.	R (right)	Duration
	Other sniffing	Both nostrils vibrating at the Plexiglas beside, in a 5 cm radius to the plastic tube with odour.	O (other)	Duration
	Last sniff	Last sniff (nostril wing vibrating) at odour before the dog moves nose at least 10 cm away from the Plexiglas or turned its head to the side, and did not start sniffing again within two seconds.	L (left) or R (right)	Event

Three dogs were eliminated from the study because of fear of the test apparatus or constant barking behaviour. This left 928 recordings for analyses (29 dogs x 4 treatments x 8 trials).

An external blinded experimenter analysed video recording of 3 randomly chosen dogs, to compare the scores done by the internal experimenter. The scoring of the two experimenters was identical.

## 2.6. Statistical analyses

SAS Stat Software Version 13.2 (Copyright © 2014 SAS Institute Inc., SAS Campus Drive, Cary, North Carolina 27513, USA) was used for the statistical analyses.

Generalized linear mixed models (The GLIMMIX Procedure in SAS) were used.

Poisson distribution gave the best fit to the analyses, as the data were not always normally distributed.

Type III test for fixed effects was used to investigate the effect of fixed factors and covariates (Table 3) on the measurements:

- Total duration sniffing: the total duration of sniffing with left nostril, right nostril and other sniffing, used as an indication of the dogs motivation to investigate the odour.
- Proportion of sniffing with left nostril of time sniffing with the left and right nostrils, to see laterality effects.
- First nostril used at the odour (left or right) and last nostril used at the odour (left or right), both as indications of laterality and to see if the dogs shifted from one nostril to another for each sniffing bout.

Appendix 3 shows the questionnaire all the dog owners filled out.

**Table 3: Information about the factors considered in the analyses.**

<b>Factor</b>	<b>Description</b>
Treatment	Effect of the different odour samples; calm, immediate stressed, later stressed and control.
Trial	Effect of trial 1, 2..., 7, 8, to see novelty effect.
Stress score	The odour givers answered on a scale from 1-10 how stressed/scared they were when they gave their immediate stressed odour sample, where 1 is not stressed at all and 10 is extremely stressed. The number they gave is considered as their stress score and could have an effect for how "aversive" the odour sample is.
Sex	The effect of different gender (male or female).
Age	Age effect in years.
Nose length	Effect of the dog's nose/snout size (measured in millimetres).
Nostril distance	Effect of the dog's distance between its nostrils (measured in millimetres).
Obedience	The dog's owner gave their dog a number from 1-10, where 1 is not obedient at all, 5 is "listens sometimes" and 10 are extremely obedient.
Physical training	The dog's owner described how much physical exercise they gave their



---

	dog normally per day, with the options; 20 minutes or less, 40 minutes, 1 hour, 2 hours, 3 hours, 4 hours or more.
Mental training	The dog's owner described how much mental exercise (e.g. learning exercises) they gave their dog normally per day, with the options; none, 10 minutes, 20 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours or more.
Previous experience	The dog's owner informed if the dog was trained for any nose work, as tracking, searching, ID-search etc.) or not.

---

Analyses were done trials with data pooled over eight trials per dog to see treatment effects (Table 4). Next, analyses were done across and treatments with data pooled for all four treatments per dog to see trial effects (Table 5). Random effects included dog, treatment or trial, respectively, and residual error. Pairwise comparisons were based on differences in least squares means, adjusted for multiple comparisons using the Tukey-Kramer method. Effects were considered significant at  $P < 0.05$ .

### 3. Results

#### 3.1. Treatment effects

There was no significant effect of the treatments odour of calm, immediate stressed or later stressed or the control on any of the sniffing (Table 4).

There was found no significant effect of age, nostril distance or mental training, as shown in Table 4 and Table 5.

**Table 4: Effects of treatments and covariates on sniffing behaviour variables. P-values for analyses of data pooled over 8 trials per dog.**

Sniffing behaviour	Treatment	Stress-Score Effect	Sex	Age	Nose length	Nostril distance	Obedience	Physical training	Mental training	Previous experience
Total duration of sniffing	0,512	0,360	0,416	0,177	0,004	0,680	0,951	<0,001	0,838	0,006
Proportion sniffing with left nostril	0,922	0,002	0,127	0,312	0,015	0,997	0,002	0,045	0,125	0,653
First sniff	0,913	0,013	0,034	0,933	0,036	0,732	0,012	0,003	0,572	0,570
Last sniff	0,552	0,006	0,312	0,323	0,349	0,381	0,650	0,074	0,375	0,619

#### 3.2 Trial effects

There was found a highly significant trial effect for total duration of sniffing ( $p < 0,001$ ), where the duration of sniffing was significantly higher at first trial (a) compared to the next seven trials (b) ( $p < 0,001$ ) as shown in Table 5. There was no trial effect on laterality.

**Table 5 Effects of trials and covariates on sniffing behaviour variables. P-values for analyses of data pooled over 4 treatments per dog.**

Sniffing behaviour	Trial	Stress-Score Effect	Sex	Age	Nose length	Nostril distance	Obedience	Physical training	Mental training	Previous experience
Total duration of sniffing	<0,001	0,315	0,372	0,138	0,0014	0,651	0,946	<0,001	0,823	<0,001
Proportion sniffing with left nostril	0,868	<0,001	0,072	0,226	0,027	0,654	0,0011	0,011	0,186	0,788
First sniff	0,763	0,007	0,020	0,927	0,022	0,709	0,006	<0,001	0,538	0,535
Last sniff	0,895	0,002	0,249	0,260	0,286	0,318	0,606	0,0410	0,312	0,571

Trial 2 was significantly different from trial 1 and trial 7 ( $p = 0,049$ ) and 8 ( $p = 0,047$ ), shown in Figure 3.

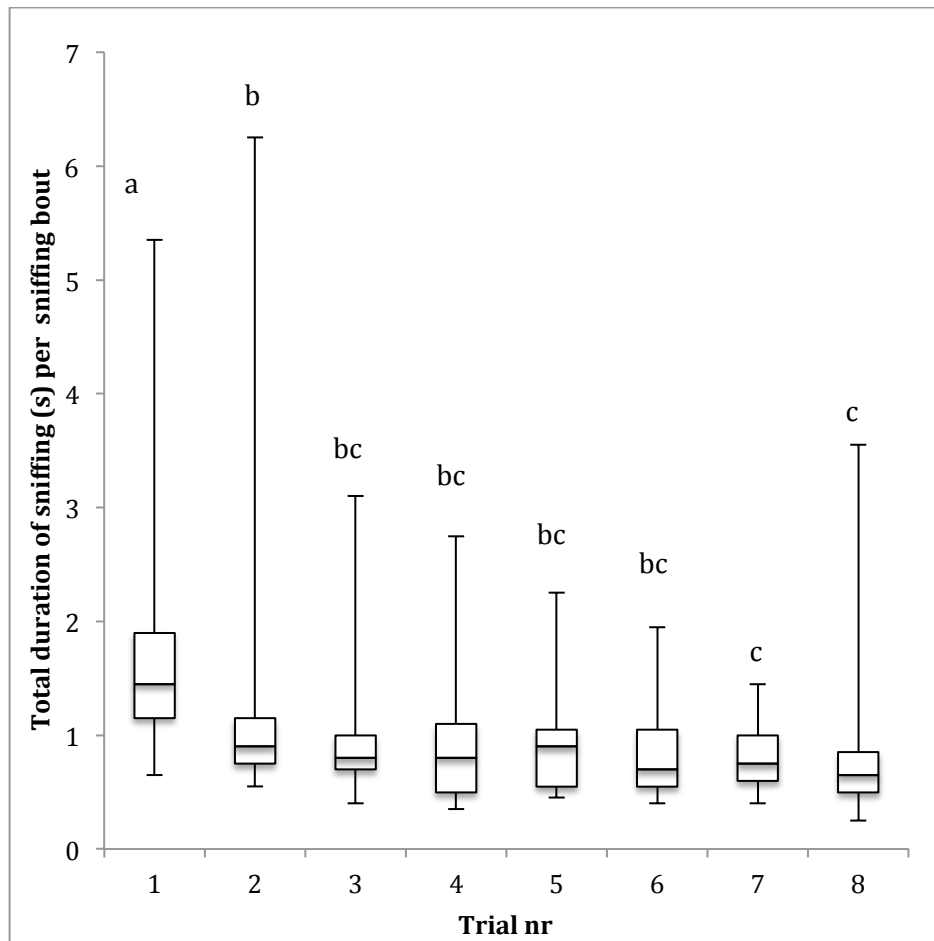


Figure 3: Trial effect on duration of sniffing (in seconds) for each sniffing bout ( $n=928$ ).

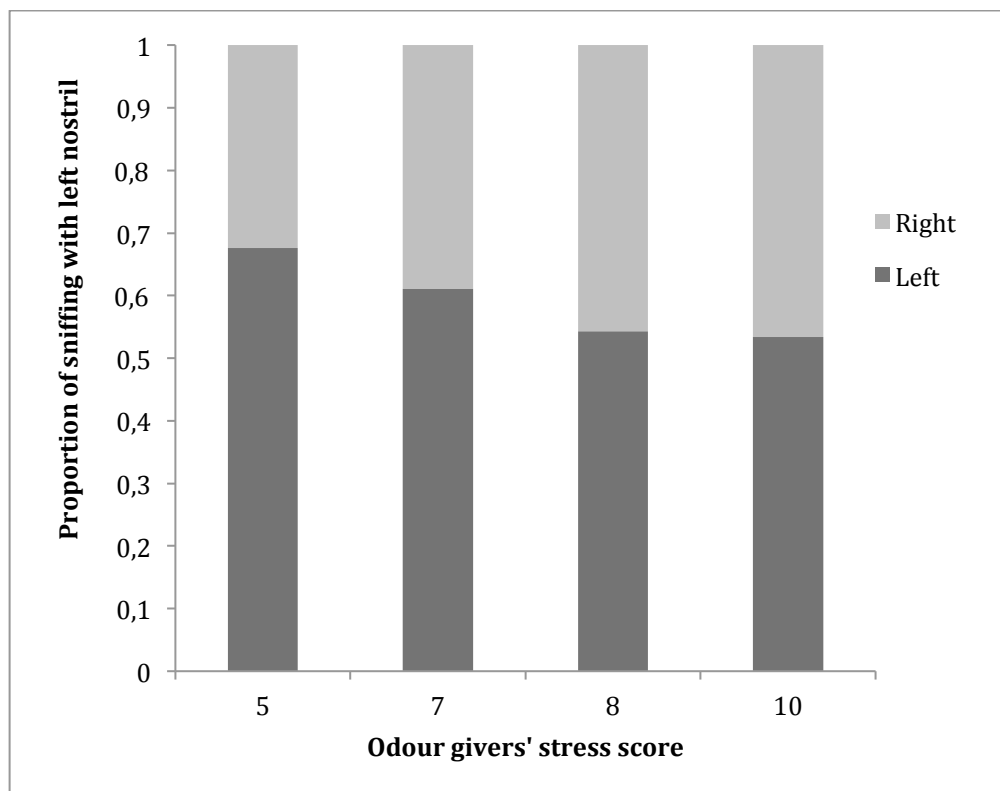
### 3.2 Stress score effects

The odour givers ( $n=8$ ) gave a score from 1-10 indicating how stressed they were when they gave their odour sample for immediate stressed, where 1 represents not stressed at all and 10 represent extremely stressed. None of the odour givers gave a score lower than 5 and no one gave the score 6 or 9. The scores represented are therefore 5, 7, 8 and 10.

Odour givers' stress score had a significant effect on proportion of sniffing with left nostril both across trials ( $p=0,002$ ) (Figure 4) and across treatments ( $p<0,001$ ).

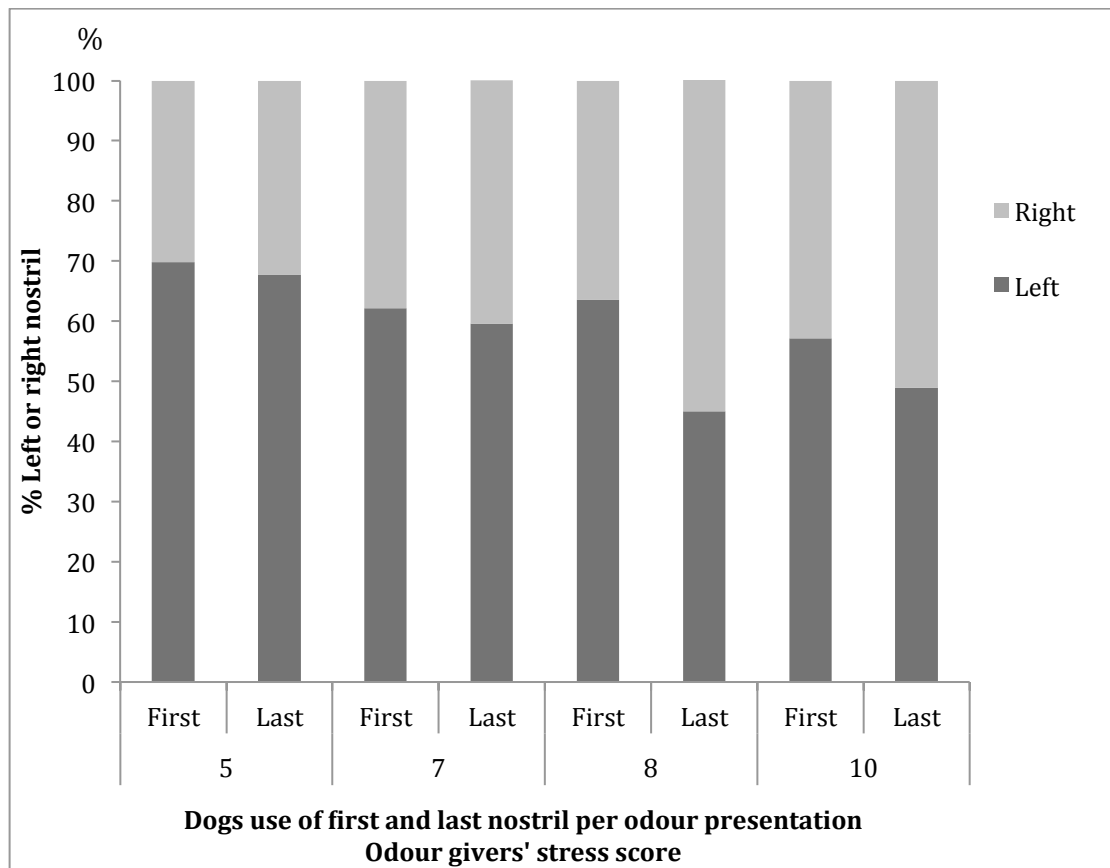
Proportion of left-bias decreased with increased stress score from odour giver. The proportion of sniffing with left nostril decreased with increased stress score from the odour givers.

Odour givers stress score had no significant effect on total duration of sniffing (Table 4 and 5).



**Figure 4:** Effect of odour givers stress score on proportion of sniffing with left nostril, where stress score 5 represent odour from medium stressed odour giver and stress score 10 represent extremely stressed odour giver.

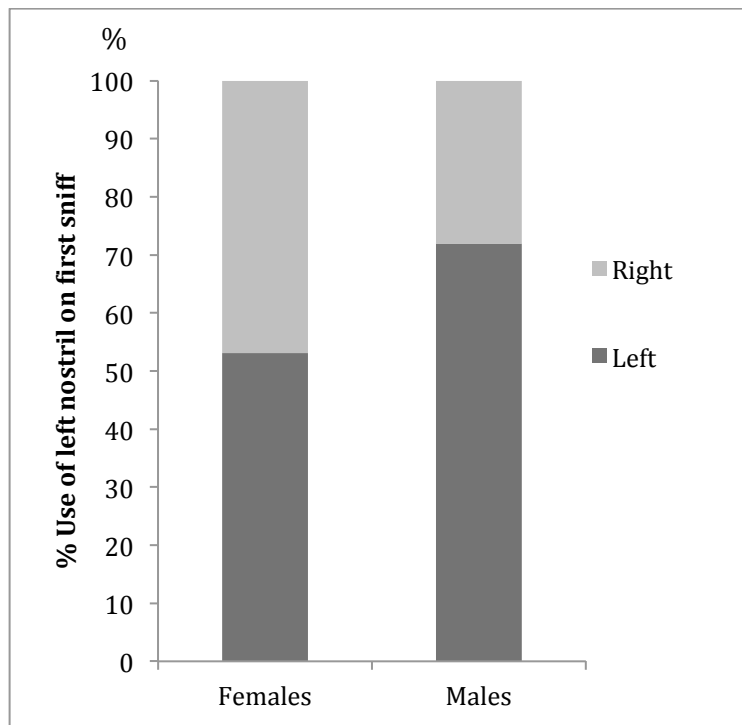
Odour givers stress score had a significant effect on percentage use of left nostril on both first sniff ( $p=0,013$ ) and last sniff ( $p=0,006$ ) at each sniffing bout/odour presentation (Figure 5). Across all dogs and treatment the significance of odour givers stress score was  $p=0,007$  for first sniff and  $p=0,002$  for last sniff. There was more sniffing with left nostril for those dogs given an odour from an odour giver with lower stress score, and more right nostril sniffing for dogs given an odour from an odour giver with higher stress score.



**Figure 5:** Effect of odour givers stress score on percentage use of left nostril for first sniff for each sniffing bout, and last sniff for each sniffing bout.

### 3.3 Sex effects

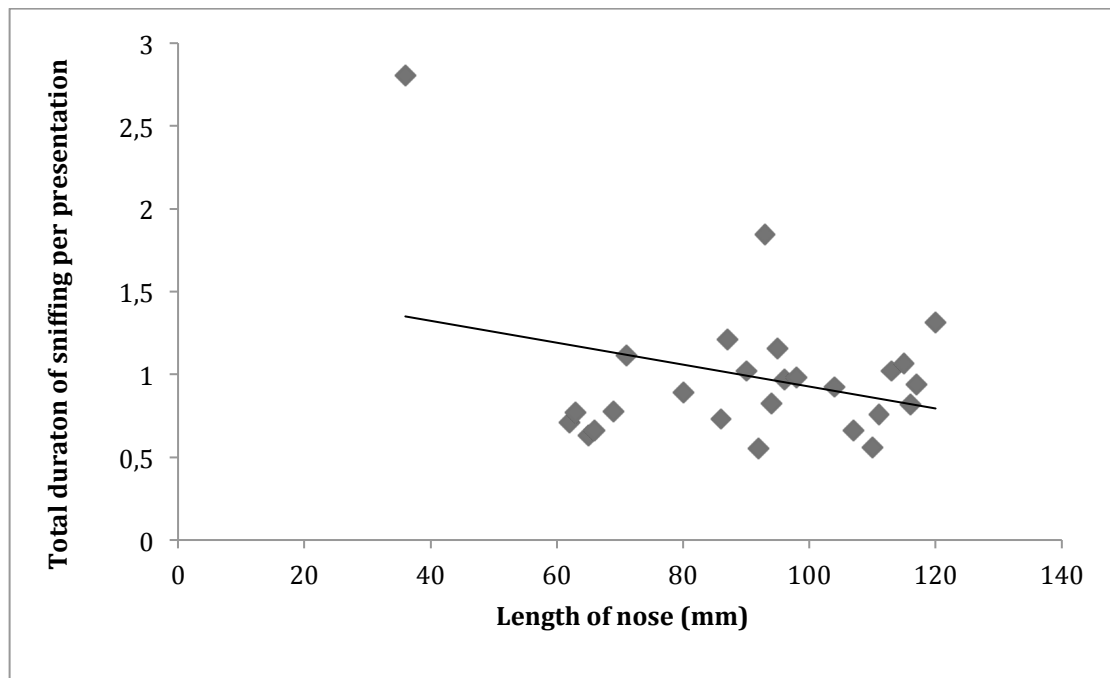
There was 16 females and 13 males included in the study. Sex had a significant effect on first sniff ( $p=0,034$ ). Females used left nostril on first sniff for 53% of the sniffing bouts, while males used left nostril on first sniff for 73% of the sniffing bouts, as shown in Figure 6. There was no significant sex effect on total duration of sniffing, proportion of sniffing with left nostril or last sniff (Table 4 and 5).



**Figure 6:** Sex effect on percentage use of left or right nostril on first sniff on each sniffing bout. 16 females and 13 males were included in the study.

### 3.4. Nose length effects

Nose length had a significant effect of total duration of sniffing both across trials ( $p = 0,004$ ) shown in Figure 7, and across treatments ( $p < 0,001$ ). The duration of sniffing decreased with longer nose length (Figure 7). The outlier in Figure 7 with the shortest nose was a cavalier king charles spaniel which had the shortest nose in the study.



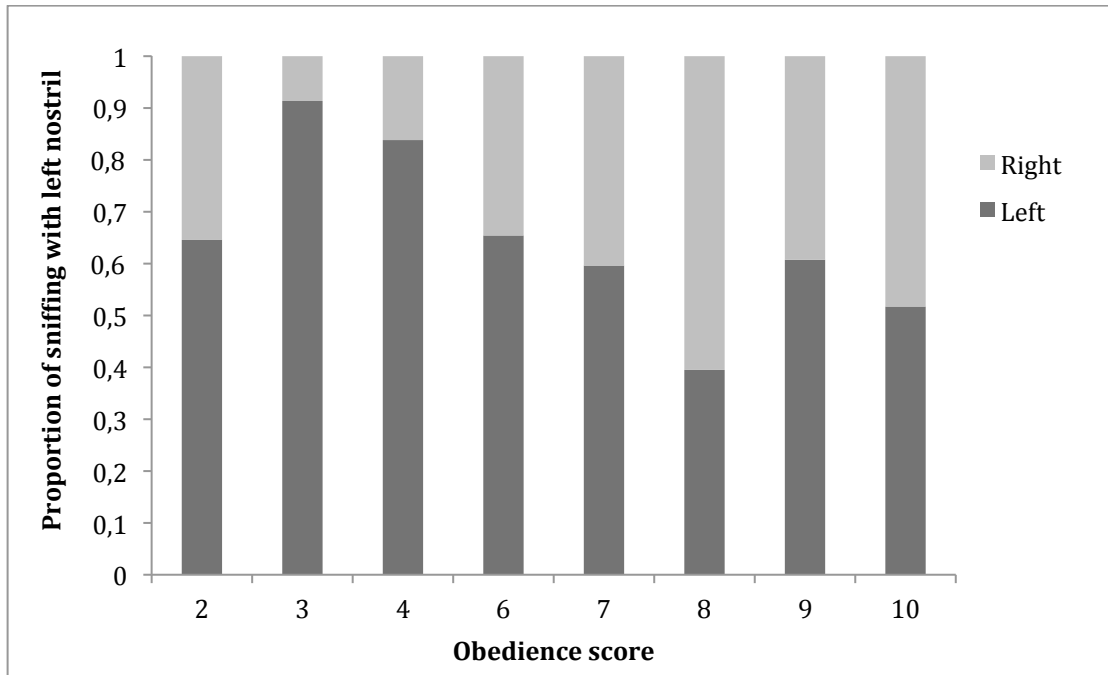
**Figure 7: Effect of nose length (millimetres) on total duration of sniffing (seconds) per sniffing bout. Each spot indicates a nose length and the average sniffing duration for the nose length. The figure is indicating lower duration of sniffing correlated with longer nose.**

Nose length had a significant effect on proportion of sniffing with left nostril ( $p=0,015$ ). There was variability across nose length and individual differences.

### 3.5 Effects of obedience

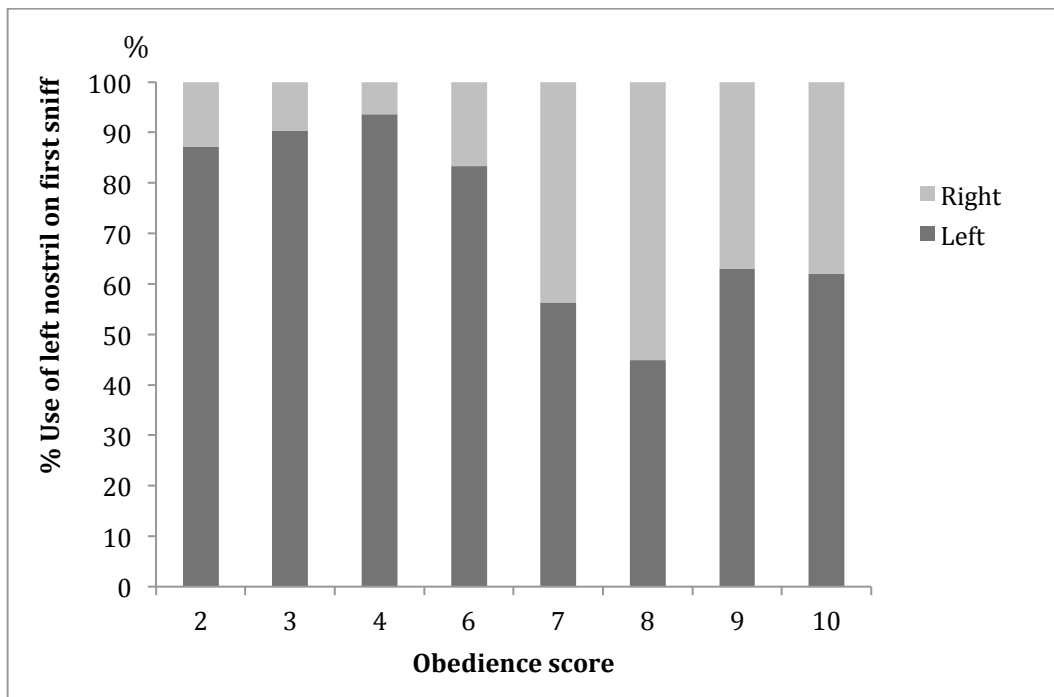
How obedient the dog was (scored by its owner) had an effect on proportion of sniffing with left nostril ( $p=0,002$ ) across trials and across treatments ( $p=0,001$ ).

Figure 8 show that proportion of sniffing with left nostril decrease with higher obedience score.



**Figure 8: Obedient effect on proportion of sniffing with left and right nostril. The dogs' owner rated the obedience of the dogs from 1-10, where 1 is not obedient at all and 10 is extremely obedient.**

Obedience did also have an effect on first sniff across trials ( $p=0,012$ ) and across treatments ( $p=0,006$ ). Figure 9 show that dogs with higher obedience score used left nostril on first sniff less than dogs with lower obedience score.

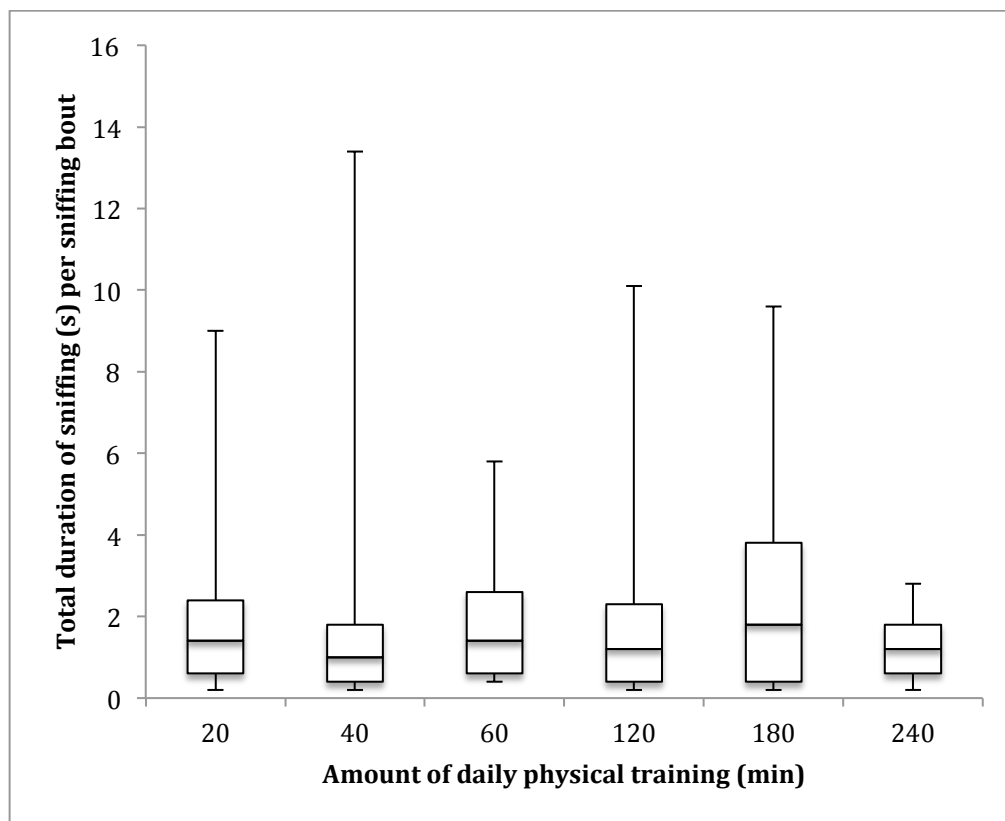


**Figure 9: Effect of how obedient the dog was on percentage use of left and right nostril on first sniff for each sniffing bout. The dogs' owner rated the obedience of the dogs from 1-10, where 1 is not obedient at all and 10 is extremely obedient.**



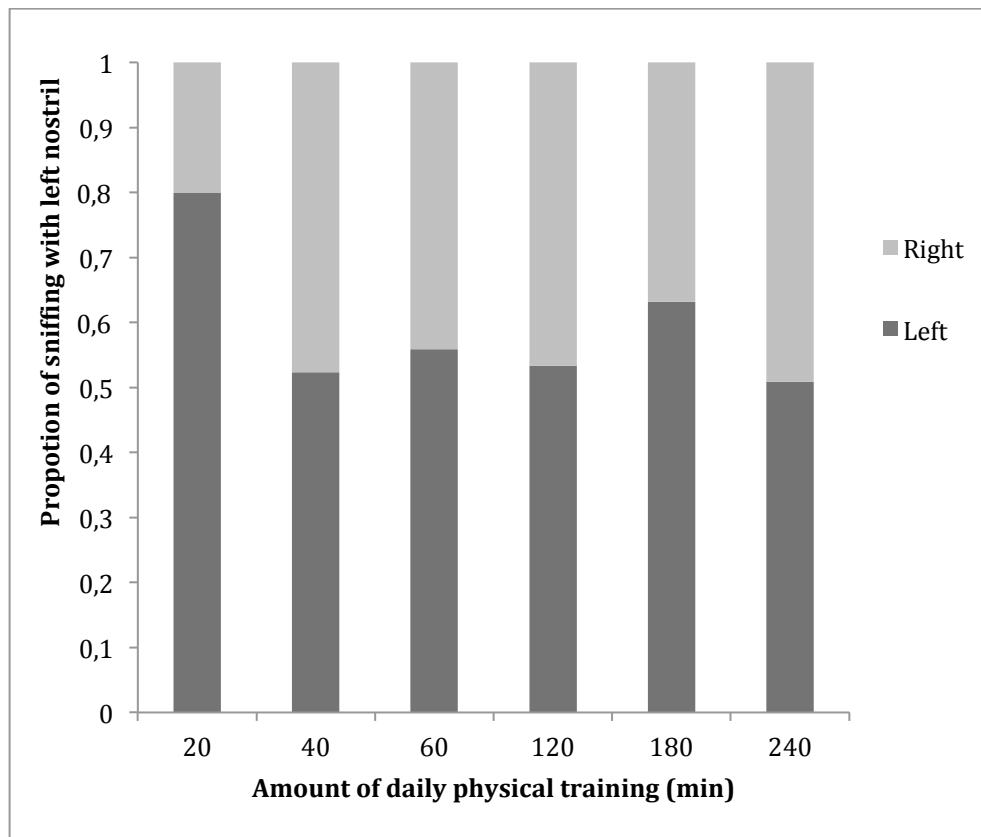
### 3.6. Effects of daily physical training

Daily physical training had highly significant effect on total duration of sniffing ( $p < 0,001$ ), both across trials and across treatments (Figure 10).



**Figure 10: Effect of daily physical training (minutes) on total duration of sniffing (seconds) per sniffing bout, across all dogs, treatments and trials.**

Physical training had significant effect on proportion of sniffing with left nostril across trials ( $p < 0,002$ ) and across treatments ( $p=0,011$ ), shown in Figure 11.



**Figure 11: Effect of daily physical (minutes) training on proportion of sniffing with left nostril per odour presentation.**

Physical training did also have significant effect on first sniff across trials ( $p=0,012$ ) and across treatments ( $p < 0,001$ ), shown in Figure 12. Also last sniff showed a significant effect of physical training ( $p=0,041$ ) across treatments.

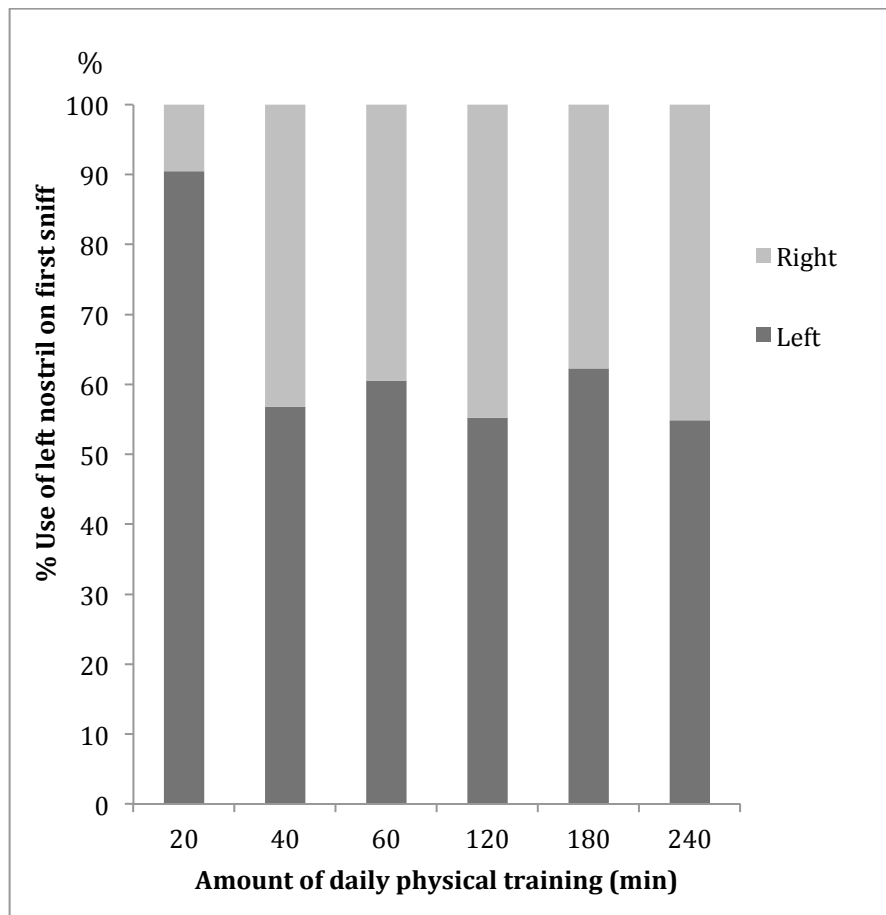
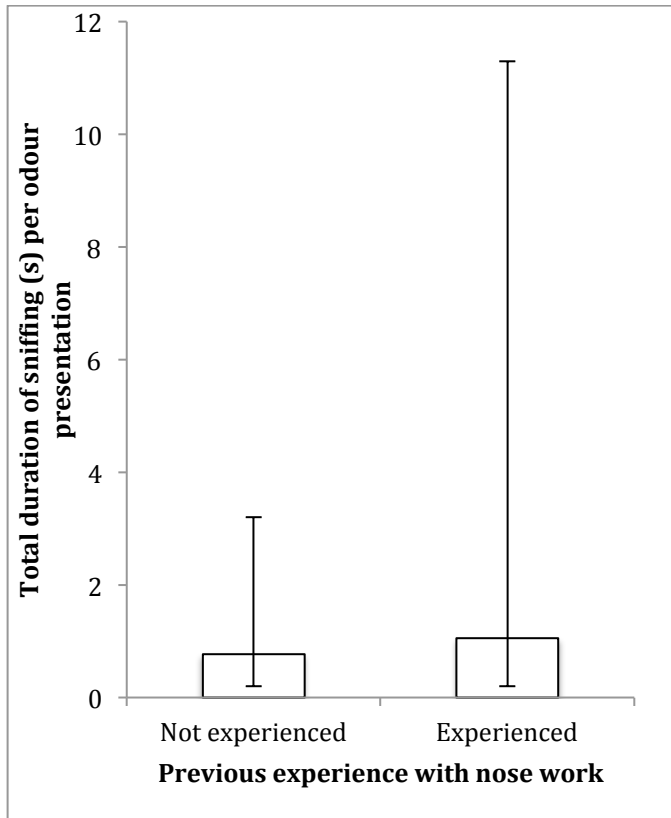


Figure 12: Effect of daily physical training (minutes) on percentage use of left nostril on first sniff.

### 3.7 Effects of previous experience with nose work

Previous experience with nose work had a significant effect on total duration of sniffing both across trials ( $p=0,006$ ) and across treatments ( $p<0,001$ ). Figure 13 shows that experienced dogs had longer investigation time per sniffing bout than inexperienced dogs.



**Figure 13: Effect of the dogs' previous experience with nose work (searching, tracking, ID-tracking, rescue etc.). 23 of the dogs were experienced and 6 of the dogs were inexperienced.**

## 4. Discussion

### 4.1. Discussion of results

There was no significant effect of the odour stimulus “calm”, “immediate stressed”, “later stressed” or control. This means that the dogs did not show any difference in investigation time for these stimuli and that no treatment effect on laterality related to stress odour for humans was found. One possible explanation for this is that the odour samples smelled almost the same and that the dogs could not distinguish between them because all of them smelled human. But in that case, the control sample should have been possible to distinguish from the other samples, because the control was never in contact with human skin. Another possibility is that other covariates were more decisive for laterality effects and investigation time.

There was a clear trial effect on duration of sniffing, where the duration of sniffing was longer at first trial compared to the next seven trials (Figure 3). Trial number one and two was significantly different from trial number seven and eight. The duration of sniffing was higher at the first trials and decreased with repeated trials. The interest for the odours and need to investigate the odours was probably higher at first trial, where all four presented odours were novel to the dog.

The stress score from odour givers had a significant laterality effect shown in Table 4 and Table 5, as well as in Figure 4 and Figure 5. Figure 4 showed that there was a lower proportion of sniffing with left nostril as the stress score from the odour giver increased. This indicates that dogs show laterality effect related to human stress. Odours from an extremely stressed odour giver gave lower probability of left sniff, which mean that dogs use right nostril more when they sniff at an odour from an extremely stressed human. Based on previous findings by Siniscalchi et al. (2011), it was predicted that odour of stress from human would be an aversive odour to the dogs and that this should be shown with a higher probability of right sniff, than for non-aversive odour stimuli. Odours from odour givers with a low stress score seem to be less aversive than odours from odour givers with high stress score. Figure 5 showed how odour givers stress score affected percentage use of left nostril for first sniff at each sniffing bout and for the last sniff at each sniffing bout. The difference between first sniff and last sniff was little, but the difference between first sniff and last sniff

increases when the odour givers stress score is high. More interestingly, there was also here lower proportion of left sniff in odours that had high stress score, than odours with low stress score. This supports that odours with a higher stress score are more aversive than odours with a lower stress score.

There was no significant effect of interaction between treatment and stress score, but the control sample should have been giving different results from the odour treatments. The stress score results are therefore not particularly reliable, but give interesting indications to continue to study.

Laterality effect on sex is shown in some laterality studies on dogs (Quaranta et al., 2004; Wells, 2003). There was a significant sex effect on first sniff with left nostril (Figure 6). Females did not show a clear preference for left or right nostril used for first sniff, as they used left nostril 53% at first sniff and right nostril 48% on first sniff. Males on the other hand, used left nostril 73% on first sniff and right nostril only 28 % on first sniff. This means that males show a significant left bias on first sniff. The same tendencies have been reported in other laterality studies on dogs, where males shows a higher left bias than females in paw preference tests (Poyser et al., 2006; Quaranta et al., 2004; Wells, 2003). This finding is consistent with males being bolder than females on average, if a left bias in sniffing is associated with less fearfulness.

Laterality effects were found in relation to nose length. Nose length had a clear effect on total duration of sniffing (Figure 7). Dogs with large nasal cavity have more olfactory sensory neurons in the olfactory epithelium than dogs with small nasal cavity (Bear et al., 2007). Dogs with a large nasal cavity can thereby receive more sensory information for each sniff. It is possible that this is why the duration of sniffing tended to decrease with increasing nose length. Nose length did also have an effect on proportion of sniffing with left nostril. It is reported that dogs use their right nostril for non aversive stimuli and left nostril for aversive stimuli, as long as the stimuli is novel (the differences are reduced with repeated stimuli presentations) (Siniscalchi et al., 2011). Dogs with longer nose might receive more odour information and easier receive enough information to habituate more rapidly to the

novel odours. This could explain why dogs with long nose do not show the same left bias as dogs with shorter nose.

Obedience had a significant effect on laterality, shown as proportion of sniffing with left and right nostril, and for percentage use of left and right nostril on first sniff. The obedience score is not confirmed by any behavioural tests and is therefore not particularly objective. The tendencies are that more obedient dogs have a lower proportion of using left nostril than less obedient dogs (Figure 8). Also on first sniff, dogs with higher obedient score have a lower percentage of left nostril use than what is found among dogs with a lower obedience score (Figure 9). According to the hypothesis, these findings suggest that less obedient dogs may be more confident and independent. This means that obedience could have an effect on laterality in dogs and that this factor needs further investigation.

Physical training had highly significant effect of total duration of sniffing, (Figure 10). A laterality effect of physical was shown on proportion of sniffing with left and right nostril (Figure 11) and on first sniff (Figure 12). Dogs that were given more than 20 minutes of physical training daily used more right nostril on first sniff than dogs who got 20 minutes or less physical training on an average day. How daily physical training affects laterality in dogs is hard to say and needs further investigation. One hypothesis is that dogs that get minimal physical activity have less experience with human stress odours and therefore are less affected or find it less aversive. It is possible that some of these covariate effects are due to correlations with other untested effects, such as breed differences. Perhaps dogs receiving little exercise were more motivated to explore odours because they have fewer opportunities in their daily lives.

Dogs that had previous experience with nose work showed significantly higher duration of sniffing per odour presentation (Figure 13). Only 6 dogs were inexperienced with nose work, while 23 dogs were experienced to various degrees. Dogs that are trained for nose work might have higher motivation to sniff on objects, as sniffing behaviour is rewarded in nose work training.

There was no effect of age, nostril distance or amount mental training for any of the measurements.

## **4.2. Challenges of studying dogs**

Dogs show a great variation in morphology, genetics and behaviour (Svartberg, 2006) and can be defined as “intraspecies semi-closed breeding populations that show relatively uniform physical characteristics developed under controlled conditions by human action” (Irion et al., 2003). But dog breeds are changing all the time, due to artificial selection by humans, genetic drift and influx from other dog populations (Miklosi, 2007). Dog breeds differ in both behaviour and morphology, because dogs mostly have been selected for a wide range of functions and looks. This might also affect their sensitivity to odours and thereafter the occurrence of laterality related to the olfactory sense. A large inter-individual variation within a breed is also known, indicating that breeds tend to differ only in selected traits (Coppinger & Coppinger 2001), which makes it hard to compare breeds. Breed differences are not investigated in this thesis project, but it could be worth further investigation because there is found effects of morphological traits like nose length.

To study dog behaviour is challenging because of the large differences in species- and individual history. Use of working dogs, pet dogs or unwanted dogs from a kennel may show differences in behaviour even when the test situation is performed the same way for all the dogs. The environment the dogs live in could also affect laterality (Poyser et al., 2006), as these animals have a consistent environment around them (for instance the organization of rooms and furniture) that can lead to expression of learned behaviour instead of constitutional neurobiological lateralization. To limit the individual differences, all dogs in this study did all live in the house with their humans and were exercised daily. Furthermore, “rules” and learned skills for each individuals could “mask” lateralization behaviour by other responses, as if the dog in extremely “well behaved”, stressful or show extreme excitement or fear when presented a novel stimuli. Dogs who live with their human family could be more sensitive to the behaviour of the experimenter or owner because they are used to close human contact. Sensitivity to signals given unconsciously by the experimenter or the dogs’ owner could have an effect on the dogs’ attitude and thereby affect laterality.



### **4.3. Consequences of rewarding sniffing behaviour**

In the study performed in this thesis project, it was decided that sniffing should be rewarded with food to motivate the dogs to sniff. However, rewarding sniffing behaviour may have an effect on the dogs' attitude and also laterality. It is possible to avoid the learned responses by letting the animals do blocks of sniffing before getting a reward. The animals will then have problems to assign the reward to particular stimuli or behaviour as long as the timing and frequency is varying, which was done in the presented experiment. When every presentation is rewarded, an association to stimuli-response pairing can be created that can give a negative or positive outcome (Smith et al., 2008). We could for instance get "fake" sniffing behaviour if the dog misunderstand and did a nose-touch instead of sniffing. This happened with some of the dogs, but the behaviour was revealed in the video analyses in slow motion, as the nose was in close contact with the Plexiglas and nostril vibration was not present when the dogs showed nose touch behaviour.

### **4.4. Discussion of previous studies on olfactory laterality in dogs in relation to current findings**

There are only a few studies published on brain lateralization related to the olfactory sense and even fewer studies on dogs for this topic. The study on lateralization of response to odour stimuli by dogs, by Siniscalchi et al. (2011), was the basis for this thesis. As mentioned in the "Preliminary study" section, different methods were tested before the design for this research was landed, because I could not make the dogs sniff at the odour with the method from Siniscalchi et al. (2011). The research in this paper was not able to show laterality of different odours in the same matter as Siniscalchi et al. (2011) did.

Siniscalchi et al. (2011) gives an inadequate description of their methodology. It is not described whether the dogs are trained for the task or not, or if they are rewarded or not. Training could have a huge impact on the duration of sniffing and also the dogs' emotions related to the task. The design in Siniscalchi et al. (2011) consisted of a stick with odour sample at the tip of this stick. This design was tested in preliminary studies for my thesis, but lead to biting at that stick, sniffing at the side of the stick or

sniffing other places. Sniffing at the tip of the stick seemed very unnatural for the dogs in my preliminary studies. It would have been very useful to get an explanation of how Siniscalchi et al. (2011) made the dogs sniff at the tip of the stick. Moreover, it is never mentioned whether the video analyses was performed blinded or not or if the analyses was done by multiple experimenters to control for unconscious bias. Unconscious study bias may occur when analyses is unblinded (Morissette et al., 2011). Siniscalchi et al. (2011) did not have an “other” category for sniffing. This means that we cannot know how they have categorized sniffing that is neither clear left nostril nor clear right nostril. Chances of unconscious study bias may increase when the analyses is unblinded and without “other” category. Three minutes presentation for each odour was used in Siniscalchi et al. (2011), while results showed that the dogs seldom sniffed more than 2 seconds. It was not described how they motivated the dogs to sniff at the odour during these 3 minutes or what they had as criteria for “first sniff” and “last sniff” within the time limit. I chose to record and analyse one “sniffing session” instead of having a fixed presentation time, because I wanted to register what nostril that was used first and last at each odour for each time the dog sniffed at the odour. This means that only one sniffing session (with continuous sniffing) was registered per odour in my study. These differences between my study and the study by Siniscalchi et al. (2011) could attribute to the different outcome from my studies compared to Siniscalchi et al. (2011).

The presented odours in Siniscalchi et al. (2011) were divided into aversive (vet odour and adrenaline) and non-aversive stimuli (food, lemon, vaginal secretion and cotton). The odours in this study (sweat from humans at different stress levels) might not be aversive at all, or the difference of smell between the three stress levels could have been minimal (all of them could have smells just “human”). The plastic tube was held by the experimenter in the current study to motivate the dog, which could have resulted in the dog sniffing the experimenter rather than the odour sample.

The predictions of the current study was that odour from immediate stressed and later stressed human would be aversive for the dog, while odour from calm human would be non-aversive. However, we cannot control what the dog can actually smell. It is possible that the dog only smells odour of human, regardless of stress level, or that most odour of stress disappeared during the handling. This can explain why there was

no effect of treatments in this study. It could also be that individuals are interested in different odours, so that some dogs reacts to, for instance, the smell of cotton, while other individuals reacts to the smell of human, stress, plastic tube or plastic gloves (used when handling the odour samples before they were frozen). Maybe more time was needed for cortisol to accumulate in sweat before collecting the samples.

Siniscalchi et al. (2011) also spread their trials over many days, which may have increased attention. All trials were performed the same day in the current study, which could have resulted in loss of interest or carry over effects from previous samples.

## **5. Conclusions**

The study supports laterality effects of odour stimulation on dogs. Dogs seem able to detect how stressed humans are, based on the results found in the stress score analyses. However, dogs did not show laterality effects related to the treatments groups or the control sample. In future studies, I would recommend to measure cortisol levels of odour givers when they give a stress odour, instead of basing the stress level on the odours givers own experience of stress (the stress score). The treatment groups did not affect investigation time. Trial effects show that the interest of investigating odours decreased over time, but it was not found any lateralization effects related to trial effects. Laterality in dogs based on olfaction did not show any relation to age, distance between nostrils or mental training. Laterality effects of sex in showed, where males show a clear left bias while females showed a very little left bias. Covariates like obedience, physical training and previous experience with nose work have never before been related to laterality in dogs and merit further investigation.

## 6. Literature

- Andrew R. J. 2002. Memory formation and brain lateralization. In: Rogers, L. J. & Andrew, R. J., editors. *Comparative vertebrate lateralization*. New York: *Cambridge University Press*, 582-626.
- Bear, M., Connors, B. & Paradiso, M. 2007. *Neuroscience: Exploring the Brain*. USA: Lippincott Williams & Wilkins, 265-275.
- Berns, G. S., Brooks, A. M. & Spivak, M. 2014. Scent of the familiar: An fMRI study of canine brain responses to familiar and unfamiliar human and dog odors. *Behavioural Processes*, In Press, March 6th, 2014, 1-10.  
<http://dx.doi.org/10.1016/j.beproc.2014.02.011>
- Bradshaw, J. L. & Rogers, L. J. 1993. *The evolution of lateral asymmetries, language, tool use, and intellect*. New York: Academic Press, 463.
- Collins, R. L. 1975. When left-handed mice live in right-handed worlds. *Science*, 187, 181-184.
- Collins, R. L. 1985. On the inheritance of direction and degree of asymmetry. In S. Glick, D. editors. *Cerebral lateralization in nonhuman species*. New York: *Academic Press*, 41-71.
- Coppinger, R. & Coppinger, L. 2001. *Dogs: a New Understanding of Canine Crigin, Behavior and Evolution*. University of Chicago Press, Chicago.
- Davidson, R. J. & Haugdal, K. 1998. *Brain Asymmetry*. 2.ed. Cambridge, MA: MIT Press.
- Deckel, A. W. 1995. Lateralization of aggressive responses in *Anolis*. *Journal of Experimental Zoology*, 272, 194-200.
- Denenberg, V. H. 1981. Hemispheric laterality in animals and the effects of early experience. *Behavioral and Brain Sciences*, 4, 1-49.
- Diekamp, B., Regolin, L., Güntürkün, O., & Vallortigara, G. 2005. A left-sided visuospatial bias in birds. *Current Biology*, 15, 372-373.
- Elalmis, D. D., Ozgunen, K. T., Binokay, S., Tan, M., Ozgunen, T., Tan, U. 2003. Differential contributions of right and left brains to paw skill in right- and left-pawed female rats. *International Journal of Neuroscience*, 113, 1023-1042.
- Guo, K., Meintis, K., Hall, C., Hall, S. & Mills, D. 2009. Left gaze bias in humans, rhesus monkeys and domestic dogs. *Animal Cognition*, 12, 409-418.
- Hauser, M. D. & Anderson, K. 1994. Left hemisphere dominance for processing vocalizations in adult, but not infant rhesus monkeys: field experiments. *Proceeding of National Academy of Sciences*, 91, 3946-3948.
- Hauser, M. D. 1993. Right hemisphere dominance for the production of facial

expression in monkeys. *Science*, 261, 475-477.

Hummel, T., Mohammadian, P. & Kobal, G. 1998. Handedness is a determining factor in lateralized olfactory discrimination. *Chemical Senses*, 23, 541-544.

Irion, D.N., Schaffer, A.L., Famula, T.R., Eggleston, M.L., Hughes, S.S. & Pedersen, N.C. 2003. Analysis of genetic variation in 28 dog breed populations with 100 microsatellite markers. *Journal of Heredity*, 94, 81-87.

Lobue, V. & DeLoache, J. S. 2008. Detecting the snake in the grass: attention to fear-relevant stimuli by adults and young children. *Psychological Science*, 19, 284-9.

Martinez, B. A., Cain, W. S., de Wijk, R. A., Spencer, D. D., Novelly, R. A. & Sass, K. J. 1993. Olfactory functioning before and after temporal lobe resection for intractable seizures. *Neuropsychologia*, 7, 351-363.

McManus, C. 2002. *Right Hand, Left Hand: The Origins of Asymmetry in Brains, Bodies, Atoms and Cultures*. Cambridge, Harvard University Press, 2002. ISBN: 0-674-00953-3.

Miklosi, A. 2007. *Dog Behavior, Evolution, and Cognition*. Department of Ethology, Eötvös Loránd University, Budapest. Oxford University Press.

Morisette, K., Tricco, A. C., Horsley, T., Chen, M. H. & Moher, D. 2011. Blinded versus unblinded assessments of risk of bias in studies included in a systematic review. *Cochrane Database of Systematic Reviews 2007*, 4.  
DOI: 10.1002/14651858.MR000025.pub2

Murphy, J., Sutherland, A., Arkins, S. 2005. Idiosyncratic motor laterality in the horse. *Applied Animal Behaviour Science*. 91, 297-310.

Oke, A., Keller, R., Mefford, I. & Adams, R. N. 1978. Lateralization of norepinephrine in human thalamus. *Science*, 200, 1411-1413. DOI: 10.1126/science.663623

Parr, L A. & Hopkins, W.D. 2000. Brain temperature asymmetries and emotional perception in chimpanzees, *Pan troglodytes*. *Physiology & Behavior*, 71, 363-71.

Poyser, F., Caldwell, C. & Cobb, M. 2006. Dog paw preference shows liability and sex differences. *Behavioural Processes*, 73, 216-221.

Quaranta, A., Siniscalchi, M., Frate, A., Iacoviello, R., Buonavoglia, C. & Vallortigara G. 2006. Lateralised behaviour and immune response in dogs: relations between paw preference and interferon- $\gamma$ , interleukin-10 and IgG antibodies production. *Behavioural Brain Research*, 166, 236-40.

Quaranta, A., Siniscalchi, M., Frate, A. & Vallortigara, G. 2004. Paw preference in dogs: relations between lateralised behaviour and immunity. *Behavioural Brain Research*, 153, 521-525.

- Quaranta, A., Siniscalchi, M. & Vallortigara, G. 2007. Asymmetric tail-wagging responses by dogs to different emotive stimuli. *Current Biology*, 17, 199-201.
- Rashid, N., Andrew, R. J. 1989. Right hemisphere advantages for topographical orientation in the domestic chick. *Neuropsychologia*, 27, 937-948.
- Regolin, L., Vallortigara, G., 1996. Lateral asymmetries during responses to novel-coloured objects in the domestic chick: a developmental study. *Behavioral Processes*, 37, 67-74.
- Robins, A., Lippolis, G., Bisazza, A., Vallortigara, G., & Rogers, L. J. 1998. Lateralisised agonistic responses and hind-limb use in toads. *Animal Behavior*, 56, 875-881.
- Roches, A., Richard-Yris, M., Henry, S., Mohammed Ezzaouia, M. & Hausberger, M. 2008. Laterality and emotions: Visual laterality in the domestic horse (*Equus caballus*) differs with objects' emotional value. *Physiology & Behavior*, 94, 487-490.
- Rogers, L. J. 2000. Evolution of hemispheric specialisation: Advantages and disadvantages. *Brain and Language*, 73, 236-253.
- Rogers, L. J. & Andrew, R. J. 2002. *Comparative vertebrate lateralization*. New York: Cambridge University Press.
- Rogers, L. J. & Vallortigara, G. 2008. From antenna to antenna: lateral shift of olfactory & memory in honeybees. *PloS ONE*, 3, 2340, DOI:10.1371/journal.pone.0002340.
- Royet, J. & Plailly, J. 2004. Lateralization of Olfactory Processes (Review). *Chemical Sciences*, 29, 731-745.
- Scheumann, M. & Zimmermann, E. 2008. Sex-specific asymmetries in communication sound perception are not related to hand preference in an early primate. *BMC Biology*, 6. DOI: 10.1186/1741-7007-6-3.
- Siniscalchi, M., Lusito, R., Sasso, R. & Quaranta, A. 2012. Are temporal features crucial acoustic cues in dog vocal recognition? *Animal Cognition*, 15, 5, 815-821.
- Siniscalchi, M., Sasso, R., Pepe, A., Dimatteo, S., Vallortigara, G. & Quaranta, A. 2011. Sniffing with the right nostril: lateralization of response to odour stimuli by dogs. *Animal Behaviour*, 82, 399-404.
- Siniscalchi, M., Sasso, R., Pepe, A. M., Vallortigara, G. & Quaranta, A. 2010. Dogs turn left to emotional stimuli. *Behavioural Brain Research*, 208, 516-521.
- Siniscalchi, M., Quaranta, A. & Rogers, L. J. 2008. Hemispheric specialization in dogs for processing different acoustic stimuli. *PloS One*, 3, 3349. DOI: 0.1371/journal.pone.0003349.
- Smith, J. D., Beran, M.J., Couchman, J. J. & Coutinho, M. 2008. The comparative study of metacognition: Sharper paradigms, safer inferences. *Psychonomic Bulletin &*

*Review, Springer, 15, 679–691.*

Sovrano, V. A. 2004. Visuallateralization in response to familiar and unfamiliar stimuli in fish. *Behavioural Brain Research*, 152, 385–91.

Svartberg, K., 2006. Breed-typical behaviour in dogs - historical remnants or recent constructs? *Applied Animal Behaviour Science*. 96, 293-313.

Vallortigara, G. 1992. Right hemisphere advantage for social recognition in the chick. *Neuropsychologia*, 30, 761-768.

Vallortigara, G. 2000. Comparative neuropsychology of the dual brain: a stroll through left and right animals' perceptual worlds. *Brain and Language*, 73, 189-219.

Vallortigara, G. 2006. The Evolution of behavioral and brain asymmetries: Bridging together neuropsychology and evolutionary biology. In Y. Malashichev & W. Deckel, editor. *Behavioral and morphological asymmetries in vertebrates*, 1-20. Austin, TX: Landes Bioscience.

Vallortigara, G. & Andrew, R. J. 1991. Lateralization of response to change in a model partner by chicks. *Animal Behaviour*, 41, 187-194.

Vallortigara, G., Regolin, L., Bortolomiol, G., & Tommasi, L. 1996. Lateral asymmetries due to preferences in eye use during visual discrimination learning in chicks. *Behavioral Brain Research*, 74, 135-143.

Vallortigara, G., Rogers, L. J., Bisazza, A., Lippolis, G., & Robins, A. 1998. Complementary right and left hemifield use for predatory and agonistic behavior in toads. *Neuro report*, 9, 3341-3344.

Wells, D. L. 2003. Lateralised behaviour in the domestic dog, *Canis familiaris*. *Behavioural Processes*, 61, 27-35.

Wittling, W. 1995. Brain asymmetry in the control of autonomic-physiologic activity. In: Davidson RJ, Hugdahl K, editors. *Brain asymmetry*, 305-57. Cambridge, MA: MIT Press.

Youngentob, S. L., Kurtz, D. B., Leopold, D. A., Mozell, M. M. & Hornung, D. E. 1982. Olfactory sensitivity: is there laterality? *Chemical Senses*, 7, 11-21.

## Appendix 1

Dogs included in the study. The table shows the dogs name, sex, age and breed.

---

Hundens navn	Hundens kjønn	Hundens alder	Rase
Nanok	Hannhund (ikke kastret)	2 år	Hvit Gjeterhund
Chocco	Hannhund (ikke kastret)	6 år	Border collie
Fix	Hannhund (ikke kastret)	6 år	Portugisisk vannhund
Kip	Hannhund (ikke kastret)	7 år	BC
Spin	Hannhund (ikke kastret)	2 år	Border Collie
Boris	Hannhund (ikke kastret)	6 år	Labrador
Billy	Hannhund (ikke kastret)	5 år	Portugisisk vannhund
Django	Hannhund (ikke kastret)	4 år	Rhodesian ridgeback
Noah	Hannhund (ikke kastret)	6 år	Tervueren
Get Weilers Frekke			
Kvikk	Hannhund (ikke kastret)	3 år	Border Collie
Capo	Hannhund (ikke kastret)	3 år	Japansk Spisshund
Takiro	Hannhund (ikke kastret)	7 år	Japansk Spisshund
Bamse	Hannhund (ikke kastret)	4 år	Japansk Spisshund
Vega	Tispe (ikke sterilisert)	2 år	Border collie
Cava	Tispe (ikke sterilisert)	2 år	Mellompuddel
Soki	Tispe (ikke sterilisert)	3 år	Belgisk fårehund Tervuren
Cava	Tispe (ikke sterilisert)	4 år	Cavalier King Charles Spaniel
Lexi	Tispe (ikke sterilisert)	5 år	Vorsteher
Luna	Tispe (ikke sterilisert)	3 år	Border Collie
Kida	Tispe (ikke sterilisert)	3 år	Sheltie
Dina	Tispe (ikke sterilisert)	5 år	Riesenschanuzer
Akita	Tispe (ikke sterilisert)	2 år	Chodsky Pes
Gnizt	Tispe (ikke sterilisert)	4 år	
Dina	Tispe (ikke sterilisert)	5 år	Groenendael
Ixi Dee	Tispe (ikke sterilisert)	4 år	Storpuddel
Sara	Tispe (ikke sterilisert)	3 år	Shetland sheepdog
IBA	Tispe (ikke sterilisert)	4 år	Hvit Gjeterhund
Montmorenjas Ivrige			
Jemilla (Milla)	Tispe (ikke sterilisert)	5 år	Tervueren
litchi	Tispe (ikke sterilisert)	6 år	Border collie

---



## Appendix 2

The table is showing odour identity (Odour from person nr 1.8) and order code of the presented odours for each dog.

Odour from person	Dog nr	test 1	test 2	test 3	test 4	test 5	test 6	test 7	test 8
1	1	a	b	c	d	e	f	g	h
3	2	b	c	d	e	f	g	h	a
2	3	c	d	e	f	g	h	a	b
4	4	d	e	f	g	h	a	b	c
5	5	e	f	g	h	a	b	c	d
6	6	f	g	h	a	b	c	d	e
7	7	g	h	a	b	c	d	e	f
8	8	h	a	b	c	d	e	f	g
1	9	a	b	c	d	e	f	g	h
2	10	b	c	d	e	f	g	h	a
3	11	c	d	e	f	g	h	a	b
4	12	d	e	f	g	h	a	b	c
5	13	e	f	g	h	a	b	c	d
6	14	f	g	h	a	b	c	d	e
7	15	g	h	a	b	c	d	e	f
8	16	h	a	b	c	d	e	f	g
1	17	a	b	c	d	e	f	g	h
2	18	b	c	d	e	f	g	h	a
3	19	c	d	e	f	g	h	a	b
4	20	d	e	f	g	h	a	b	c
5	21	e	f	g	h	a	b	c	d
6	22	f	g	h	a	b	c	d	e
7	23	g	h	a	b	c	d	e	f
8	24	h	a	b	c	d	e	f	g
1	25	a	b	c	d	e	f	g	h
2	26	b	c	d	e	f	g	h	a
3	27	c	d	e	f	g	h	a	b
4	28	d	e	f	g	h	a	b	c
5	29	e	f	g	h	a	b	c	d
6	30	f	g	h	a	b	c	d	e
7	31	g	h	a	b	c	d	e	f
8	32	h	a	b	c	d	e	f	g

## Appendix 3

Application form and questionnaire filled out by the dog owners. The form was online, made in Google Drive (© Google.com).

Page 1 of 1

### Verving av hund til atferdstest

Form Description

Ditt navn

Epostadresse

Gjenta epostadresse

Telefonnummer

Hundens navn

Hundens kjønn

Hunden må være fertil for å kunne brukes i prosjektet

- Tispe (ikke sterilisert)  
 Hannhund (ikke kastret)

Rase

Hunden må være renrasert for å kunne brukes i prosjektet

Hundens alder

Velg alderen hunden er nærmest i november 2014

- 2 år  
 3 år  
 4 år  
 5 år  
 6 år  
 7 år

**Hvor lydlig/veloppdragen er hunden din**

Der 1 er problematisk ulydig, 5 er hører av og til og 10 er svært lydlig

1 2 3 4 5 6 7 8 9 10

**Har hunden tidligere vært brukt til søksoppgaver?**

- Nei
- Ja, godbitsøk
- Ja, neseprøve (LP)
- Ja, spor
- Ja, rundering
- Ja, feltsøk/finne gjenstander
- Ja, spesialsøk
- Ja, ANNET
- Vet ikke

**Hvor mange timer DAGLIG trener du hunden din FYSISK (gjennomsnitt)**

Husk: det er ikke om å gjøre å gi mye mosjon her - svar ærlig og kryss av det som er nærmest en vanlig hverdag

- Under 20 minutter
- 20-50 min
- 1 time
- 2 timer
- 3 timer
- over 4 timer

**Hvor mange timer DAGLIG trener du hunden din mentalt (lydighetstrening, triks, agility, søk osv)**

Svar ærlig og sett kryss på alternativet som er nærmest en gjennomsnittsdag hos deg

- Ikke noe
- 10 min
- 20 min
- 30 min
- 1 time
- 2 timer
- 3 timer
- over 4 timer

**Hvis tisse: Hvordan ligger hunden an i forhold til løpetid?**

Når løp hunden sist og når forventes neste løpetid

Hvilke dager har du mulighet til å komme?

- 9. oktober
- 10. oktober
- 13. oktober
- 14. oktober
- 15. oktober
- 16. oktober
- 20. oktober
- 21. oktober
- 22. oktober
- 23. oktober

Hvilke tidsrom har du mulighet de ulike dagene?

Annet

## Appendix 4

Video camera view and picture of test apparatus



Video camera  
view. Left nostril  
used



Camera view, "other  
sniffing". Experimenter  
motivating the dog to  
sniff by holding the  
plastic tube



Test apparatus



Norwegian University  
of Life Sciences

Postboks 5003  
NO-1432 Ås, Norway  
+47 67 23 00 00  
[www.nmbu.no](http://www.nmbu.no)