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Physiological and behavioural responses to fear and discomfort in dogs and goats

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Cover: A goat with her two kids and a beautiful Collie
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Abstract

Animal welfare is an issue of great public and scientific interest. In this thesis, physiological and behavioural methods were used to evaluate fear and discomfort in two different species; dogs and goats. In the first study, fear of gun shots and different floor surfaces was investigated in collie dogs. Dogs that were fearful of floors had higher heart rates than dogs that were less fearful. Dogs fearful of gunshots had higher heart rates, haematocrit and plasma concentrations of cortisol, progesterone, vasopressin and β-endorphin, than less fearful dogs, which demonstrates that fear of gunshots is a serious stressor. In the second study, it was shown that housing and company of other animals affects arterial blood pressure, heart rate, and the concentrations of β-endorphin and oxytocin in goats. However, cortisol and vasopressin concentrations did not differ between goats that were tethered respectively loosely housed in pairs. In the dairy industry, the permanent early separation of mother and offspring is of great concern. In the third study, we found no changes in heart rate, arterial blood pressure or plasma concentrations of cortisol, β-endorphin, oxytocin and vasopressin in goats after separation. However, both goats and kids vocalised intensively. In the fourth and fifth studies, kids were either permanently separated, daily separated, or kept full-time with mothers, and were subjected to an isolation test with a dog bark at two weeks and two months of age, and an arena test with a suddenly appearing novel object at two months of age. All kids had similar growth rates. Kids kept with their mothers showed more hiding behaviours at two weeks, and early separated kids were more active with another kid. Early separated kids also deviated most in the isolation test at two weeks by reducing their vocalisation earlier and having a higher heart rate before and after dog barking, and at two months by having higher heart rate throughout the test. Daily separated kids bleated comparatively more at two weeks, decreased their heart rate after dog bark and showed the strongest fear reaction in the arena test at two months. In conclusion, it is important to measure several different physiological and behavioural parameters when assessing animal welfare.

Key words: behaviour, canine, caprine, cortisol, dog, fear, goat, heart rate, rearing system, separation, welfare

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Fysiologiska och etologiska mått på rädsla och obehag hos get och hund

Abstract

I denna avhandling användes fysiologiska och etologiska metoder för att utvärdera rädsla och obehag hos två olika arter; hund och get. I den första studien undersöktes golv- och skotträdsla hos hundar av rasen collie. Golvrädda hundar hade högre hjärtfrekvens än hundar som inte var rädda. Skotträdda hundar hade högre hjärtfrekvens, hematokrit och plasmakoncentrationer av kortisol, progesteron, vasopressin och β-endorfin än hundar som inte visade skotträdsla, vilket visar att skotträdda hundar utsätts för stark stress. I den andra studien visades att inhysningssystem och social kontakt med andra getter påverkar arteriellt blodtryck, hjärtfrekvens och koncentrationerna av β-endorfin och oxytocin hos getter. Plasmakoncentrationerna av kortisol och vasopressin skilde sig däremot inte mellan uppbundna getter och lösgående getter i par. Inom intensiv mjölkproduktion separeras vanligen get och killing tidigt. I den tredje studien fann vi inga förändringar i hjärtfrekvens, arteriellt blodtryck eller plasmakoncentrationer av kortisol, β-endorfin, oxytocin och vasopressin hos getter efter separation. Dock vokaliserade både getter och killingar intensivt. I den fjärde och femte studien studerades killingar som föddes upp i tre olika system; separerade från mamman, separerade dagtid men tillsammans med mamman under natten, eller tillsammans med mamman dygnet runt. Vid två veckors och två månaders ålder utfördes ett isoleringstest med ett hundskall och vid två månaders ålder även ett arenatest med ett okänt objekt. Tillväxten var lika i alla behandlingar. De killingar som gick med sina mammor visade mer gömmarbeteende vid två veckors ålder. De killingar som var permanent separerade från sina mammor var mest aktiva tillsammans med andra killingar. Dessa killingar avvek mest från de andra behandlingarna genom att vokaliseringsfrekvensen minskade tidigare under isoleringstestet vid två veckors ålder, hjärtfrekvensen var högre före och efter hundskallet vid två veckors ålder, samt under hela testet vid två månaders ålder. De killingar som separerades dagligen vokaliserade mer vid två veckor, hjärtfrekvensen minskade efter hundskallet och de visade den starkaste rädslereaktionen under arenatestet vid två månader. Sammanfattningsvis är det viktigt att mäta flera olika fysiologiska och etologiska parametrar vid bedömning av djurvälfärd.

Keywords: beteende, djurvälfärd, get, hjärfrekvens, hund, kortisol, rädsla, separation

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Preface

It started with a dog, Mollie. She was afraid of gun shots, and I was an ambitious dog owner that wished to participate in competitions. So, I trained and trained my dog to ignore gun shots. Finally, when we managed to pass the test that included gun shots, and the assessor said to me: “You can tell she is not afraid at all. It’s not possible to train a dog to hide her fear.”

Well, as all dog-nerds know, it’s quite possible to train a dog to cope with frightening situations. But what is going on inside an animal, invisible from the outside? My curiosity was aroused. Twenty years later, this thesis is complete.

So, what happened? Well, life happened.

I had four beautiful children. When the youngest was three years old, in 2010, I defended my licentiate thesis. The idea was to complete my PhD thesis shortly after. But four lovely kids, and a great full-time job, with focus on welfare in dairy cattle, proved a handful. But it also gave me experience and practical knowledge about welfare issues in dairy production.

And now, thanks to my amazing supervisors who never gave up on me, this thesis is complete. And you know what? The subject of these studies has only grown more topical.

Dedication

To my kids Hampus, Frida, Sofia and Fabian, I love you.

To my dear late friend, Annika, who passed away too early. Annika, despite her belief in veganism, encouraged me to finish this thesis. We agreed that different roads may lead to improved animal welfare. I miss you.

"Rivers know this: there is no hurry. We shall get there some day"
Winnie-the-Pooh ~ A.A. Milne

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. E. Hydbring- Sandberg, L. Winblad von Walter, K. Höglund, K. Svartberg, L. Swenson and B. Forkman (2004). Physiological reactions to fear provocations in dogs. *Journal of Endocrinology* 180, 439-448.

II. E Hydbring-Sandberg*, L Winblad von Walter and B Forkman. (2021). Cortisol is not enough - complex stress reaction in tethered goats. Submitted.

III. L. Winblad von Walter, L. Lidfors, A. Madej, K. Dahlborn, E. Hydbring-Sandberg (2010). Cardiovascular, endocrine and behavioural responses to suckling and permanent separation in goats. *Acta Veterinaria Scandinavica* 52 (51).

IV. M. Högberg, L. Winblad von Walter, E. Hydbring-Sandberg, B. Forkman, and K. Dahlborn*. (2021). Growth rate and behaviour in early, daily or not separated kids and the corresponding milk production of their goats. Submitted.

V. L. Winblad von Walter*, B. Forkman, M. Högberg, E. Hydbring-Sandberg (2021). The effect of mother goat presence during rearing on kids response to isolation and to an arena test. Submitted.

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The contribution of Louise Winblad von Walter to the papers included in this thesis was as follows:

I. Shared responsibility for design of the study, took part in all data acquisition, shared responsibility for data analysis, shared responsibility with first author for summarising results and writing and critically revising the article with input from co-authors.

II. Shared responsibility for design of the study, took part in data acquisition, shared responsibility for data analysis, shared responsibility with first author for summarising results and writing.

III. Shared responsibility for design of the study, took part in all data acquisition, shared responsibility for data analysis. Main responsibility for writing and critically revising the article with input from co-authors.

IV. Shared responsibility for design of the study, took part in all data acquisition, responsible for behavioral analysis.

V. Shared responsibility for design of the study, took part in all data acquisition, main responsibility for data analysis. Main responsibility for writing and critically revising the article with input from co-authors.

1. Introduction

1.1 Animal welfare

Animal welfare is an issue of great interest among consumers, pet-owners, producers, and professionals, but is often affected by a polarised debate. Although scientific studies have contributed to the development of valuable improvements to the housing, management and care of animals dependent on humans, there remain many issues of concern, such as for example excessive fear in pet dogs, and aversive management procedures in livestock, e.g., early permanent separation of mothers from offspring.

Already in the Sixties, the Brambell Report (Brambell & Barbour, 1965) stated that animals should have the freedom to "to stand up, lie down, turn around, groom themselves, and stretch their limbs." These were referred to as 'Brambells' Five Freedoms' and the report also stressed that, "welfare is a wide term that embraces both physical and mental well-being of the animal". Just over a decade later, in 1979, the Farm Animal Welfare Committee (FAWC, 1979) presented the 'five freedoms' that are widespread around the world: freedom from hunger and thirst, discomfort, pain, injury and disease, fear and distress. Hence, the Brambell report was highly influential on the European regulations to protect farm animals (Veissier *et al*., 2008). Two decades later, in 2002, the World organisation for animal health (OIE) presented a resolution on animal welfare, and at presence it is stated that "animal welfare means the physical and mental state of an animal in relation to the conditions in which it lives and dies", (OIE, 2019). The increasing concern and interest in animal welfare has resulted in a vast research considering different aspects of animal welfare to date. However, according to Fraser (2008) different concepts of the bases for animal welfare influence the interpretation of animal welfare in a given situation (Figure 1).

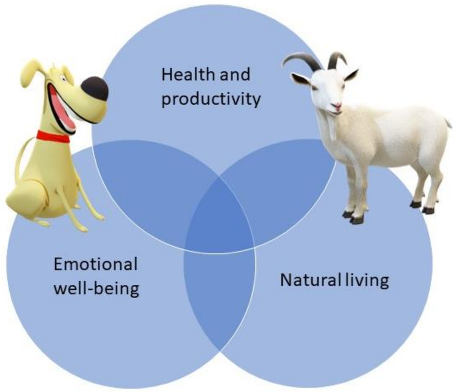

Figure 1. Three different views of animal welfare, adapted from Fraser, 2008. One prioritises the animal's health and freedom from pain, another the animal's happiness or distress, and the third the animal's ability to live a natural life within the system where it is kept. The different starting points imply that different individuals can judge the same situation to be good or bad for an animal's welfare.

Another influential definition of animal welfare is the one by Broom (1996): "The welfare of an individual is its state as regards to its attempts to cope with its environment". In the current thesis I will mainly focus on the view of animal welfare that emphasises how individuals perceive their environment (Duncan 1993; Veissier & Boissy 2007).

1.2 Measuring animal welfare

To define animal welfare as individual experience is an intuitively appealing thought, but is it possible to measure an animal's subjective feelings? Animal welfare has commonly been evaluated by focussing on the resources available, e.g. stocking density or access to food. An alternative approach that is becoming more common is to use animal based measures, both in science and in practical animal welfare assessment systems (EFSA, 2012). In the Welfare Quality system, principally animal-based measures are used to assess animal welfare on farm. The identified main principles of welfare are good feeding, good housing, good health, and appropriate behaviour, all considered to affect the probability of a good experience for the animal (Botreau *et al*., 2009). In a similar way Mellor (2020) has constructed the ‘five domains model’ that is based on the four principles, or domains, of nutrition, physical environment, health, and behavioural interactions. These four together constitute the fifth domain, that of mental state. As can be seen from the above, many researchers in animal welfare of today argue that feelings are a central part of animal welfare.

Feelings are subjective experiences and not possible to measure directly, but by combining physiology and behaviour, we can get a little closer. Simultaneous measurements of behavioural and physiological parameters aim at improving our understanding of the reactions of animals to different situations. In this thesis, we have combined behavioural and physiological methods to study two aspects of animal welfare, fear and discomfort, in two completely different species, the dog and the goat.

1.3 Dogs and goats

Dogs are predators and predominantly carnivores, used in many areas of society today: as companions, in sport, as rescue, police, or military dogs, and, not least, to aid disabled individuals. This put great demands on the dogs’ mental health. Dogs of many breeds are however so fearful of noises, or unfamiliar individuals and environments, that they cannot cope with the demands of today’s society. In addition, fearfulness may develop into aggressive behaviour if the dog is cornered or trapped. Frightened dogs with no possibility to escape, i.e. perfom a flight response, try to defend themselves, i.e. switch to a fight response. This is important to consider in breeding for mentally stable dogs that function in modern society.

Goats are abundant over the world, especially in the developing countries were three-quarters of the 700 million domestic goats are found (Dwyer, 2017). Hence, goats are important for both meat and milk production. Goats are usually kept as production animals and not companion animals. An understanding of how they react to fear or pain is important, not only to be able to recognize them and thus prevent them, but also to facilitate the handling in production

Goats are active and highly social animals (Dwyer, 2017; Zobel *et al*., 2019). They establish a strong and exclusive bond between goat and kid immediately after parturition (Collias, 1956; Klopfer, 1971; Poindron *et al*., 2003; Poindron *et al*., 2007a, 2007b; Miranda-de la Lama & Mattiello, 2010). Therefore, management procedures that lead to behavioural restrictions, disrupt social contacts between flock mates, or disrupt the bond between mother and young, may affect the welfare of the goats.

1.4 Fear and discomfort

Fear, an internal emotional state, is a reaction caused by the perception of actual danger (Gray, 1987; Boissy, 1995; Forkman *et al*. 2007; Sherman & Mills, 2008). Fear is caused by threatening stimuli and functions as the link between the stimuli and patterns of behaviour and physiology (Adolphs 2013). When an individual perceives danger or a threat, physiological reactions and behavioural responses are activated (Sherman & Mills, 2008), mediated by the amygdala in the brain (Adolphs, 2013) and may end with the so-called 'fight or flight' (Cannon, 1929; Steimer, 2002) or freezing reaction (Steimer, 2002). Hence, behavioural responses to fear vary and consist of both active and passive strategies: active defence (attack, threat) or active avoidance (flight, hiding, escape) and passive avoidance (immobility) (Erhard & Mendl 1999). Fear is a highly adaptive, self-protective response, helping the individual to cope with a challenging environment by organising the behavioural response to a truly dangerous situation (Panksepp, 1998; Koolhaas *et al*., 1999; Steimer, 2002; Adolphs, 2013; Lloyd, 2017). However, an animal that is frequently subjected to fear, with no possibility to escape, will suffer from stress (Lloyd, 2017), and may develop behavioural problems (King *et al*., 2003) impairing the long-term health of the animal (Koolhaas, *et al*., 1999).

Discomfort is an expression for mental and/or physical unease. In the five freedoms, it is stated that an animal should be provided with an appropriate environment and shelter to be free from discomfort. Discomfort can be experienced for example by lame cows (O'Callaghan *et al*. 2003), during heat stress in cattle (Mandal *et al.*, 2021) or when subjected to management procedures as for example accumulation of milk in the udder due to dry-off (Rajala-Schultz., *et al* 2018).

1.4.1 Dogs' fear of noises and surfaces

In dogs, fear-related behaviour is a common problem (Wells & Hepper, 2000; Gähwiler *et al*., 2020; Hakanen *et al.*, 2020). Fearfulness is reported to be the most common cause for failing behavioural tests among shelter dogs (Poulsen *et al*., 2010) and fear of noises is highly prevalent in pet dogs (Engel *et al*., 2019; Riemer, 2019; Gähwiler *et al*., 2020; Hakanen *et al*., 2020). In a modern society, there are several fear-eliciting situations to which dogs may not be adapted, e.g., loud noises such as fireworks or gunshots. Fear of gunshots is reported to be the third-most common noise aversion, after fireworks and thunderstorms (Sherman & Mills 2008). This type of fear can cause problems for dog owners and can also be a severe welfare problem for affected dogs.

Another problem for dogs, but not as well known, is fear of walking on different types of flooring. To our knowledge, this phenomenon was not studied before our study (Paper I). However, in a recent study, Hakanen *et al*., (2020), identified fear of surfaces and heights as multifactorial, and the rough collie as one of the breeds with the highest incidence of this type of fear.

1.4.2 Aversive events in goats

Tethering

In general, goats are kept in extensive systems (Dwyer, 2017) or housed in large open barns (Zobel *et al*., 2019). Tethering of goats occur in about five percent of dairy goat herds in Sweden, but in 45-50% of dairy cattle herds (Jordbruksverket, 2019). Goats are social and active animals with high cognitive functions (Miranda-de la Lama & Mattiello, 2010). Tethering of goats implies considerable behavioural restrictions, and is in conflict with their behaviour in a natural habitat. Therefore, in this thesis, we studied several physiological variables during tethering vs. loose housing in the same goats.

Mother and young separation

Goats are generally considered as hiders, i.e., after the initial few hours of caring for a new-born kid, the mother goat rejoins the herd to forage, only returning to nurse (Lickliter, 1984; O'Brien, 1984) . However, strategies vary among different goat populations depending on habitat and other factors

(O´Brien, 1988). In free living goats, kids may stay with their mothers for up to twelve months (Miranda-de la Lama & Mattiello, 2010). However, in today's dairy production, early mother and kid separation is the most common management practice, intended to achieve maximum milk yield for commercial production (Miranda-de la Lama & Mattiello, 2010; Lu & Potchoiba, 1988). This procedure can be considered aversive and stressful for both goats and kids (Boivin & Braastad, 1996; Bergamasco *et al*., 2005; Newberry & Swanson 2008; Miranda-de la Lama & Mattiello, 2010; Winblad von Walter *et al*., 2010) since it involves disruption of the mutual bond between goats and kids, and is therefore often considered a welfare problem. However, little is known about how it affects the development of different traits such as, for example, fear. Therefore, the effects of early separation on physiology and behaviour were studied in this thesis.

Social isolation

The social group is important for survival in providing protection from predators and help in caring for the young, as well as assistance in finding food (Dwyer, 2017). Therefore, it's highly relevant for goats to react adversely when isolated from conspecifics, and isolation is considered a highly negative event for goats (Carbonaro *et al.*, 1992; Al-Qarawi & Ali 2005; Forkman *et al*., 2007; Siebert *et al*., 2011; Chojnacki *et al.*, 2014). In this thesis, we tested both social isolation and performed an arena test (with an added startle effect), which also contains the component isolation but with a larger area than in the isolation test.

1.5 Fear and discomfort: physiological and behavioural responses

All species have developed certain behavioural strategies for survival, but while there is a large diversity in behaviours between species, the physiological reactions are more conserved even across a range of species.

The nervous system and the endocrine system of the individual interact in response to different situations with the aim of maintaining homeostasis. When an individual experiences excitement, fear, or stress, the brain receives information both from the body and from the environment. The limbic system and hypothalamus are structures in the brain involved in emotions and motivations. The hypothalamus is located in the middle, basal part of the brain, and controls and coordinates the autonomic nervous system, the endocrine system, and the behaviour of the individual (Figure 2).

1.5.1 The autonomic nervous system

The hypothalamus responds to physical and emotional challenges by activating the autonomic nervous system, which concist of the sympathetic nervous system and the parasympatethic nervous. In stress situations, the sympathetic nervous system tends to become activated, which for example leads to release of adrenaline and noradrenaline from the adrenal medulla. Noradrenaline and adrenaline are also transmitter substances in the postganglionic sympathetic neurons. Sympathetic activation prepares the individual for fight, flight, and fright, i.e., increases arterial blood pressure, heart rate, blood flow to the muscles, cellular metabolism, blood glucose levels, glycolysis, muscle strength, mental activity and blood coagulation. In addition, activation of the sympathetic nervous system may in some species, such as dogs (Reeve *et al.*, 1953) and goats (Eriksson *et al*., 1994), stimulate the spleen to contract and thus release erythrocytes into the blood, This is measured as haematocrit, e.g. percent erythrocytes of the total blood. The parasympathetic nervous system (PNS) has an effect opposite to the sympathetic stimulation, e.g., decreases heart rate and stimulates the digestion. But the PNS is also involved in passive behavioural responses to fear, e.g., immobility and freezing. The hypothalamus can alter the activation of the two systems rapidly depending upon the situation.

In earlier studies in goats, basal values of heart rate and blood pressure have been recorded telemetrically during different reproductive periods and feeding (Hydbring *et al*., 1999) as well as during blood sampling (Hydbring *et al*., 1997). The results showed that heart rate, but not always blood pressure, varies considerably according to management routines. Heart rate is also reported to be affected according to positive anticipation (Boissy *et al*., 2007).

1.5.2 The hypothalamus-anterior pituitary-adrenal-axis

The hypothalamus also controls the endocrine system and the pituitary gland and releases corticotropin releasing hormone (CRH) to the anterior pituitary. The anterior pituitary stimulates release of adrenocorticotropic hormone

(ACTH) which in turn stimulates the adrenal cortex to release steroid hormones such as glucocorticoids, mineral corticoids and androgens.

Cortisol

One of the main effects of cortisol is to make energy available in challenging situations by mobilizing protein, fat and glucose. Cortisol stimulates gluconeogenesis which make glucose available. In addition, cortisol has important anti-inflammatory and immunosuppressive functions. The major part of circulating cortisol in the blood is bound to corticosteroid-binding globulin (CBG) and some to albumin, and only small quantities are in the biologically active free form (Sjaastad *et al.*, 2016). Cortisol is a commonly used stress parameter in many species, and is released during both physical activity and mental alertness

In dogs, the cortisol concentration is for example reported to increase in dogs trained with aversive-based methods compared to reward-based methods, and in dogs entering a pet-hotel (Vieira de Castro *et al*., 2020) and as a response to transportation (Bergeron *et al.*, 2002; Frank *et al*., 2006; Herbel *et al*., 2020). Reduced cortisol levels in dogs is also reported to be linked to play and exploratory behavior (Rossi *et al.*, 2018), human interaction (Shiverdecker *et al.*, 2013) and petting in shelter dogs (Hennessy *et al*., 1998; Dudley *et al*., 2015) .

In goats, cortisol is widely used, and has been measured in such different situations as for example isolation (Kannan *et al.*, 2002; Al-Qarawi & Ali, 2005), parturition (Hydbring *et al.*, 1999), transportation (Sanhouri *et al*., 1989; Greenwood & Shutt, 1992; Kannan *et al*., 2000; Ali *et al.*, 2006) restraint (Al-Qarawi & Ali, 2005), stomach intubation and fluid administration (Eriksson *et al.*, 1994), disbudding (and food deprivation as well as catching sight of food *(*Olsson *et al*., 1995). Cortisol is essential to milk synthesis (Bomfim *et al*., 2018) and released in conjunction with milking in goats (Olsson & Högberg, 2009; Högberg *et al*, 2014).

Progesterone and testosterone

Progesterone is a reproductive hormone primarily released from the ovaries and transported in blood plasma bound to proteins. However, progesterone has been reported to be secreted from the adrenal in male rats (Schaeffer & Aron, 1987) and humans (Elman & Breier 1997; Wirth *et al*., 2007) but also in ovariohysterectomised cats (Chatdarong *et al*., 2006) and ovariohysterectomised cows (Yoshida & Nakao, 2005). The administration of ACTH to ovariohysterectomised gilts resulted in the elevation of cortisol, progesterone, and prostaglandin $F_{2\alpha}$ metabolite levels (Mwanza *et al*., 2000), which indicates that cortisol and progesterone may be released at the same time from the adrenal cortex.

Testosterone is mainly produced in the testes and is necessary for spermatogenesis and male characteristics. However, testosterone may also be released from the adrenal cortex in both sexes (Sjaastad, 2016). Testosterone has been reported to be connected to aggression and physical activity (Olweus *et al*. 1988; Williams *et al*. 2000; Simpson, 2001). Recently, in a study on women, it was suggested that an aggressive response to provocation was correlated to high levels of testosterone in saliva (Probst *et al*., 2018).

1.5.3 Hypothalamus-anterior pituitary: β-endorphin

In the anterior pituitary, the same hormone precursor that generate ACTH also generate *β*-endorphin. (Figure 1). ß-endorphin is released into the peripheral circulation due to stress or exercise (Lee & Wardlaw, 2007) and is involved in pain inhibition by binding to opioid receptors. They are therefore important in the endogenous pain-control system. ß-endorphin has also been reported to mediate euphoria that can be experienced in prolonged exercise (Smith, 2006). In a recent study on stress in shelter dogs (Righi *et al*., 2019), ß-endorphin is suggested to play a role in modulating the stress response in dogs.

1.5.4 Hypothalamus-posterior pituitary: Vasopressin and Oxytocin

Vasopressin and oxytocin are synthesized in and secreted from neurons in the hypothalamus. They are transported directly via axons to the posterior pituitary, from which they are released into the blood stream (Figure 1). Oxytocin stimulates milk ejection and uterine contraction during parturition. The milk ejection reflex is induced by tactile stimuli of the mammary glands which send impulses via sensory nerves to the hypothalamus. Oxytocin is secreted and then released from the pituitary to the blood and transported to the mammary glands where it induces contraction of myoepithelial cells around the alveoli (Sjaastad, 2016). Oxytocin is also synthesized in the ovaries, testes, uterus, and several other organs. Oxytocin is also released as a neurotransmitter in the brain and has, along with other hormones, an important function in developing and maintaining maternal attachment to offspring and promoting maternal care (Uvnäs-Moberg, 1998; Newberry & Swanson 2008). It also has a dampening effect on social anxiety and fear (Insel and Young, 2001).

Vasopressin, also known as antidiuretic hormone (ADH), is secreted during dehydration to decrease water excretion by the kidneys. However, ADH is also a potent vasoconstrictor and is, as such, called vasopressin. The vasoconstrictor effect of vasopressin can elevate blood pressure when concentrations reach high levels. Vasopressin is also a neurotransmitter and involved in aggressive behaviour (Stribley & Carter, 1999; Caldwell *et al*., 2008) and enhances arousal, attention and vigilance (Carter & Altemus, 1997).

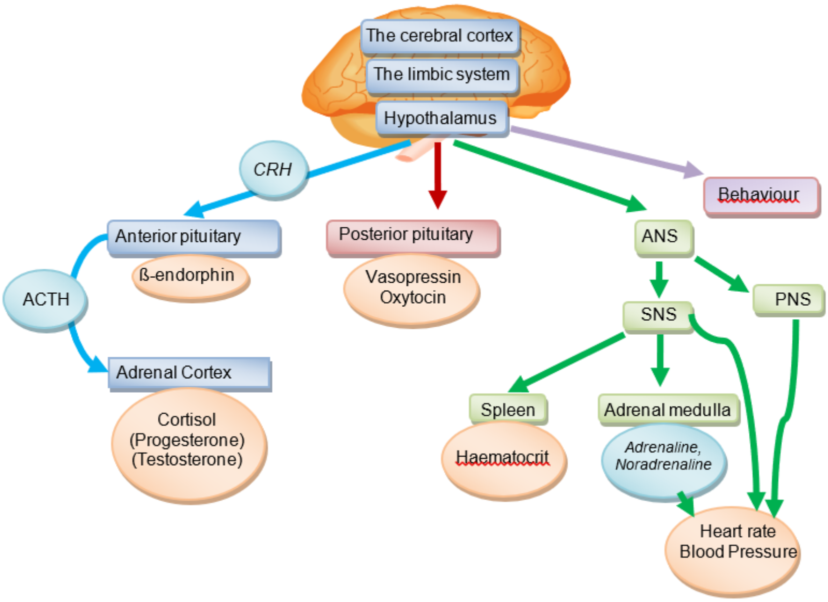

Figure 2. Modified from Winblad von Walter, 2010. A simplified schematic drawing of the stress response. The figure illustrates the physiological and behavioural stress response, e.g. the hypothalamic-pituitary-adrenal axis (blue pathway), release from the posterior pituitary (red pathway), stimulation of the autonomic nervous system (green pathway) and behavior (purple)

1.5.5 Behaviours related to fear and discomfort

There are a diverse array of behavioural patterns indicating a fear reaction in animals. Avoidance, flight, and immobility are examples common in several species and observable in both dogs and goats. However, behavioural patterns related to fear can be contradictory since both proactive and reactive strategies can be observed, such as active defence and avoidance and passive avoidance. Active defence includes attack or threat while active avoidance can be expressed as flight, hiding, or escape. Passive avoidance can be expressed as immobility or freezing (Boissy, 1995; Erhard & Mendl, 1999). In dogs, several behavioural expressions of fear have been identified. Dogs fearful of sounds may pant, pace, tremble or perform eliminative behaviours (Sherman & Mills, 2008), but hiding or escape behaviour is also indicative of fear in dogs. In goats, locomotor activity, rearing, exploration and vocalisations (Price & Thos, 1980; Forkman *et al*., 2007) but also sniffing (Carbonaro *et al.* 1992) are examples of behaviour recorded in fearful situations.

When mother and young are separated early and abruptly, the behavioural response for reinstatement can be locomotion (searching) and vocalisations (Newberry & Swanson 2008) which under natural conditions would increase the probability of reunion (Panksepp, 1998; Manteuffel *et al*., 2004) Acoustic signals communicate that animals are in need of something and Goats use different types of vocalisations and exhibit increased frequencies of vocalisation when isolated from social partners (Price & Thos, 1980).

1.6 Early separation: effect on milk production and growth

Early separation often leads to reduced growth rate in goat kids (Miranda de la Lama & Mattiello, 2010). The main argument for early separation of goats and kids is the increased amount of milk that can be collected for processing and consumption (Delgado-Pertíñez *et al*., 2009a, 2009b). However, there is a great diversity in housing and management systems of goats, and the time period that goats and kids are kept together varies in Sweden from one day up to five months (Brandt, 2009), but it has been recommended that weaning should not occur before six to seven weeks of age (Miranda-de la lama & Mattiello, 2010). In addition, it has been shown that an early interruption of the bond between mother and kid affects both lactation persistency and oxytocin release during milking (Marnet & McKusick, 2001).

Figure 3. One of the goats in the experimental herd with her triplets in home pen. Goats and kids develop at strong bond early after parturition.

2. Aims of the thesis

The overall aim of the present thesis was to study physiology and behaviour during fear and discomfort in dogs and goats and the interactions between the different variables.

Specific aims were to:

- Investigate the physiological response, as measured by heart rate, haematocrit, cortisol, progesterone, testosterone, vasopressin and ß-endorphin concentrations, in dogs to a floor test and a gunshot test (Paper I).
- Establish whether it is possible to separate dogs that are behaviourally fearful of walking on different types of floors and fearful of gunshots from less fearful dogs, by measuring physiological variables (Paper I).
- Investigate physiological responses of tethering versus loosely held goats in pairs, by recording heart rate, blood pressure, and plasma concentrations of cortisol, β-endorphin, vasopressin, and oxytocin (Paper II).
- Investigate physiological response of blood pressure, heart rate, cortisol, β-endorphin, vasopressin, and oxytocin and behaviour of dairy goats during suckling and permanent separation of goats and kids with established bonding (Paper III).
- Investigate home-pen behaviour and growth rate in kids that were early separated, daily separated or kept full time with mother goat (Paper IV).
- Investigate how milk yield and milk composition in goats are affected by daily separation or no separation from kids (Paper IV).

- Establish whether kids reared full-time with mother, part time with mother or without mother differed in their physiological and behavioural responses to an aversive situation (Paper V).

3. Hypothesis

The overall hypothesis was that measuring several physiological and behavioural parameters is important when evaluating animal welfare.

The following specific hypothesis were tested:

- It is possible to separate fearful dogs from less fearful dogs by measuring physiological response to two fear eliciting tests; a floor test and a gun shot test (Paper I)
- Tethering in goats cause a physiological stress response and affect welfare (Paper II)
- Suckling is a peaceful event, while acute separation causes stress in goats with established bonding to their kids (Paper III)
- Keeping goats and kids together increase welfare of both mother and kid, and have a positive effect on milk composition (Paper IV).
- Kids reared with mother for two months are less fearful in their physiological and behavioural reactions when compared to early separated kids. The reaction of daily separated kids will fall between the other two treatments (Paper V)

4. Materials and methods

In this section, a summary of methods and experimental procedures are given. Detailed descriptions can be found in the corresponding sections within each paper (I-V).

4.1 Animals

4.1.1 Dogs (Paper I)

Thirteen privately owned male dogs of the Collie breed were studied. The collie breed was chosen for the breed's known problems with fearfulness. The dogs were selected from a questionnaire. The questionnaires were distributed at a meeting for interested dog owners where they were informed about the study, and contained questions about the behaviour of the dogs that concerned everyday life. All dogs classified as fearful of gunshots in this study were described as such by their owners. In addition, the owners stated whether they perceived their dogs to be fearful or not fearful of walking on different types of floors. Seven dogs were considered as fearful and seven as less fearful. Since fear of floors did not always occur concomitantly with fear of gunshots, there were consequently four different groups of dogs. One of the less fearful dogs was taken out of the study because of difficulties with the blood sampling. The use of privately owned dogs was approved by the National Board of Agriculture and the experimental design was approved by the local Ethics committee for Animal Experiments in Uppsala.

4.1.2 Goats and kids (Paper II-IV)

All goats and kids in this thesis were of the Swedish domestic breed (*Capra hircus*) and belonged to an experimental herd at the Swedish University of

Agricultural Sciences. They were housed in indoor pens enriched with boxes as hideouts and tables to climb on. Straw and wood shavings were used as bedding material. The goats and kids were fed hay and concentrates. Water and mineral stones were available ad libitum. They were well adapted to handling and the adult goats were accustomed to blood sampling procedure. The care of the animals and the experimental design for all studies was approved by the local Ethics committee for Animal Experiments in Uppsala. In the study presented in Paper II, eight non-pregnant, non-lactating goats, aged 2-6 years participated. Six of the goats had implanted transmitters that registered heart rate and arterial blood pressure telemetrically.

In the study presented in Paper III, seven goats with similar transmitters as described above and one kid each participated. The goats were moved to individual boxes before parturition and kept there during the study.

Eleven goats *(Capra hircus)* and 22 kids participated in the studies presented in paper IV and V. The goats were milked between 07.00 h and 08:00 h in the morning and between 15:00 and 16:00 h in the afternoon.

4.2 Experimental procedures

4.2.1 Dogs: Floor test and gunshot test (Paper I)

The dogs were subjected to a floor test and a subsequent gunshot test (Figure 4). During the floor test, the dogs walked over a total of seven different types of floors in sequence: plastic, parquet, marble stairs, clinker, concrete, iron grids, and wobbling boards. Before the test, and after the parquet, marble stairs and the wobbling boards, blood samples were taken. After the floor test was finished, the dogs were walked to a fenced meadow where they rested for 30 minutes. Two gunshots were then fired with five minutes in between. Heart rate was measured throughout the experiment by telemetric Polar equipment and blood samples were taken through a permanent catheter in the cephalic vein before, during, and after the tests. In this study, several behavioural parameters were recorded, but all measured behaviours were not possible to include in Paper I. For classification of the dogs as fearful or not fearful during the floor test, an observer scored whether dogs were unaffected, a little hesitant, or very hesitant to enter the passage, and whether dogs tended to move towards the wall. After both the first and second gunshots, the initial startle reaction and degree of fear was estimated as described in Paper I.

Figure 4. One of the participating dogs passing iron grids during floor test, and one dog in the meadow during gunshot test

4.2.2 Goats: Tethered alone vs loose-housed in pairs (Paper II)

The goats were randomly divided into two groups and the experiment was performed in a cross-over design. The goats were either kept in a pen with another goat or tied up individually in a metabolism cage. All goats were housed in the same stable and could see and hear each other. Before the registrations started, the animals were housed in the new system for four days. Thereafter, telemetry registrations were made every 30 minutes for 24 hours. Blood samples were taken during a separate day by venipuncture at 08.00, 10.00, 12.00, 14.00 and 16.00h, and plasma cortisol, β-endorphin, vasopressin and oxytocin concentrations were analysed.

4.2.3 Goats: Suckling and permanent separation (Paper III)

Four studies were performed in goats kept with their first-born kid in individual boxes (Figure 5):

- Study 1: Heart rate and blood pressure were analysed around an undisturbed suckling as visualized on the videotape recordings.

- Study 2: Blood samples were taken before, during, and after suckling.
- Study 3: On the day of goat and kid separation, blood samples were taken before and after separation, 3-4 days after parturition. In addition, vocalisations were recorded after separation.
- Study 4: Heart rate and blood pressure were analysed the first and second nights after parturition, and the nights after study 2 and 3. Time spent lying down was estimated from the video recordings.

The goats were videotaped for behavioural analyses and heart rate and blood pressure were recorded by telemetry during all four studies. Similar to paper II, the blood samples were analysed for plasma concentrations of cortisol, β-endorphin, vasopressin and oxytocin

Figure 5. One of the goats with her first born kid in home pen. All goats and kids were kept together during colostrum period.

4.2.4 Kids: Effect on kids of mother goat presence (Paper IV-V)

Before parturition, twelve goats were randomly assigned to two treatments: ‘daily separated,’ where goats and kids (n=6) were kept together but separated daytime between 07.30-15.00h (DAY-SEP), and ‘no separation,’ where goats and kids (n=6) were kept together 24h (NON-SEP). The kids in the latter group were only allowed to suckle one teat, as the other was covered with a bra to prevent suckling. After parturition, one kid was allocated into the same treatments as their mothers (DAY-SEP, NON-SEP) and the rest of the kids (n=10) were allocated to a third treatment where the kids were permanently separated from their mothers after the colostrum period (SEP). Due to udder problems one goat was euthanised, and therefore one of the goats in DAY-SEP had two suckling kids.

In Paper IV, we focused on kids' behaviour in the home pen, kids' growth, and the mother goats' milk production. Body weight of all kids was recorded once weekly from birth until 9 weeks of age (Figure 6). At two weeks (range 12-16 days), and 2 months (range 59-69 days), instantaneous recordings of kids' behaviour in the home pen were made every 10th minute by direct observations from 7:00 until 19:00h by two observers. The goats were machine milked twice daily at 7:30 and 15:30h, and milk samples were collected over 70 days. Both udder halves were milked separately with a specially designed "separation-milker 8L" bucket-milking machine (provided by DeLaval international AB, Tumba, Sweden). Samples of fresh milk were analysed for fat, lactose and total protein by a mid-infrared spectroscopy method (Miris farm milk analyser, 2001). The casein concentration was determined by a rennet coagulation method and finally measured by the same mid-infrared spectroscopy method as above.

In Paper V, focus was on kids' reactions to challenges. All kids were subjected to an isolation test at two weeks and two months of age. The isolation test lasted for twelve minutes with the sound of a dog bark at ten minutes. In addition, a 20-minute arena test was performed at two months of age. At 10 minutes, a novel object appeared in the shape of a plastic bag filled with cans that fell from the ceiling. During all tests, heart rate was measured every 5 seconds by telemetric Polar equipment and saliva samples were taken. During the isolation test, the first sample was taken in the home pen before the test, the second sample immediately after the test, the third sample ten minutes after reunion with mother, respectively kids group, and the fourth sample one hour after reunion. During the arena test, the first sample was taken in the home pen before the test, the second sample immediately after the test was finished, and the third sample one hour after the test. Recordings of vocalisations were made continuously by the same person by direct observation during all tests. The arena-test was videotaped and analysed for behaviours using Boris 4.1.1. (Friard & Gamba, 2016).

Figure 6. The kids were weighed weekly.

4.3 Aspects of materials and methods

4.3.1 Methods for physiological measures

Blood sampling

Measuring physiological variables can affect the animal and influence the results. It is therefore important that the people involved knows the proper way of handling the animal to mimimize effects of the sampling per se. Analyses of blood give much information and therefore blood sampling is commonly used.

To minimize the disturbing of the animals in the test situation in Paper I, and goats in Paper III, a catheter was inserted under local anaesthesia into the cephalic vein in the dogs and in a jugular vein in the goats (Figure 7). In the present experiments, these procedures were done calmly and with gentleness and the animals did not show aversive reactions and appeared calm. After insertion the animals rested, and thereafter blood samples could be withdrawn repeatedly without any reactions from the individual.

Figure 7. A dog with a catether inserted in the cephalic vein and blood sampling in a goat from a catether inserted in the jugular vein.

In Paper II, the goats were in some parts of the experiment loose-housed in pairs. Goats are curious and active animals, and there was a risk that the goats would try to nip on each other's permanent catheters. Therefore, blood samples were taken with venipuncture. Since the goats were used to experiments and to be restrained, they did not seem to react much to the sampling.

Alternatives to blood sampling include collection of saliva. This method works for some parameters, for example cortisol. The advantages with saliva sampling it that it is non-invasive and easy to perform. In cases of privately owned animals, it is possible for the owner to collect the samples by themselves. However, despite being non-invasive, some animals may resist this method, and it may be difficult to collect enough amount saliva. In addition, not all hormones are possible to analyse in saliva.

Blood plasma and saliva cortisol analyses

All blood and saliva samples were analysed in our laboratory by the same experienced person. The methods used were validated for each variable and each species. Dilutions of plasma were parallel to standard curves in all radioimmunoassay used. The analyses performed also had high recovery, low intra-assay variation (<10%) and were sensitive as judged by the low least detectable values.

Recordings of heart rate and blood pressure

A useful method when studying fast autonomic changes is telemetric measurements, which make it possible to continuously measure heart rate and blood pressure in undisturbed animals.

Heart rate measurements in the dogs in Paper I and the kids in Paper V were performed by a Polar heart rate monitor (Polar Vantage NV; Polar Ltd Bromma). Before the experiments started, a heart rate monitor was strapped around the chest of the dogs/kids. The Polar heart rate monitor is a non-invasive method for recording heart rate enabling recordings of the heart rate of freely moving animals. The receiver is a watch that can be fastened on the animal (Figure 8).

Figure 8. The Polar Sport Tester in one of the dogs in Paper I and in one of the kids in paper V. The goat kid is sniffing on a harness that was used on goats in Paper IV-V.

The goats in Papers II and III had a surgically implanted telemetric devices for recording both heart rate and blood pressure (Figure 9). The device consists of a sealed transmitter body (Data Sciences Inc., St Paul, MN, USA) placed subcutaneously on the side of the goats' neck connected to a fluid-filled catheter which ends in the carotid artery. The operation technique has been described by (Hydbring *et al.*, 1997) . The transmitter sends signals to a computer via a receiver placed over each box. This method makes it possible to register both blood pressure and heart rate in unrestrained animals over long time periods of time. By comparing recordings made simultaneously with telemetry and conventional methods, this method has been validated in goats.

Figure 9. The telemetric device and an illustration of the catether (Paper II and III).

Both telemetric methods have advantages and disadvantages. The surgically implanted telemetric device gives the possibility to record both heart rate and systolic and diastolic blood pressure values in conscious freely moving animals. However, the area the animals can move around is restricted by the distance to the receivers. An obvious advantage with the Polar Sport Tester is that it is non-invasive and that it can be used in the field since the receiver is carried by the animal. The method is used in several species, and has been validated for dogs (Essner *et al*., 2013) cows However, it is not possible to register blood pressure and the belt moves, the transmission may be disrupted.

4.3.2 Methods for behavioural measures

Even though behavioural studies are always non-invasive, it must be taken into consideration that animals can be affected solely by the presence of an observer or by a behaviour test *per se*.

Since the dogs used in this study were living as family members, the dog owner stayed with the dog throughout the whole experiment to avoid effects of separation from the owner. The dog owners were instructed to be passive and not interact with their dog during the floor test and gunshot test. The owners responded carefully to this. Throughout the dog study, one observer did direct observations of the behaviour of the dogs, but the whole experiment was also videotaped.

The goats in Papers II and III were observed for several days and nights in some experiments. For practical reasons, direct observations at all times were not possible, which was why the behaviour of the goats was observed from videotapes, and due to missing data the behavioural data were not included in Paper II. The arena test in Paper V was also videotaped. Documentation of behaviour on videotapes is valuable since the tapes can be checked many times, reducing the risk of missing valuable information. On the other hand, small behavioural expressions, such as for example trembling or ear position, may be difficult to detect from a videotape.

The registrations on home-pen behaviour in Paper IV, as well as registrations of vocalization at separation in Paper III, and the isolation and arena tests in Paper V, were performed by direct observation. For the long-term registrations of several behaviours for home pen behaviour, we chose instantaneous sampling every 10th minute, but for the registration of vocalization during the tests that lasted 12-20 minutes, we performed continuous observations. Continuous recording gives a more accurate picture of the performed behaviours but is not possible to manage through several hours and numerous behaviours.

4.3.3 Methods for production measures

In the goats that were kept full time with their kids (NON-SEP), one teat was machine milked and the kids suckled the other. To prevent the kids from suckling the teat intended for machine milking, the goats had a specially designed bra suspended in a harness that allowed the kids to suckle only one teat (Figure 10). Daily separated kids (DAY-SEP) suckled both teats.

Figure 10. The NON-SEP goats in Paper IV-V had a specially designed bra preventing the kids from suckling the teat intended for machine milking.

5. Main results

This section gives a summary of the results of Papers I – V. Detailed information of the results can be found in the corresponding sections within each paper.

5.1 Fear provocation in dogs (paper I)

Seven of thirteen dogs were classified as fearful of floors. The single physiological variable affected by the floor test was the heart rate, which was higher in fearful dogs during the floor test than in less fearful dogs. Seven of thirteen dogs were classified as fearful of gunshots. The physiological reaction to a gunshot was striking in fearful dogs. Almost all measured variables, heart rate, haematocrit, plasma cortisol, progesterone, vasopressin and ß-endorphin, were higher in fearful dogs compared to in less fearful dogs. In dogs fearful of gunshots, the behavioural expression of fearfulness showed a large variation between individuals. Some dogs responded to this challenge by escape, while others stayed passive in the same spot, trembling or shaking.

5.2 Complex stress reaction in goats (paper II and III)

Comparisons between tethered goats and goats kept penned in pairs, in Paper II, revealed that heart rate and arterial blood pressure were higher when the goats were tied up. In contrast, the β-endorphin and oxytocin concentrations were significantly higher in the loose-housed goats. In addition, oxytocin concentrations fluctuated more when the goats were kept in pairs. However, cortisol and vasopressin concentrations did not differ between the two systems. In line with earlier studies, heart rate was affected by feeding

(Hydbring *et al.*, 1999), and increased around feeding in both tethered and loose goats held in pairs.

As ascertained in Paper III, the separation of goats from their kids did not cause any physiological changes in the goats. During the 20 minutes of observations after separation, both goats and kids vocalised intensively, but it took only 11 minutes on average for the goats to lie down after separation. Both heart rate and blood pressure were significantly lower, and the goats spent more time lying down the night after separation than the first and second nights after parturition.

5.3 Effects of Suckling in goats (paper III)

Suckling did not induce any significant cardiovascular changes in the goats tested in this study. Plasma concentrations of cortisol and ß-endorphin increased during suckling and five minutes after, while oxytocin and vasopressin remained unaffected by suckling.

5.4 Milk yield and composition in goats kept with their kids full-time or with daily separated kids (paper IV)

The mean daily milk yield were higher in DAY-SEP goats (2420 g ± 119 g) compared to NON-SEP goats (2149 ± 79 g). In addition, the fat concentration was also higher in DAY-SEP goats (4.9 ± 0.1 %) than NON-SEP goats (4.4 ± 0.1%). However, there were no differences between DAY-SEP and NON-SEP goats in total protein, lactose, or casein concentration.

Regarding udder halves, the, milk yield was as expected higher from the milked teat than from the suckled one in the NON-SEP goats while no differences were found between right and left udder halves in DAY-SEP goats, indicating that both teats were suckled at a similar amount (Figure 11).

Figure 11. Kids suckling. The NON-SEP goats had a bra in a harness to prevent the kids from suckling the teat intended for milking, while the DAY-SEP suckled both teats.

5.5 The effect of mother goat presence on kids growth and home-pen behaviour (paper IV)

The growth rate was similar in all kids (180 ± 9 g/day). At two weeks, kids that stayed with their mothers full-time showed more hiding behaviour than the other treatments. There were no differences between treatments in total active or resting behaviour, but kids that were permanently separated were more active with another kid at both two weeks and two months than kids kept full time with mother .

5.6 The effect of mother goat presence on their kids response to isolation and an arena test (paper V)

There were no clear-cut differences between treatments in the kids' responses to isolation or arena test. However, the kids that were permanently separated from their mothers deviated most from the other treatments in the isolation tests. They reduced their vocalization earlier and had a higher heart rate before and after the sound of a dog bark at two weeks and a higher heart rate throughout the test at two months. On the other hand, the daily separated kids bleated more at two weeks compared to the other treatments, and showed a clear decrease in heart rate after dog barking at two months. In addition, the daily separated kids showed the strongest fear reaction in the arena test, at two months of age. They performed more escape attempts before 'startle' and showed a clear drop in heart rate and more passivity after the novel object. The permanently separated kids vocalized more before the novel object compared to the other treatments but showed more explorative behaviour after the test. However, this was not significant compared to kids kept full-time with mother. There were no differences between rearing systems regarding latency to sniff object or exploring the startle object.

Figure 12. Pictures from the different home pens for permanently separated kids, daily separated kids and in kids kept full time with mother.

5.6.1 Reunion after isolation (unpublished results)

After the isolation test, we measured the kids response to reunion either with their mothers or the kids group. For the separated kids, we defined “drinking milk from the milk bar with an artificial teat” as suckling. At two weeks of age, the daily separated kids suckled more than kids in the other treatments ($P<0.05$), but at two months, there were no difference between treatments in suckling (Figure 13). At two weeks, kids kept full-time with mother picked more hay than separated kids, and at two months, the daily separated kids and kids kept full-time with mother picked more hay than separated kids.

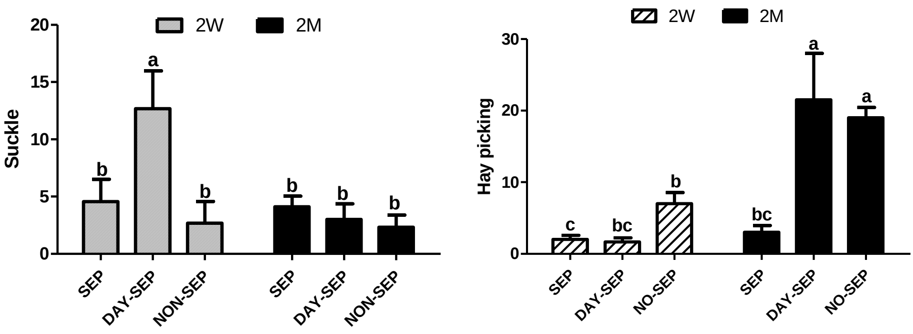

Figure 13. Suckling (a) and eating behaviour (b) registered every 10^{th} seconds for ten minutes during the reunion phase (after isolation test). The kids were either permanently separated from their mothers (SEP, n=10), separated for 8h during daytime (DAY-SEP, n=6) or not separated (NON-SEP, n=6). The kids were observed for ten minutes each at two weeks of age and for two months of age. Values are presented as least square means (LSM) and standard error of mean (SEM). Different superscript letters indicate significant differences (P<0.05).

At two weeks, the overall mean heart rate was higher during reunion than isolation but at two months, the overall mean heart rate was higher during isolation than reunion for all treatments (Figure 14).

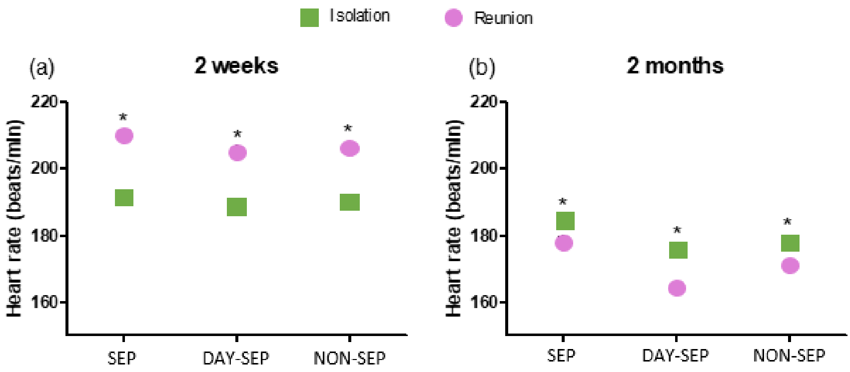

Figure 14. Overall means of heart rate during isolation and reunion at 2 weeks (a) and 2 months (b) of age for all treatments. The kids were either permanently separated from their mothers (SEP, n=10), separated for 8h during daytime (DAY-SEP, n=6) or not separated (NON-SEP, n=6). At 2 weeks, the overall mean heart rate was higher during reunion than isolation, but at 2 months the heart rate was higher during isolation than reunion for all treatments. *indicate significant differences in mean heart rate between isolation and reunion. Level of significance was ($P<0.05$).

5.7 Further observations (unpublished results)

5.7.1 Individual differences

The individual differences in heart rate and vocalisation were large in both Paper IV and V. During isolation test at two weeks of age, eight kids showed a pattern where heart rate and vocalisation followed each other, while thirteen kids showed a different pattern with a negative relationship between heart rate and vocalisation (Figure 15). Twins that received the same treatment (in SEP and DAY-SEP), showed different reactions according to vocalization and heart rate pattern.

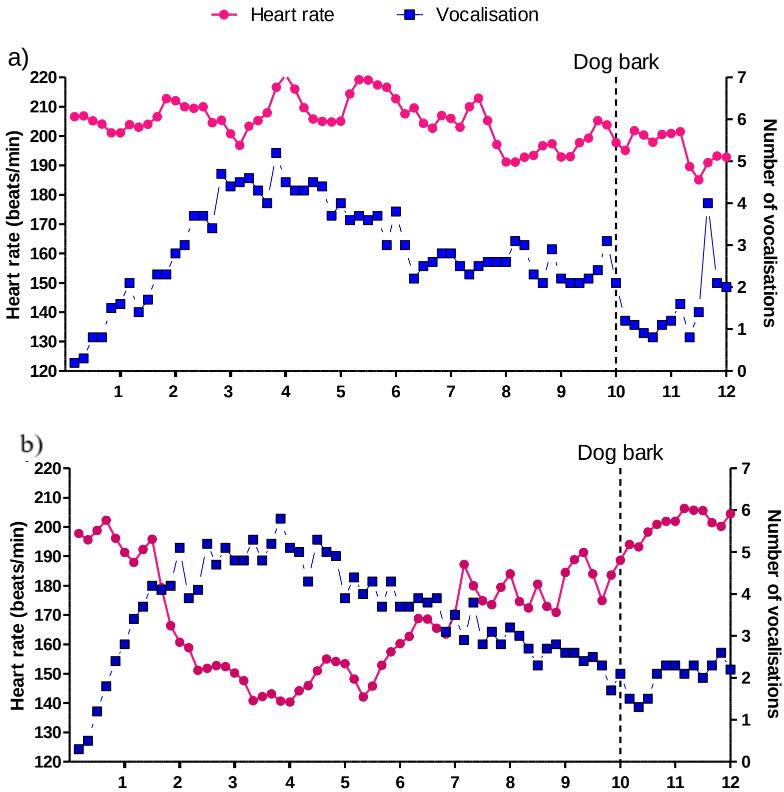

Figure 15. Heart rate and vocalization rate in kids during isolation test at two weeks of age. Eight kids showed a pattern where heart rate and vocalisation followed each other (a), while thirteen kids showed a negative relationship (b).

5.7.2 Gender differences

In Papers I-III, we studied one gender. In Papers IV-V, the kids were both females and males. In those studies, primarily the female kids were kept with their mothers, which resulted in a skewed distribution of gender between treatments. A single overall difference between gender was found. The latency to sniff object was longer for females than males (Figure 16).

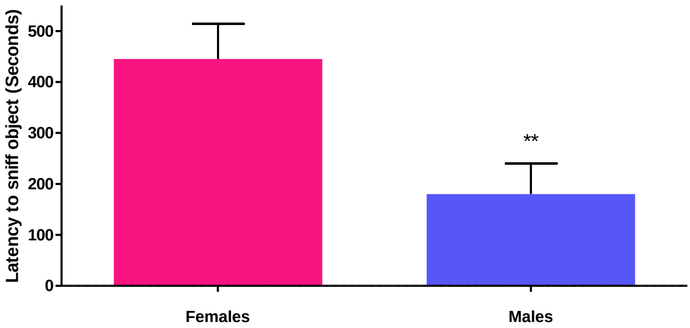

Figure 16. Latency to sniff object during the arena test in 22 kids. The latency to sniff object was longer for females than males ($P \leq 0.03$).

5.8 Methods and results in summary

The methods used for behavioural and physiological measures aimed to disturb the animals as little as possible, but also be possible to perform in the different studies. Therefore, different methodologies were used in the different papers. In Table 1, all parameters used in the respective papers are presented, as well as whether they significantly increased, decreased or were remained unchanged during the study.

Table 1. Summary of methods used and results of the studies included in this thesis. (↑, ↓) indicate significant differences, (↔) no significant differences, (-) not measured

Variables	**I (Dogs) Floor test/ Gunshot test**	**II (Goats) Tethering**	**III (Goats) Suckling/ Acute separation**	**IV (Kids) Separated/ Daily Separated/ Not separated**	**V (Kids) Separated/ Daily Separated/ Not separated**
Physiological					
Heart rate	↑, ↑	↑	↔	-	↑↓
Haematocrit	↔, ↑	-	-	-	-
Blood pressure	-	↑	↔	-	-
Cortisol	↔, ↑	↔	↑, ↔	-	↔
β-endorphin	↔, ↑	↓	↑, ↔	-	-
Oxytocin	-	↓	↔	-	-
Vasopressin	↔, ↑	↔	↔	-	-
Progesterone	↔, ↑	-	-	-	-
Testosterone	↔	-	-	-	-
Behavioural					
Vocalisation	-	-	↑	-	↑
Active behaviour	-	-	-	↑	↑
Passive behaviour (resting/laying)	-	-	↑	↑	↔
Exploring	-	-	-	-	↑↓
Startle-response	↑ ↔ (gunshot)	-	-	-	↑↓
Immobility	↑↓	-	-	-	↑↓
Avoidance/escape	↑↓	-	-	-	↑↓
Behavioural tests					
Isolation test	-	-	-	-	↑↓
Separation	-	-	↔	↑	↑
Tethering	-	↑	-	-	-
Arena test	-	-	-	-	↑↓
Production					
Milk production	-	-	-	↑	-
Growth rate	-	-	-	↔	-

Heart rate was measured in all papers but Paper IV, and an elevated heart rate was found in fearful dogs (Paper I), tethered goats (Paper II) and a mixed response was shown in kids during the tests (Paper IV). However, heart rate was not altered in goats according to suckling or acute separation from kids (Paper III). Blood pressure was measured in goats in Paper II and III, and was elevated as a response to tethering (Paper II), but not altered due to suckling or separation (Paper III). Haematocrit response was measured solely in the dogs (Paper I), and increased as a response to gun fire in dogs fearful of gunshots. Blood plasma concentrations of cortisol, β-endorphin and vasopressin were measured in Paper I-III. In addition, oxytocin was measured in goats in Paper II and III. Progesterone and testosterone were measured solely in dogs (Paper I). In the kids in Paper V, saliva cortisol concentration was measured, but there were no differences between treatments. However, plasma cortisol and β-endorphin concentrations were elevated in dogs fearful of gunshots (Paper I) and during suckling in goats (Paper III), while tethered goats showed lower concentrations of β-endorphin than loose housed (Paper II). This was also true for oxytocin (Paper II).

Vocalisation was measured as a response to separation in goats (Paper III) and social isolation in kids (Paper V). It was shown to be the only reaction in goats that were acutely separated from their offspring's, and could also help in distinguish the reaction to isolation in kids reared in different systems. Passive behavior was measured in Paper III-V and active behavior in Paper IV-V. The goat mothers were laying down more after separation (Paper III), but there were no differences between treatments in total time spent resting or being active in home-pen. However, differences were found in time spent resting or being active close to social companion or alone (Paper IV). In Paper V, the behavioural tests revealed differences between treatments. The daily separated kids were more active than the separated kids before novel object. Exploring was measured in kids during the arena test (Paper V). The separated kids and the non-separated kids spent more time exploring than the daily separated after novel object although the difference was not significant between daily separated and non-separated. The startle response to gun-shot in dogs and novel object in kids was measured as escape behavior as an indicator of fear. This was used as a measure to classify the dogs as fearful respectively less fearful of gun-shots.

Potentially stressful events or tests were used in all Papers but Paper IV, and was effective to detect treatment differences. The production measures milk yield and milk composition were measured in goats, and growth rate in kids in paper IV. Growth rate did not differ between treatments, but milk composition was positively affected by suckling in both daily separated and goats kept full time with their kids.

Figure 17. Goat and kid in NON-SEP treatment resting together

6. General discussion

6.1 Discussion of main results

Measuring several physiological and behavioural parameters, preferable simultaneously, has in this thesis proved positive, when it comes to assessing fear/discomfort and animal welfare. Even though the work with this thesis began twenty years ago, the subject of measuring and assessing animal welfare has become even more in focus to meet society and consumer demand, and to secure a sustainable food production system. The management systems that form the basis of the studies in goats in this thesis, early mother-young separation and tied-up housing systems, were of high interest when this work started. At present, these management practices are still common in dairy cattle in many parts of the world, although it is forbidden according to Swedish legislation to build new facilities for tied-up systems. In fact, on behalf of the Swedish government, the Board of Agriculture recently presented a report on the consequences of a prohibition of tied-up animals in Sweden (Jordbruksverket, 2019). The interest in animal welfare issues of the second species of this thesis, the domestic dog, has increased during recent years (Chmelíková *et al*., 2020). Hence, the results of our dog study, that was published already in 2004, are topical issues today.

The results presented emphasise the importance of using several behavioural and physiological measurements to understand animal reactions and evaluate animal welfare. In addition, different behavioural reactions to the same stimuli may result in similar physiological response. However, the more we learn about interactions between physiology and behaviour the closer we get to the animal's emotions. Hence, it is important that evaluations of stress situations in animals do not exclusively rely on measurements of one stress hormone alone. This became obvious in this thesis where isolation, arena test and tethering did not cause a cortisol response, although glucorticoid concentrations are often used as a measure of stress (Ralph & Tilbrook, 2016).

Heart rate and activity/passivity showed strong significance in the present thesis, which indicates that measures of heart rate, in combination with the individual's physical activity, can make it possible to interpret if an elevated heart rate is caused by physical activity, mental arousal or a combination of both. However, to interpret whether the individual experiences positive or negative feelings, it is necessary to consider the situation in which the measures are performed. Hence, the commonly used stress markers heart rate and cortisol measurements should always be combined with measurements of physical activity and behavioural studies of fight / flight, positive expectation and exploration. It is also important to consider if the individual is in a social situation.

6.1.1 Fearfulness in dogs

The strongest fear response in this thesis was seen in dogs fearful of gunshots, where almost all physiological parameters measured were elevated and the dogs also showed strong behavioural reactions that nevertheless varied between individuals. The gunshot test caused a massive physiological stress response, a fear reaction, while the floor test just increased heart rate in fearful dogs. The increased heart rate may be partly due to the novel situation and the locomotion performed. In dogs fearful of gunshots, the behavioural expression of fearfulness showed a large variation between individuals. Some dogs responded to this challenge by trying to escape, while others stayed passive at the same spot, trembling or shaking. Despite the different responses, all measured physiological variables except testosterone increased in fearful dogs compared to dogs that were less fearful, indicating a massive activation of both sympathetic nervous system and hypothalamus-pituitary-adrenal axis. When analysing the behavioural recordings, it turned out that the measures, hesitance and tendency to move towards the wall in the floor test and initial reaction and degree of fear for the gunshot test, were the measures that best correlated to the mental states of fear and fearlessness.

In this thesis, observer ratings were used as a tool to score the degree of fear of gunshots. The use of observer ratings has become more common in recent years and is considered a useful scientific tool (Meagher, 2009). This was combined with the initial reaction which, in our opinion, correlated well to the response to gunshots.

The difference in cortisol concentration between dogs fearful of gunshots and less fearful dogs was striking—the plasma peak level was four times as high in fearful dogs as in less fearful dogs. It has been suggested that a doubling of plasma cortisol concentrations indicate stress or poor welfare (Houpt, 2004) . The elevation of progesterone concentration simultaneously to cortisol in the dog study, indicate an ACTH stimulated release of progesterone from the adrenal cortex. Progesterone release in response to stress is in accordance with earlier studies in for example steers (Cooper *et al.*, 2011), rodents (Deis *et al.*, 1989; Romeo *et al*., 2004), and humans (Breier & Buchanan, 1992; Herrera *et al*., 2016).

Both testosterone and vasopressin concentrations were elevated in both less fearful and fearful dogs already before the gunshots. Both these hormones are reported to be related to physical activity (Campbell *et al*., 1982; Wade, 1984; Tharwat *et al.*, 2014) and the increased levels in all dogs may be explained by the physical activity during the walk to the fenced meadow where the gunshot test took place. However, there were no differences between fearful and less fearful dogs in testosterone concentration. Stimulation with ACTH has been reported to increase testosterone concentrations in female dogs but not in males (Frank *et al*., 2003), which may explain the lack of differences in testosterone between fearful and less fearful male dogs. Vasopressin on the other hand, was elevated after gunshot in fearful dogs, which is in accordance with the involvement of vasopressin in acute stress responses in cooperation with corticotropin releasing factor (Beurel & Nemeroff, 2014).

β-endorphin is involved in inhibiting pain during acute stress. Hence, the elevated β-endorphin levels after gunshots in fearful dogs may have been caused by pain in the dogs' ears. However, it may also simply be an effect of the fearful dogs' experience of the situation as extremely stressful. β-endorphin is suggested to be involved in regulating long-term stress response in dogs by working together with the immune system, trying to prevent the adaptive response from becoming maladaptive (Righi *et al*., 2019), but is also released during acute stress.

According to the results of this thesis, fear of gunshots constitutes a severe stress for the individual. However, anyone exposed to an unexpected loud noise can react with transient fear, and when exposed several times during a short time range, the fear can either fade or become more intense. For this reason, we chose to fire two gunshots to be able to identify dogs that were truly fearful of gunshots and not just surprised by a sudden loud noise. Fear of loud noises is recognised as a problem in dogs (Engel *et al*., 2019; Riemer, 2019; Gähwiler *et al*., 2020; Hakanen *et al*., 2020), where fear of gunshots is the third most common fear reported (Sherman & Mills, 2008). In addition, (Blackwell, *et al*., 2013) found that fear of fireworks and thunder frequently co-occurred with fear of gun shots, which suggest that fear of gunshots is a welfare problem in pet dogs. Fear of other loud noises as fire works and thunder are more prevalent within certain breeds than others (Hakanen *et al.*, 2020). Most likely, dogs fearful of gunshots are also more prevalent within some breeds.

In dogs fearful of floors, the single elevated parameter was heart rate. Increased heart rate caused by emotional stimuli or stress is known to decrease rapidly in dogs (Caraffa-Braga *et al.*, 1973), but the heart rate remained at a high level during the whole floor test, demonstrating an activation of the sympathetic nervous system for a long time period. However, the elevated heart rate was probably an effect of mental arousal rather than physical activity, since there was a difference between fearful and less fearful dogs and all dogs were physically active to the same extent. Hakanen *et al*., (2020) studied factors related to fear of surfaces and heights, and found breed as one factor. The Rough Collie, used in this thesis was among the breeds with the highest prevalence. Therefore, both fear of floors and fear of gun shots should be considered in dog breeding.

6.1.2 Stress and discomfort in goats

Goats are active, curious and gregarious animals, and develop an exclusive mother-young bond rapidly after parturition (Romeyer *et al*., 1994; Poindron *et al*., 2007a, 2007b). Therefore, we hypothesized that both tethering alone and early separation from kids would induce a stress response in the goats and that rearing with or without contact to mother affect kids' response to a challenge. However, the only obvious behavioural reaction of the goats to separation from their kids was the intensive vocalisation for a short time period, and this was not accompanied by physiological changes. On the other hand, tethering resulted in both cardiovascular and hormonal changes. In a recent article by (Katzenberger *et al.*, 2020), it is pointed out that tie-stalls have an adverse effect on animal welfare in cattle. In goats, the behavioural restrictions in tied-up systems, that limit locomotion and social contact (Miranda-de la Lama & Mattiello, 2010), probably means impaired welfare. This is supported by the findings in Paper II, where cardiovascular as well as hormonal effects of tethering were found when compared to loose-housed goats kept in pairs. The loose-housed goats could move around and perform social behaviours, while tethering implies behavioural restrictions. Hence, the cardiovascular response, with an elevated heart rate and blood pressure in tethered goats, was rather an effect of mental stress than physical activity.

In paper III-V, we aimed at an overall picture of changes in physiology, behaviour and production of early separation and different rearing systems, and the consequent impact on welfare in goats and kids. The acute phase of the separation of goat and kid in paper III, did not alert the sympathetic nervous system or activate the hypothalamus-pituitary-adrenal axis in the goats, as indicated by the absence of a physiological response. However, the separation caused an intensive, but short lived, vocalisation that was expressed in all goats. In cows, the vocalisation rate is higher if separation from calves is performed a few days after parturition compared to immediate separation (Lidfors, 1996; Weary & Chua, 2000; Stěhulová *et al*., 2008). In the studies in Papers III-V, the goats and kids were together during the colostrum period for four days before separation, which might have strengthened the maternal bonding. In addition, the goats and kids could hear each other after separation in Paper III - IV, which also may have influenced the vocalisation. Cows that can see and hear their calves after separation vocalise more than cows with no visual or auditory contact (Stěhulová *et al*., 2008). Increased vocalization rate is an indicator of arousal (Briefer, 2012), but it is suggested that goats can distinguish between contact calls of positive or negative valence, i.e. contact calls express the callers emotional state which the receiver goats can interpret (Baciadonna *et al*., 2019). In addition, it´s been shown that kids can recognize and locate their mothers based on vocalisation cues (Ruiz-Miranda *et al*., 1993). Hence, it’s possible that the vocal contact between the goats and their kids after separation had an influence on the lack of sympathetic response to separation in the goats.

Although the study in Paper III did not show any physiological responses in the goat mothers to early separation, the rearing of kids with or without their mothers seems to have an impact on the kids reactions to aversive situations (Paper V). Isolation is considered as a stressful situation for goats

(Price & Thos, 1980; Kannan *et al*., 2002), and isolation and the arena test are considered reliable tests of fear and discomfort in goats (Forkman *et al*., 2007). Social isolation is a potent stressor for social species and the separation anxiety probably cause more suffering than a fear-eliciting event when social animals are tested individually (Forkman *et al.*, 2007) Hence, reactions to an arena test are probably elicited rather by the component of isolation than the new environment (Price & Thos, 1980).

Several of the results show that the permanently separated kids were more affected by system at two weeks of age. They did not show hiding behavior in home-pen, as measured by resting alone. During the hiding period, it is the mother who initiates contact between mother and kid by coming to the area of the kid's hiding place and calling for her kid. After the hiding period, it is the goat kid that seeks contact when it is time to suckle (Lickliter, 1984). The lack of mother initiative to suckling and the need to come out of the hiding place to suckle from an artificial teat may have affected the time spent hiding in separated kids. Another effect of the mother's presence, is that the more time kids had access to mother, the more time they spent together. This indicates that although kids start to form social groups with other kids after the hiding period (Lickliter, 1987; O'Brien,1988), and may spend considerable time with other kids, the presence of the mother is important. As hiders, kids spend more time together with mother as it matures (Lickliter, 1984b).

In addition, the permanently separated kids did not vocalise to the same extent as the other treatments during isolation test, but had a higher heart rate. At two months of age these kids vocalised as the other treatments, but had a higher heart rate. A possible explanation may be that they at that age had established social contact and security in the kids' group in which they were reared, and bleated as an attempt to reinstate contact. Due to technical reasons, we could not measure other behaviours than vocalization, and therefore we do not know if for instance higher physical activity was the cause of elevated heart rate in the separated kids. This stress the importance of measuring several physiological and behavioural variables.

At two months of age, the daily separated kids seemed to be most affected, although they were habituated to the daily separation procedure. Both goats and kids gave a "restless" impresson and bleated, especially when the door between the dams' and kids' rooms was opened up (personal observations). In addition, the daily separated kids showed the strongest fear reaction in the arena test. They performed most escape behaviour before ‘the fear eliciting stimuli’, and showed a clear drop in heart rate and more passivity after. The combination of immobile behaviour and a decrease in heart rate is indicative of a parasympathetic activation and a passive avoidance behaviour (Roelofs, 2017). The daily separation may have functioned as a repeated stressor for the kids, although it´s tempting to believe that daily separation is of advantage for the kids well-being compared to permanent separation. Hence, daily separation in dairy production need further studies.

Kids kept full time with their mother reacted to in between the other treatments. However, in overall, they always reacted less compared to the most deviant treatment group.

6.1.3 Responses to aversive practices or experiences

Surprisingly, although we studied fear- and stressful situations in Papers I-III and V, significant elevations in cortisol were only found in dogs fearful of gunshots and in goats suckling their offsprings. Neither tethering or acute separation, nor isolation caused a cortisol response. Hence, cortisol should be supplemented with other stress markers when assessing stress. Vasopressin was measured in both dogs (Paper I) and tethered as well as suckled and separated goats (Paper II-III), but was solely released in dogs fearful of gunshots.

Progesterone and testosterone were measured in dogs in Paper I, and progesterone was elevated in dogs fearful of gunshots. The simultaneous release of progesterone and cortisol indicate an ACTH stimulated progesterone release, thus indicative of a stress-related response.

The causation of β-endorphin release is complicated to interpret. β-endorphin is reported to increase as a response to a diverse array of emotions, i.e. pleasure and wellbeing in humans (Barchas & Berger 1980, Hawkes 1992) and stress and pain (Amir *et al*., 1980; Greenwood & Shutt, 1990; Hydbring Sandberg *et al.*, 1999; Sprouse-Blum *et al*., 2010). Oxytocin on the other hand, is involved in social bonding (Uvnäs-Moberg, 1998; Kendrick, 2004; Lim & Young, 2006). In addition, both β-endorphin and oxytocin have been reported to increase during physical activity (Kasting 1988; Sforzo, 1989; Malinowski *et al*., 2006). Hence, the higher β-endorphin and oxytocin concentrations in loose goats compared to tethered may be explained by better possibilities to move around and perform social behaviours and thereby increased well-being.

Cortisol and β-endorphin were released concomitantly as a response to two completely different events, gunfire in fearful dogs and suckling in goats. This was probably an effect of pain or stress in the dogs respectively a metabolic effect in the goats.

Hence, the array of blood plasma hormones analysed in dogs in Paper I and adult goats in Paper II and III is a strength in evaluating the impact of events used in this thesis. The diverse endocrine responses in this thesis strengthens our hypothesis on the importance not to limit the physiological measures to one single hormone, e.g. cortisol, when evaluating potentially stressful situations.

The cardiovascular parameters heart rate and blood pressure are commonly used to evaluate stress reactions. An obvious advantage is the possibility to register ‘at distance’ without handling, and thereby affecting the animals. Heart rate and blood pressure respond quickly to changes in environment and/or frame of mind, and change in response to both positive and negative events. However, when using cardiovascular variables to evaluate stress, it is important to consider that cardiovascular variables fluctuate as a response to everyday events in goats, and also what could be considered as positive events, e.g., feeding and milking (Hydbring *et al*., 1997; Hydbring *et al*., 1999). In addition, age effects and physical activity must be taken into consideration. When kids were reunited with their mother or the kids group at two weeks, the overall mean heart rate was higher than during isolation. This indicate that reunion caused mental arousal to a larger extent than isolation. However, reunion was most probably experienced as positive, demonstrating that heart rate may increase as a response to positive events. At two months, the heart rate was lower during reunion than isolation, indicating that the stress response to isolation ceased when the kids were reunited to their mother respectively the kids group.

In dogs, hesitation to walk and walk close to the wall during floor test were used as indicators of immobility respectively avoidance/escape. The response to gun shots in fearful dogs was diverse, and ranged from freezing behaviour to extreme flight. Active behaviours in goats during an arena test have been measured as time spent running, number of squares entered, number of jumps (Roussel *et al*., 2005). Jumps are common in isolated goats (Price & Thos, 1980) and are indicative of trying to escape from the situation.

The novel object that dropped from the ceiling during arnea test caused a flight response in all kids, and was most certainly a highly fear eliciting event. Regarding passive behaviour, there is an obvious difference between resting behaviour and fear induced immobility or freezing behaviour. The goats in Paper III were standing less after separation. However, the increased lying behaviour after separation is rather indicative of resting than a freezing response, while some of the fearful dogs responded by immobility induced by fear. Exploring in kids was measured as sniffing on the floor, walls or novel object. The behaviour exploring, in the sense of gathering information, is an important feature of survival in animals (Carson, 1985) and has been used as a measure of lower level of fear in goats (Roussel *et al.*, 2005). Exploring was not measured in the dogs, but would have been of interest as a complement to differ between fearful and less fearful dogs.

Separation from kids and tethering alone elicited different stress responses, solely a behavioural response respectively a cardiovascular response. Fear of walking different types of floors caused an elevation of heart rate while fear of gun shots caused a massive physiological and behavioural response in dogs. The complexity of the responses to the different situations in this thesis strengthen the need for using a wide variety of physiological and behavioural measures when evaluating stress and fear in animal welfare research.

6.1.4 Production measures

In the past, before the five freedoms were formulated, it was generally believed that animals that had a high production rate also had a good welfare. Today, we know that this is not always the case. However, it is still important that livestock used for food production have a high production. Therefore, it was of interest to investigate milk yield and composition in the goats, and growth rate in kids that was either early permanently separated, daily separated or kept full time with the mother.

The daily milk yield in Swedish dairy herds has been estimated to be 2.8 L per day and goat (Brandt, 2009). The goats in this study had lower milk yield in comparison, but only with small differences (2.4 kg in daily separated and 2.1 kg/day in non-separated goats). However, the goats in this study produced more milk in comparison to other goat breeds kept together with their kids. For example, the daily production of marketable milk for Payoya dairy goats was 2.0 L during the suckling period and 2.1 L after weaning (Delgado-Pertíñez, Guzmán-Guerrero, Caravaca, *et al*. 2009) (Delgado-Pertinez *et al*., 2009 b) and Florida dairy MIX-goats yielded 1L marketable milk (Delgado-Pertinez *et al.*, 2009 a). (Delgado-Pertíñez, Guzmán-Guerrero, Mena, *et al*. 2009) Hence, the results in paper IV confirms that it is possible to maintain marketable milk yield during 70 days in Swedish dairy goats kept together with one kid. This is in contrast to (Fernández *et al.,* 2019) who found that marketable milk production was higher during six weeks after parturition in separated goats compared to goats kept with their kids for 24 hours. However, after weaning at six weeks, they found no differences in marketable milk production. In that study, they found no effect on milk fat content. Despite the drop in marketable milk production, they concluded that artificial rearing of kids increase rearing costs, which meant a final economic return in favour of rearing kids together with the mother goat. In that study, the live weight at 42 days was less in artificially reared kids despite ad libitum access. In contrast to our study, they were fed milk replacer and received a lesser amount than suckling kids. In our study, the growth rate of the kids was similar regardless of separation strategy (paper IV), most certainly a result of free access to goat milk in separated kids. However, growth varied more in daily separated kids compared to the other treatments. After the isolation test at two weeks, the daily separated kids also spent more time suckling when reunited with mother. This indicate that even if this group could compensate milk intake when together with their mothers, they were probably suffering from hunger early in lactation indicating lower welfare.

Can our results in goats be transferred to apply to dairy cows and their calves? Differences in anatomy and physiology in the mammary gland speaks against it. Goats have large cisterns and store between 50- 80% in the gland cistern compared to dairy cows that store about 20% in the cistern. (Marnet & McKusick, 2001; Salama *et al*., 2004; Bruckmaier, 2005). The cisternal milk can easily be emptied without milk ejection and milk stored in the alveolar compartment can only be expressed into the cistern after a contraction of the myoepithelial cells (Andersson, 1951; Soloff *et al*., 1989; Marnet & McKusick, 2001; Bruckmaier, 2005). Animals with large cisterns are able to store the milk more effectively between milkings (Marnet & McKusick, 2001) and therefore goats, in contrast to dairy cows, are less dependent on milk ejection (Peris *et al*., 1997). In small ruminants, this is evident, since the milk flow has no correlation with oxytocin in plasma during milking (Marnet & McKusick, 2001). Studies in goats (Olsson & Högberg, 2009) and ewes (Negrão & Marnet 2003) confirm that oxytocin levels in plasma only increase during suckling and not during milking. In dairy cows oxytocin is released both during suckling, hand- and machine milking (Gorewit *et al.* 1992; Negrão, 2008) and therefore, suckling has less positively effects on milk composition. The positive effects on growth rate has been shown in calves (Meagher *et al.*, 2019), however, to investigate if keeping cows and calves together have similar effects on calf behavior as in kids need further studies.

6.1.5 Effects of suckling in goats

In paper III, the goats stood more when they were together with their kids than after separation, probably an effect of suckling. The number of suckling bouts was on average two times per hour, which is slightly higher than the frequency found by (Högberg *et al*., 2016). However, this difference is probably an age effect, regarding the kids in the study by Högberg *et al*., (2016) were 8-11 weeks old. Suckling, was accompanied by elevated concentrations of cortisol and β-endorphin, and a small but not significant increase in blood pressure in undisturbed goats. Elevated cortisol concentrations have been reported during suckling in cows (Dunlap *et al*., 1981; Ellicott *et al*., 1981), sheep (Marnet & Negrão, 2000) and goats (Olsson & Högberg, 2009) and is probably rather a metabolic effect, although though some arousal may have occurred. In contrast to the study by Olsson & Högberg (2009), no oxytocin or vasopressin release was found in our study. A possible explanation is that we may have missed the peaks due to the short suckling bouts. In addition, the goats in this study were in early lactation, while the goats in the study by Olsson & Högberg (2009) had been lactating for several weeks. Milk composition in Paper IV was positively affected by suckling. Most likely this is an endocrine effect (oxytocin, prolactin) that affects the entire udder, since milk composition was similar in the suckled teat and the teat that was only machine milked.

6.1.6 Individual differences

The behavioural physiology approach used in this thesis emphasises that individual variation is of great significance when evaluating potentially aversive situations. This was true for both the privately owned dogs and the goats in our experimental herd. Individual variation is necessary in the natural habitat for evolutionary fitness (Koolhaas *et al.*, 2010) and is important to take into consideration in most research fields.

There were individual differences in the majority of measures. However, heart rate increased in all fear- and stressful situations in both species (I, II, V) except for acute separation in goats despite intensive vocalisations (III). At two weeks, the vocalisation rate in all kids during isolation (Paper V), followed a similar overall pattern. However, the heart rate varied between individuals, and stayed at a high level throughout the test for half of the kids, i.e. the heart rate and vocalisation rate were positively correlated, while in the other half, the heart rate and vocalisation rate were negatively correlated, i.e. heart rate decreased when vocalisation increased. The somatic and autonomic nervous system change in response to emotions, which cause tension of muscles that are used for voice production (Briefer, 2012). In humans, the impact of the autonomic nervous system on vocalisation differs between emotions and depend on which of the sympathetic and parasympathetic systems that dominate (Zei Pollermann, 2008). Hence, vocalisation is a parameter that has potential to describe the individual's experience. Possibly, registrations of valence of the kids vocalization during isolation would have been helpful to understand the different patterns of heart rate responses in the kids.

6.1.7 Gender differences

Gender may partly have affected our results. Chojnacki *et al*., (2014) found that female offspring to goat mothers exposed to prenatal high density housing, showed a more pronounced fear response to isolation than males. In our study, the female kids showed a longer latency to sniff object than males. A long latency to approach a novel object can be indicative of a non-curious or indifferent animal rather than a fearful reaction (Forkman *et al*, 2007). However, in this case, the sudden appearance of the novel object probably elicited a surprise effect and therefore a fear reaction in all kids. This is supported by the initial flight reaction in all kids. They may all have been initially scared to the same extent, but the most curious individuals overcame their fear and approached the novel object in a shorter time than the less curious individuals. In that case, the males may have been curious to a larger extent than the females.

The distribution of gender between treatments was skewed in our study as an adaptation to farming practices. Goats usually deliver twins, and few goats have enough production to suckle two kids and still have milk for human consumption. Hence, in the case of twins with different gender, the female kids was kept with their mothers as an adaptation to dairy goat farming where the female kids are most likely kept with their mothers for later recruitment to the herd. However, if very high yielding goats are used, it may be possible for the goats to suckle two kids and still produce enough milk for human consumption. In the present study, even when the dams were kept together with one kid for 24 h, some individuals yielded 3-4 kg milk daily.

6.1.8 Welfare implications

The occurrence of fearful dogs is of concern for modern society from both dogs' and humans' point of view. As already discussed, fear of gunshots puts a severe strain on fearful dogs and therefore affects their welfare. It is also likely, that fearfulness of one loud noise generalize to other loud noises (Blackwell *et al*., 2013), and an online survey showed that more than fifty percent of the dog owners considered their dogs as fearful of fireworks (Riemer, 2019).The handling of fearful dogs places great demands on dog owners, since fearful dogs may develop aggressive behavior (King *et al*., 2003; Rooney *et al*., 2016). Fearfulness in dogs is one reason for dogs being handed over to dog shelters (Segurson *et al.*, 2005). Since fearfulness has been reported to be a hereditary trait in dogs (Overall *et al.*, 2008; Riemer, 2019), this should be taken into consideration in breeding.

For animals in dairy production, tied-up systems and early separation of mother and young are controversial subjects. The physiological response to tethering in the goats in this thesis clearly shows that this system, which implies restricted possibilities to perform behaviours and have social contact, had a negative impact.

In this thesis we have shown that even when the goats show minor reactions to early separation, there are effects on the kids. Kids housed with access to their mothers spent considerable time with them. Hence, it seems like kids that have the possibility to choose, they choose to be with their mothers. This most likely affects the welfare of both kids and goats. For future research and development, it is desirable to focus on housing and management systems, with special consideration of the positive effects of keeping mother and offspring together, a topical issue for dairy cattle

(Ventura *et al*., 2013; Johnsen *et al*., 2016; Agenäs, 2017; Busch *et al*., 2017; (Mikuš *et al*., 2020; Placzek *et al*., 2020).

Animal welfare is to a large extent a controversial issue, despite a consenus regarding the objectives of animal welfare: all individual animals should fare well in life. This is how Broom defined welfare in 1996. It is tempting to believe that animals living a natural life have the best welfare. But a natural life contains many things that we, as humans, do not connect to welfare, like starvation, predators, illness, and injuries. Is the elimination of those threats, in human care, worth the price? That is a hypothetical question with no answer. The results of the studies in this thesis, show how important it is to use several different measures of behaviour and physiology to be able to interpret the animals' reactions and evaluate animal welfare. The results also emphasizes the importance of considering the individual differences of the animals, both in how animals experience different situations but also in how they cope with different situations physiologically and behaviourally.

Photo: Lena Holm

7. Conclusions

The results of this thesis emphasise the importance of using a wide variety of behavioural and physiological parameters when trying to interpret animal’s reactions and assessing animal welfare. It is important that evaluations of stress situations in animals don´t rely on measurements of one stress hormone alone. Furthermore, it is not always behavioural reactions of fear or discomfort that are accompanied by physiological changes and vice versa. In addition, both behavioural and physiological reactions to the same stimuli may differ considerably between individuals.

The following specific conclusions can be drawn from the work presented:

- Dogs fearful of walking on different floors had higher heart rate than less fearful dogs during the floor test. Fear of gun shots is a serious stressor for dogs. Dogs fearful of gunshots showed a massive physiological response, e.g. elevated high heart rate, haematocrit and plasma concentrations of cortisol, progesterone, vasopressin and β-endorphin, during the gunshot test despite different behavioural reactions.

- Dogs fearful of gunshots showed a strong physiological response that the less fearful dogs did not show. It was therefore possible to separate fearful dogs from less fearful by physiological variables.

- Tethered goats had higher heart rate and blood pressure, but lower concentrations of β-endorphin and oxytocin than loose goats kept in pairs, indicating that tethering may be experienced as negative in goats compared to when held loose in pairs.

- In contrast to the cardiovascular response, the commonly used stress-indicators cortisol and vasopressin concentrations were not affected by tethering. This indicate that the use of single measures, e.g., cortisol concentrations, is not sufficient to give an accurate picture of an animal's response to management systems.

- Neither suckling, nor permanent separation of goats and kids with established bonding was accompanied by cardiovascular changes.

- Suckling caused elevated plasma cortisol and β-endorphin concentrations but did not affect oxytocin or vasopressin in the goats, while permanent separation was not accompanied by endocrine changes despite intensive vocalisations, earlier suggested to indicate stress.

- Kids' growth rates were similar regardless of rearing with full-time, part-time or no contact with mother.

- It is possible to maintain milk yield with one kid present and that the milk composition is positively affected by keeping goats and kids together.

- Separated kids showed an altered behaviour, *e.g.* less hiding behaviour, than the other treatments and kids that had access to mother chose to spend considerable time with them.

- Kids permanently separated from their mothers seemed to have developed a bond to their flock mates at two months of age, and thereby adapted to live in a group with other kids, while those separated daily demonstrated the strongest stress response at this age.

- Kids separated early, separated daily, or kept full-time with their mothers showed different responses to a challenge. This demonstrates the importance of if and how the mother is present, as well as the importance of using a several physiological and behavioural measures when evaluating stress in animal welfare research.

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Popular science summary

Animal welfare is a topical issue in today's society, engaging pet owners, consumers and citizens as well as food producers. Animal welfare is at present even more relevant than when the first studies in this thesis were done 20 years ago. During this time period, a vast number of research projects have been published on different animal species, which has contributed to an increased understanding of what affects animal welfare, which has made possible improvements in the care and housing of livestock. Despite this, many important questions remain. What animals really experience and feel can be difficult to know. However, by studying a wide array of different physiological and behavioural changes in the same individual, it is may be possible to evaluate the animal's experiences of different situations. Different individuals may experience the same situation in different ways, which may lead to diverse physiological and behavioural reactions. The type of stress response shown in different individuals and situations may differ, as may the relation between behaviour and physiology. Increased knowledge about how physiology and behaviour interact is therefore important to better interpret the animals' experiences and thereby improve their welfare.

Both the involuntary nervous system, i.e. the autonomic nervous system, and the hormonal system are controlled by the hypothalamus. The hypothalamus is an area in the brain that is superior to both the autonomic nervous system and the pituitary gland, which is a hormone-producing gland, releasing several different hormones that in turn affect other hormone-producing organs. The pituitary gland affects, among other things, the adrenal cortex to release the hormone cortisol (hypothalamic-pituitary-adrenal cortex axis). In addition, the sex hormones testosterone and progesterone can be released via the adrenal cortex. The hypothalamus also produces the hormones oxytocin and vasopressin, which are transported to the posterior lobe of the pituitary gland from which they are released.

Oxytocin is a hormone that is released in connection with parturition and milk let-down, but also in connection with physical contact and is sometimes called the feel-good hormone. Vasopressin is a water-saving hormone, that at higher concentrations also act to elevate blood pressure. The pituitary gland can also release the hormone β-endorphin, which acts as the body's own morphine and is important in pain relief, but which also can be released during stress. The autonomic nervous system is divided into the sympathetic and the parasympathetic nervous system. The sympathetic nervous system (fight, flight and fright system), generally increases the body's performance by, among other things, raising blood pressure and heart rate and stimulating the release of the hormones adrenaline and noradrenaline from the adrenal medulla. In some species, the sympathetic nervous system also stimulates the release of red blood cells from the spleen, i.e. increases the haematocrit. The parasympathetic nervous system, also called the rest and digest system, is most active at rest, decrease heart rate and stimulates digestion.

Fear is a natural and necessary part of life, intended to protect the individual from harmful situations by triggering certain behaviours such as avoidance or defensive behaviours, and activate physiological stress reactions such as the sympathetic nervous system and the hypothalamic-pituitary-adrenal cortex. However, recurring, strong fears may put both physical and mental strain on the animal and adversely affect its welfare. Fear can be expressed in different ways, and there are both active and passive strategies for dealing with fear. Through escape behaviour, the animals can flee from a threat. However, animals that are afraid can also react by "freezing" behaviour, i.e. being completely immobile. The behavioural reactions are adapted to different situations, making it possible for the animal to cope with the situation. The individual may also change strategy if it turns out that the first strategy is not appropriate. The overall goal of this dissertation was to study the physiology and behaviour of fear and discomfort in dogs and goats and to investigate the physiological and behavioural changes in the situations included in the thesis.

In the first study, fearfulness of floors and gun shots in dogs of the Collie breed was studied. In some dog breeds, it is common for individuals to be afraid of, or even refuse to walk on, different types of floors. This can imply major problems not only for the dogs, but also for the dog owners. Fear of gun shots is another type of fear, that causes a lot of suffering for the dogs and also is very problematic for the dog's owner. Fear of gun shots is also difficult to train as this fear is often very strong, and even tends to become worse over time. The results from the first study showed that dogs fearful of floors subjected to a floor-test, and dogs fearful of gun shots that were subjected to a gun shot test, had an elevated heart rate compared to dogs that were less fearful. In addition, the dogs fearful of gun shots had higher levels of red blood cells (haematocrit) in the blood, as well as higher levels of the hormones cortisol, progesterone, vasopressin and β-endorphin after the gun shot test compared to dogs that were not fearful of gun shots. There was no difference in the hormone levels in the fearful/non-fearful dogs in the floor-test however. The dogs fearful of gun shots, reacted differently to the gun shot test in terms of behaviour. Some dogs fled, while others were paralyzed and stood completely still, but in all fearful dogs the physiological variables increased to a large extent. The study showed that it is possible to detect fear with physiological variables, while it can be difficult to assess fearfulness from solely behavioural observations.

In dairy production, certain management routines and housing systems are questioned, such as early separation of mother and young as well as tethering of animals. Goat and goat kids establish strong bonds early after parturition. Goats are considered as hiders, e.g. the first hours after birth, the goat leaves the kid lying hidden. However, the length of the hiding period varies from days to weeks between different goat populations. In intensive dairy goat production, goats and kids are often separated early. In more extensive systems, it is more common that goat and kids are separated during daytime, while the goats graze, and are kept together during the nights. However, little is known about how these different routines affects the development of various characteristics, such as fearfulness. Strong fears can negatively affect the production in milk-producing animals. When goats and kids are separated, the possibility to reunite increases if they actively search for each other and bleat to find each other.

In the second study, we examined how goats are affected by being tied up compared to loose housed in a pen together with another goat. In tethered goats, blood pressure and heart rate increased, while the levels of the hormones β-endorphin and oxytocin were lower compared to loose housed goats. This indicates that the tied-up goats up experienced the situation as stressful. However, the hormones cortisol and vasopressin were not affected by the different housing systems, once again emphasizing the importance of measuring several physiological variables when evaluating animal welfare.

In the third study, we studied the effects of goat kids suckling their mother and how goats react to early separation from their kids. When the kids suckled, the hormones cortisol and ß-endorphin increased, but the heart rate was not affected. No physiological changes were seen in the goats when they were separated from their kids, but both goats and kids vocalised intensively. The goats did not seem to be adversely affected by the separation, even though they had spent enough time with their kids to have developed a strong bond. This could be explained by the natural hiding behaviour in goats, which implies that both goats and kids are adapted to be separated in periods.

In the fourth and fifth studies, the goat kids' reactions to separation from mother were examined further. The kids were kept in three groups; together with the mother, together with the mother at night but without the mother during the day, respectively separated from the mother but together with other kids. For two months, the kids' growth was measured, as well as milk yield in the goats and whether the composition of the milk differed between the groups. We also examined whether there were differences in the kids' behaviour depended on whether they were kept with or without their mother. To investigate whether the systems affected the kids' reactions in stressful situations, we performed tests at two weeks and two months of age. In these tests, the kids were isolated from their mothers respectively the other kids, and in an unknown environment for a short period. Kids’ growth was similar regardless of system, but the kids' behaviour was affected. The kids kept full-time with mother stayed hidden more than the others, while the kids that were completely separated from the mother and kept in a group with only other kids, spent more time with other kids when they were active. The reactions to the tests were very individual. However, at two weeks of age, the kids that were separated from the mother vocalised less than the other kids, but had a higher heart rate at both two weeks and two months of age. The kids that were separated daily from their mothers, vocalised more and showed the strongest behavioural reactions when they were two months old.

The results of the studies in this dissertation, show how important it is to use several different measures of behaviour and physiology to be able to interpret the animals' reactions and evaluate animal welfare. The behaviour of the animals and the physiological changes don’t always follow each other as expected. It emphasizes the importance of considering the individual differences of the animals, both in how animals experience different situations but also in how they cope with different situations physiologically and behaviourally.

Photo: Lena Holm

Populärvetenskaplig sammanfattning

Djurs välfärd engagerar många människor i dagens samhälle, exempelvis djurägare, konsumenter och livsmedelsproducenter. Djurvälfärd är idag om möjligt ännu mer aktuellt än när de första studierna i denna avhandling gjordes för 20 år sedan. Under denna tidsperiod har mycket forskning utförts på olika djurslag vilket har bidragit till ökad förståelse för djurs upplevelser och ökad generell kunskap vilket bland annat resulterat i förbättringar i skötsel och inhysning av livsmedelsproducerande djur. Trots detta återstår många viktiga frågor. Vad djur verkligen upplever och känner kan vara svårt att veta. Genom att studera flera olika fysiologiska och beteendemässiga förändringar hos samma individ går det ofta att utvärdera djurets upplevelser av olika situationer. Olika individer kan uppleva samma situation på olika sätt vilket kan leda till olika fysiologiska och beteendemässiga reaktioner. Det är inte alltid som dessa följer varandra och därför krävs det ökad kunskap om hur fysiologi och beteende samverkar för att bättre kunna tolka djurens upplevelser och därmed kunna förbättra deras välfärd.

Både det icke viljestyrda nervsystemet, d.v.s det autonoma nervsystemet, och det hormonella systemet styrs av hypotalamus. Hypotalamus är ett område i hjärnan som är överordnat både det autonoma nervsystemet och hypofysen som är en hormonproducerande körtel som frisätter flera olika hormoner som i sin tur påverkar andra hormonproducerande organ. Hypofysen påverkar bland annat binjurebarken att frisätta hormonet kortisol (hypotalamus-hypofys-binjurebarksaxeln). Även könshormonerna testosteron och progesteron kan frisättas via binjurebarken. Hypotalamus bildar också hormonerna oxytocin och vasopressin som transporteras ner till hypofysens baklob där de frisätts. Oxytocin är ett hormon som frisätts i samband med förlossning och mjölknedsläpp, men också vid beröring och kallas ibland för må-bra hormonet. Vasopressin är ett vattensparande hormon som vid högre koncentrationer även verkar blodtryckshöjande. Hypofysen kan även frisätta hormonet β-endorfin som fungerar som kroppens eget morfin och är viktigt vid smärtlindring men som även kan frisättas vid stress. Det autonoma nervsystemet delas in i det sympatiska och det parasympatiska nervsystemet. Det sympatiska nervsystemet (fight flight fright systemet), ökar generellt kroppens prestationsförmåga genom att bland annat höja blodtryck och hjärtfrekvens och stimulera frisättningen av hormonerna adrenalin och noradrenalin från binjuremärgen. Hos vissa djurslag stimulerar dessutom det sympatiska nervsystemet frisättning av röda blodkroppar från mjälten. Det parasympatiska nervsystemet, även kallat lugn och ro-systemet, är mest aktivt vid vila, sänker hjärtfrekvensen och stimulerar matspjälkningen.

Rädsla är en naturlig och nödvändig del av livet, avsedd att skydda individen mot skadliga situationer genom att utlösa vissa beteenden som t.ex. undvikande eller försvarsbeteenden samt aktivera fysiologiska stressreaktioner såsom det sympatiska nervsystemet och hypotalamus-hypofys-binjurebarksaxeln. Dock kan återkommande, stark rädsla innebära både fysisk och psykisk påfrestning för djuret och påverka dess välfärd negativt. Rädsla kan uttryckas på många olika sätt, och det finns både aktiva och passiva strategier för att hantera rädsla. Genom flyktbeteende kan djuren komma undan ett hot, men djur som är rädda kan också reagera med att ”frysa”, alltså vara helt orörliga. Olika beteenden är anpassade för att djuren ska kunna hantera situationen och individen kan också byta strategi om det visar sig att den första strategin inte är lämplig. Det övergripande målet med den här avhandlingen var att studera fysiologi och beteende vid rädsla och obehag hos hund och get samt studera vilka olika fysiologiska och beteendemässiga förändringar detta ger i de olika studerade situationerna.

I den första studien studerades golv- och skotträdsla hos rasen collie. Inom vissa hundraser är det vanligt att individer är rädda för, eller till och med vägrar att gå på olika typer av golv. Detta kan skapa stora problem inte bara för hunden utan även för hundägaren. Skotträdsla är en annan typ av rädsla som orsakar stort lidande för hundarna och även blir ytterst problematiskt för hundarnas ägare. Skotträdsla är också svårt att träna bort då rädslan ofta är mycket stark och snarare tenderar att förvärras med tiden. Resultaten från den första studien visade att golvrädda hundar som passerade olika typer av golv och skotträdda hundar som utsattes för ett skottprov hade högre hjärtfrekvens jämfört med hundar som inte var rädda. De skotträdda hundarna hade också högre nivåer av röda blodkroppar (hematokrit) i blodet samt högre nivåer av hormonerna kortisol, progesteron, vasopressin och β-endorfin efter skottprovet jämfört med hundar som inte var skotträdda. De skotträdda hundarna reagerade dock olika beteendemässigt på skotten. Vissa hundar flydde medan andra blev paralyserade och stod alldeles stilla men hos samtliga ökade de fysiologiska variablerna kraftigt. Studien visade att det är möjligt att detektera rädsla med fysiologiska variabler medan det kan vara svårt att se på hundarnas beteende hur rädda de verkligen var.

Inom mjölkproduktionen finns rutiner och inhysningssystem som är ifrågasatta, till exempel tidig separation av mor och unge samt uppbundna djur. Get och killing etablerar starka band tidigt efter förlossningen. De första timmarna efter födseln lämnar geten sin killing som ligger gömd. Hur länge gömningsbeteendet kvarstår varierar dock från dagar till veckor mellan olika getpopulationer. I intensiv mjölkproduktion separeras ofta get och killing tidigt. I mer extensiva system är det vanligt att get och killing separeras under dagtid så att getterna får beta för att sen vara tillsammans nattetid. Lite är dock känt hur det påverkar utvecklingen av olika egenskaper som t.ex. rädslor. Starka rädslor kan påverka produktionen negativt hos mjölkproducerande djur. Om get och killing separeras ökar möjligheten att de återförenas om de aktivt söker efter varandra och bräker för att hitta varandra.

I den andra studien undersökte vi hur getter påverkas av att vara uppbundna jämfört med lösgående i sällskap med en annan get. När getterna var uppbundna ökade blodtryck och hjärtfrekvens medan nivåerna av hormonerna β-endorfin och oxytocin var lägre jämfört med när de var lösgående. Detta tyder på att getterna när de var uppbundna upplevde situationen som påfrestande. Hormonerna kortisol och vasopressin påverkades inte av de olika inhysningssystemen vilket visar vikten av att mäta flera fysiologiska variabler vid utvärdering av djurs välfärd.

I den tredje studien studerade vi effekter av att killingen diar sin mamma samt hur getter reagerar på tidig separation från sin killing. När killingarna diade ökade både hormonet kortisol och ß-endorfin, medan hjärtfrekvensen inte påverkades. Inga fysiologiska förändringar sågs hos getterna när de separerades från sina killingar, men både getter och killingar bräkte intensivt. Getterna verkade inte påverkas negativt av separationen trots att de tillbringat tillräckligt med tid med sin killing för att ha utvecklat ett starkt band. Detta skulle kunna förklaras av att getter är gömmare, det vill säga att killingarna gömmer sig under en tid efter förlossningen, vilket skulle kunna innebära att både get och killing är anpassade till att vara separerade i perioder.

I den fjärde och femte studien undersöktes killingarnas reaktioner på separation från mamman. Killingarna hölls i tre grupper; tillsammans med mamman, tillsammans med mamman på natten men utan mamman på dagen, respektive separerad från mamman men tillsammans med andra killingar. Under två månader mättes killingarnas tillväxt, hur mycket mjölk getterna producerade och om mjölkens sammansättning skiljde sig åt mellan de olika grupperna. Vi undersökte också om det var skillnader i killingarnas beteende beroende på om de hölls med eller utan mamma. För att undersöka om systemen påverkade killingarnas reaktioner i påfrestande situationer gjorde vi tester vid två veckors och två månaders ålder som innebar att killingarna fick vara isolerade från sina mammor respektive de andra killingarna och i en okänd miljö under en kort period. Alla killingar växte lika mycket oavsett system, men killingarnas beteende påverkades. De killingar som hölls tillsammans med mamman dygnet runt låg gömda mer än de andra, medan de killingar som var helt separerade från mamman och hölls i en grupp med killingar tillbringade mer tid med andra killingar när de var aktiva. Reaktionerna på testerna var mycket individuella. Vid två veckors ålder bräkte killingarna som var separerade från mamman mindre än de övriga killingarna, men de hade högre hjärtfrekvens både vid två veckors och två månaders ålder. De killingar som separerades dagligen från sin mamma bräkte mer och visade de kraftigaste beteendereaktionerna när de var två månader gamla.

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This thesis investigate physiological and behavioural parameters to evaluate fear and discomfort in dogs and goats. The results show that fear of gunshots is a serious stressor, tethering of goats affects physiological parameters, early separation of goat and kid cause intensive vocalisation, and rearing with or without mother affect goat kids behavioural and physiological reaction to isolation. In conclusion, it is important to measure several different physiological and behavioural parameters when assessing animal welfare.

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