Physiological and Behavioural Responses to Fear and Discomfort in Dogs and Goats

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Abstract

In this thesis, behavioural and physiological methods were combined to better understand reactions to fearful situations in dogs and separation in goats. Fear of gunshots is common in dogs, but also other fears, such as fear of walking on certain floors. The aim of the dog study was to establish whether it is possible to separate dogs that are fearful of floors and gunshots from dogs that do not fear these factors. This was done by studying behaviour and changes in heart rate, haematocrit, plasma cortisol, progesterone, testosterone, vasopressin and β-endorphin concentrations in thirteen dogs during a floor test and a gunshot test. Seven dogs that were fearful of floors had higher heart rate than six dogs that were fearless. However, seven dogs fearful of gunshots had higher heart rate, haematocrit and plasma concentrations of cortisol, progesterone, vasopressin and β-endorphin than six fearless dogs which demonstrates that fear of gunshots is a serious stress for the individual. The behavioural expression of fearfulness showed a large variation between individuals. The hypothesis of the goat study was that abrupt separation affects physiology and behaviour of the goats. For comparison, seven goats with their kids were first studied during suckling, a situation considered pleasant and peaceful. 3-4 days after parturition, goats and kids were separated. Suckling did not induce any cardiovascular changes in the goats. Plasma concentrations of cortisol and ß-endorphin increased, but oxytocin and vasopressin remained unaffected. Heart rate, arterial blood pressure and plasma concentrations of cortisol, β-endorphin, oxytocin and vasopressin did not change after separation. However, both goats and kids vocalised intensively. In conclusion, behavioural signs of fear and discomfort in animals are not always accompanied by corresponding changes in physiological variables. Combining both physiology and ethology is therefore desirable in evaluation of animal welfare.

Keywords: ß-endorphin, behaviour, blood pressure, cortisol, dog, fear, goat, gunshot, haematocrit, heart rate, oxytocin, progesterone, separation, testosterone, vasopressin, welfare.

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Dedication

To my beloved kids Hampus, Frida, Sofia, and Fabian.

Till mina älskade barn Hampus, Frida, Sofia och Fabian.

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 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) [online]

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Abbreviations

CNS	Central nervous system
ANS	Autonomic nervous system
SNS	Sympathetic nervous system
HPA	Hypothalamic pituitary adrenal axis
ACTH	Adrenocorticotropic hormone
HR	Heart rate
BP	Blood pressure

Introduction

Animal welfare has been defined as "how well the individual fares in life" (Broom, 1996) or as a "state of harmony between an individual and its environment" (Désiré *et al.*, 2002). There are several other definitions of welfare, but important is that welfare depends on how individuals perceive their environment (Viessier & Boissy, 2007). Therefore, when evaluating animal welfare, the resources around the animals, *i.e.* management and housing must be considered, but more important is to assess what the animal itself can tell us about its well being. Animals cannot communicate by spoken language, but they show their status in other ways, by behavioural expressions and physiological changes. During recent years, focus on positive emotions has emerged when evaluating animal welfare (Boissy *et al.*, 2007).

In this thesis we have combined behavioural and physiological methods to understand the reactions of individual dogs and goats to different situations. Behavioural physiology is used when studying feeding behaviour, sexual behaviour, and maternal behaviour, and during the last decade when evaluating animal welfare (Houpt, 2004).

Behaviour has been described as a window on physiology (Bowdan, 1992). Behavioural physiology is the application of behavioural principles to physiological functioning and how the body processes information about itself and the environment and makes adjustments accordingly. In behavioural terms this includes attention, motivation, emotion, memory, perception and reinforcement. Learning is physiological reconfiguration involving consciousness and emerging new principles. One useful method when studying behavioural physiology is biotelemetry, which makes it possible to continuously measure heart rate and blood pressure in undisturbed animals. Behavioural studies in animals are limited in the aspect that we can only measure the behaviours animals perform but not their feelings. Simultaneous measurements of behavioural and physiological parameters aim at improving our understanding of the reactions of animals to different situations.

Animal personality can be defined as "consistent individual differences in behaviour across time and contexts" (Bergmüller & Taborsky, 2010), while coping styles have been defined as "alternative response patterns to a stressor" (Koolhaas, 2010). Already in the 1970's, it was found that individuals could differ in their response to the same set of conditions by active or passive coping (Henry & Stephens, 1977). According to that theory, active copers are more prone to activate the sympathetic-adrenal-medullary system, whereas passive copers activate the hypothalamic-pituitary-adrenal-cortical system. Today, the term proactive coping is often used for active coping, and the term reactive coping for passive coping because these terms better describe how the different individuals react to environmental stimuli (Koolhaas *et al.*, 1999). Behaviourally, reactive coping is characterized by immobilization whereas proactive coping involves an activity such as fight or flight (Koolhaas *et al.*, 1999). Proactive copers develop routines, whereas reactive copers are more flexible and react to changes in the environment (Koolhaas *et al.*, 1999). Recently, Koolhaas *et al* (2010) have suggested that animals vary on two independent dimensions which gives four different types; shy (high stress reactivity and reactive coping), panicky (high stress reactivity and proactive coping), bold (low stress reactivity and proactive coping) and docile (low stress reactivity and proactive coping).

In this thesis, dogs and goats were studied because they could be expected to react differently to potentially aversive situations. Dogs are predators and predominantly carnivores, whereas goats are prey species and are ruminating herbivores. All species have developed certain behavioural strategies for survival, but while there is diversity in behaviours between species, the physiological reactions are more conserved even across a range of species. Dogs and goats obviously differ in their behavioural expressions, but the physiological responses to fear and stress are to a large extent similar. We hypothesised that studying physiological responses in these two species would give interesting new insights into their behavioural physiology.

Fear – a life saving reaction

In 1993, Fraser defined the "five freedoms" important for animal welfare as: freedom from hunger and thirst, discomfort, pain, injury and disease, fear and distress. Fear is a reaction caused by the perception of actual danger (Forkman, *et al.,* 2007; Sherman & Mills, 2008). Alternatively, it has been defined as "an internal emotional state induced by the perception of danger during exposure to a potentially threatening stimulus" (Gray, 1987; Boissy, 1995). When an individual perceives danger or a threat, physiological reactions and behavioural responses (Sherman & Mills, 2008) start in the brain and may end with the so called fight or flight reaction (Cannon, 1929). Hence, fearfulness is a self-protective response that gives rise to avoidance or defensive behaviours and thereby helps the individual to cope with a challenging environment (Koolhaas *et al.*, 1999). However, excessive fear can cause behavioural problems (King *et al.*, 2003) and development of chronic stress, which can impair health (Koolhaas, *et al.,* 1999).

In dogs, fear-related behaviour is a common problem (Wells & Hepper, 2000) and fearfulness is reported to be the most common cause for failing dogs in behavioural tests at dog shelters (Poulsen *et al.*, 2010). In a modern society there are a number of fear eliciting situations for 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 floors in our flats or houses. This phenomenon had, to our knowledge, not been studied before and initiated the dog study in this thesis (Paper I).

Mother and young separation

Another type of situation that can be considered aversive is separation of mother and young. The procedure involves disruption of the bond between mother and offspring and is sometimes considered as a welfare problem. In goats and kids an exclusive bond between mother and young is established soon after birth (Miranda-de la Lama & Mattiello, 2010; Poindron *et al.*, 2007a; Poindron *et al.,* 2007b). In free living goats, the kids can stay with their mothers for up to 12 months (Miranda-de la Lama & Mattiello, 2010). The mother recognises its kid through olfactory cues (Collias, 1956; Klopfer, 1971; Poindron, *et al.,* 2007b) but also by acoustic and visual signals (Poindron *et al.,* 2003). After the first few hours of caring for the newborn kid, the kid leaves the goat and hides, while the goat join the herd to forage and only comes back for nursing (Lickliter, 1984). However, strategies vary among different goat populations depending on habitat and other factors (O´Brien, 1984). Early mother and young separation is the most common management practice at dairy farms, intended to achieve maximum milk yield for commercial production (Miranda-de la Lama & Mattiello, 2010; Lu & Potchoiba, 1988). Therefore, the effects of early separation on physiology and behaviour were studied in this thesis.

Physiological and behavioural responses to fear or discomfort

The brain constantly receives information from internal organs and from the environment. Without input from the senses the nervous system would not be able to control the body. Information is stored as memories in certain parts of the brain and can be used later. The nervous and the endocrine systems of the individual interact in response to different situations with the aim of maintaining homeostasis. Several hormones are also found as neurotransmitters in the brain. It is not always clear if they are released centrally and peripherally at the same time, which makes it difficult to draw conclusions of effects in the brain by analysing blood samples.

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, which are 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 and major parts of the endocrine system (Figure 1).

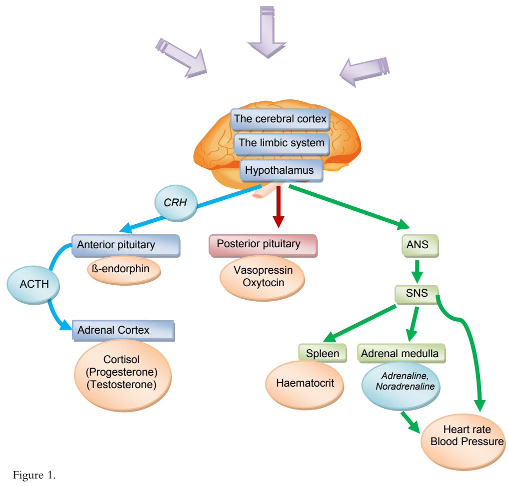

Figure 1.

Figure 1. A simplified schematic drawing of the physiological stress response. The figure illustrates activation of the hypothalamic-pituitary-adrenal axis (HPA-axis) (blue pathway), release from the posterior pituitary (red pathway), and the stimulation of the sympathetic nervous system (SNS) (green pathway). Orange marked parameters are measured in this thesis

The sympathetic nervous system

When the sympathetic nervous system (SNS) is activated, noradrenaline and adrenaline are released from the nerve endings and from the adrenal medulla (Figure 1). Sympathetic activation prepares the individual for fight, flight and fright, *e.g.* 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, in some species, activation of the SNS stimulates the spleen to contract and release erythrocytes, which increases the haematocrit (percent erythrocytes of the total blood) (Figure 1). Stimulating the parasympathetic nervous system

(PNS) mostly has the opposite effect to SNS stimulation *e.g.* decreases heart rate.

In earlier studies in goats, basal values of heart rate and blood pressure have been recorded telemetrically during different reproduction stages 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, *e.g.* heart rate increases in goats expecting food. Such knowledge is important when evaluating cardiovascular reactions to different situations in animals.

The Hypothalamus-pituitary-adrenal axis

Corticotropin releasing hormone (CRH) is released from the hypothalamus to the anterior pituitary, which in turn releases adrenocorticotropic hormone (ACTH) that stimulates the adrenal cortex to release the steroid hormone cortisol and during some circumstances enough progesterone and testosterone that can be detected in blood plasma (Figure 1).

Cortisol

One of the main effects of cortisol is to make energy available in challenging situations by mobilizing protein, fat and glucose. Gluconeogenesis is stimulated and glucose is made available for the heart, nervous system and skeletal muscles. 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.

Cortisol is a commonly used stress variable in many species. In dogs, the plasma cortisol concentration varies according to different situations such as ground transport (Frank *et al.*, 2006), air transport (Bergeron *et al.*, 2002), housing in animal shelters (Hennessy, et al., 1998) and environmental challenges (Haverbeke *et al.*, 2008). In goats cortisol is widely used and has been measured during many different situations such as 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) food deprivation as well as catching sight of food (Olsson *et al.*, 1995).

Progesterone

Progesterone is a reproductive hormone primarily released from the ovaries and transported in blood plasma bound to proteins. 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 ovariehystectomised female cats (Chatdarong *et al.*, 2006) and ovariectomised cows (Yoshida & Nakao, 2005). Therefore, it was of interest to measure in this thesis.

Testosterone

Testosterone is mainly produced in the testes and is necessary for spermatogenesis and male characteristics. As well as progesterone, testosterone is synthesized in and released from the adrenal cortex (Figure 1). Testosterone has been reported to be connected to aggression and psychic stress among others (Olweus *et al*, 1988; Simpson, 2001; Williams *et al.*, 2000).

ß-endorphin

Increased ß -endorphin concentrations have been associated with stress and pain in several species. ß-endorphin is generated from the same hormone precursor as ACTH, pro-opiomelanocortin. ß-endorphin is released from the anterior pituitary (Figure 1) but has its main functions in the brain. Endorphins are endogenous opioids that can act on opiate receptors and induce analgesia. They are therefore important in the endogenous pain control system.

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 to 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 myopeithelial cells around the alveoli. Oxytocin is synthesized in 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 & Young, 2001).

Vasopressin, also named 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 influence blood pressure when concentrations reach high levels. Vasopressin is also a neurotransmitter and is involved in aggressive behaviour (Stribley & Carter, 1999; Caldwell *et al.*, 2008) and enhances arousal, attention and vigilance (Carter & Altemus, 1997).

Behaviours related to fear, stress and discomfort

There is a diverse array of behavioural patterns that indicate a fear reaction in animals. Avoidance, flight and immobility are examples that are common in several species and can be observed in both dogs and goats. However, behavioural patterns related to fear can be contradictory since both active and passive 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 is also indicative of fear in dogs. In goats, locomotor activity, rearing, exploration, vocalisations and eliminative behaviour (Forkman *et al.,* 2007; Price & Thos, 1980) but also sniffing (Carbonaro *et al.*, 1992) have been 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 condition would increase the probability of reunion (Panksepp, 1998). Vocalisation is also a commonly used indicator of acute stress and discomfort since it is considered to express fear (Boissy, 1995). Acoustic signals communicate that animals are in need of something and vocalisation is used to call for herd members (Manteuffel *et al.*, 2004). Goats use different types of vocalisations and exhibit increased frequencies of vocalisations when isolated from social partners (Price & Thos, 1980). Dogs have a large repertoire of vocalisations, which includes whining, yelping, growling and barking. Dogs bark under several circumstances, to greet, invite to play, seek contact, when reacting to loneliness or pain, and as defence or threat (Pongrácz, *et al.,* 2010).

Aims of the Thesis

The overall aim of the present thesis was to study interactions between physiology and behaviour, with special reference to situations associated with fear or discomfort.

Specific aims:

- To study changes in the physiological variables heart rate, haematocrit, cortisol, progesterone, testosterone, vasopressin and ß-endorphin concentrations in dogs when walking on different floors and during a gunshot test.
- To establish whether it is possible to separate dogs which are behaviourally fearful of walking on different types of floors and fearful of gunshots from fearless dogs by measuring physiological variables.
- To study the response of blood pressure, heart rate, cortisol, ß-endorphin, vasopressin and oxytocin concentrations around suckling in goats; a situation considered to be peaceful and pleasant.
- To study if permanent separation of goats and kids with established bonding affects blood pressure, heart rate, cortisol, ß-endorphin, vasopressin and oxytocin concentrations in the goats.

Materials and Methods

The methods and experimental procedures are described in detail in each paper.

Animals

Paper I. Thirteen privately owned male dogs of the collie breed were studied. The collie breed was chosen since it is considered to be one of the breeds that has 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 that were classified as fearful of gunshots in this study were described to be so by their owners. In addition the owners stated if they perceived their dogs as fearful or not fearful of walking on different types of floors. Seven dogs were considered as fearful and seven as fearless. Since fear of floors did not always occur concomitantly with fear of gunshots, there were consequently four different groups of dogs. One of the fearless dogs was taken out of the study because of difficulties with the blood sampling.

Paper II. Seven goats and their kids of the Swedish domestic breed *(Capra Hircus)* were studied. They were housed together in an indoor pen but were moved to individual boxes before parturition and kept there during the study.

Preliminary study: Isolation of castrated male goats
Six castrated male goats, aged 8 months (±10 days) were used. They were housed together in an indoor pen (5.1m x 6.6 m) enriched with tables and boxes to climb on. The goats were fed hay at 7:00 and 15:00 and water was available at all times. At the time for the experiment, they weighed 32.8 ± 3.2 kg (range 27 to 35 kg).

All goats in this thesis belonged to an experimental herd at the Swedish University of Agricultural Sciences. They were well adapted to handling and the adult goats were used to the 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. The use of privately owned dogs was approved by the National Board of Agriculture.

Experimental procedures

Paper I

The dogs were subjected to a floor test and a subsequent gunshot test. During the floor test, the dogs walked over a total of seven different types of floors in the sequence; plastic, parquet, marble stairs, clinker, concrete, iron grids and wobbling boards. Before the test started and after 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 5 minutes between. Heart rate was measured during the whole 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 if the dog was unaffected or little or very hesitant to enter the passage and if it 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.

Paper II

Four studies were performed in goats kept with their first-born kid in individual boxes. The goats were videotaped and heart rate and blood pressure were recorded by telemetry during all four studies:
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: Blood samples were taken before and after the goat and kid were separated 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.

Preliminary study: Isolation of castrated male goats

Six castrated male goats were isolated for 10 minutes in a room (2.85 x 2.20 m) with no possibility to hear or see other goats. Heart rate was measured continuously with Polar Sport tester equipment (Polar Vantage NV, Polar Ltd, Bromma, Sweden). Blood samples were taken via a permanent catheter (Secalon® T, Becton Dickinson, Stockholm, Sweden) inserted into one of the jugular veins. Blood sampling were performed before isolation, immediately after and 30 and 60 minutes after isolation, and the behaviour of the goats was videotaped. Average heart rate before and after the test moments were calculated as mean values of 5 minutes of registrations. To avoid effect of blood sampling per se, heart rate values were calculated from registrations 10 min before blood sampling. The average heart rate during isolation was calculated as a mean value of the 10 min of isolation.

Comments on physiological methods

Blood sampling

Collection of 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 because every species and every individual is different. Analyses of blood give much information and blood sampling is often used. To avoid upsetting the animal in the test situation a catheter was inserted under local anaesthesia into the cephalic vein in the dogs and in a jugular vein in the goats. In the present experiments these procedures were done calmly and with gentleness and the animals did not show aversive reactions and rapidly settled down. Thereafter, blood samples could be withdrawn repeatedly without any reactions from the individual.

Alternatives to blood sampling include collection of urine or saliva. The advantages with these methods are that they are non-invasive, easy to perform and it is possible for the owner to collect the samples. However, not all parameters are possible to analyse in saliva and urine.

Blood plasma analyses

All the blood samples were analysed in our laboratory by the same experienced person. The methods used were validated for each variable and each species *i.e.* 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

Before the dog experiment started, a heart rate monitor (Polar Vantage NV; Polar Ltd Bromma) was strapped around the chest of the dog. The Polar heart rate monitor is a non-invasive method for recording heart rate, used by humans during training, but functions well in dogs (Ogata *et al.*, 2006) enabling recordings of the heart rate of freely moving animals. The receiver is a watch that can be fastened on the animal (Figure 2).

Figure 2. The Polar Sport Tester in one of the dogs in Paper I.

The goats had a surgically implanted telemetric device for recording both heart rate and blood pressure (Figure 3). 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.

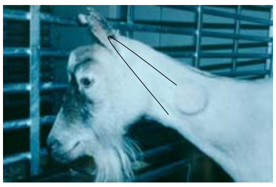

Figure 3. The telemetric device used in Paper II.

Both these telemetric methods have advantages and disadvantages. The surgically implanted telemetric device is an extraordinary method which 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. However, it is not possible to register blood pressure.

Comments on behavioural methods

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 were observed for several days and nights in some experiments. For practical reasons direct observations at all times were not possible why the behaviour of the goats were observed from videotapes. Documentation of behaviour on videotapes is valuable since the tapes can be checked many times which reduces the risk of missing valuable information. On the other hand, small behavioural expressions, such as for example trembling or ear position can be difficult to detect from a videotape.

Results and Comments

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 fearless 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 fearless 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 at the same spot, trembling or shaking.

Comment:

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 might partly been due to the novel situation and the locomotion performed. In the gunshot test all fearful dogs independent of their behavioural response showed a very strong physiological response by activating both SNS and HPA 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 fearful and fearless.

The dog-owners opinion about their dogs' fearfulness of floors did not always agree with the results of our test. As discussed in Paper 1, there is a large variation in which type of floors the dogs are accustomed to. Therefore, all dogs may not have been in contact with all types of floors in this test. It seems also to be highly individual if the dog feels uncomfortable on shiny, slippery floors because they can't get a foothold, while another reacts more to an unstable surface where they can lose their balance.

Anyone exposed to an unexpected loud noise can react with transient fear. However, 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.

In contrast to the floor test, the dog-owners opinion about the dogs' fearfulness of gunshots did highly agree with the test result showing that the dogs' earlier behaviour/reactions in similar situations could be repeated with our test and that fear of gunshots is a specific fear that dog-owners are aware of.

Suckling and permanent goat and kid separation (Paper II)

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

The separation did not cause any physiological changes in the goats. During the 20 minutes 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.

Comment: There was a small but not significant increase in blood pressure at suckling in undisturbed goats

The only obvious behavioural reaction of the goats to separation from their kids was the intensive vocalisation for a short time period. The high vocalisation was not accompanied by physiological changes. In this study the goats could not search for the kid since they were in boxes and maybe the most adaptive behaviour is "acceptance of the loss" and not using energy on trying to reunite with the kid. Probably the goats stood more when they were together with their kids because of the kids suckling.

Isolation of castrated male goats (preliminary results)

The preliminary results from the study of adult male goats showed an increase in heart rate (Figure 4a), an increase in plasma concentration of cortisol (Figure 4b) and an increase in plasma concentration of progesterone (Figure 4c) compared to the sample taken before isolation. There were no differences in haematocrit before and after isolation.

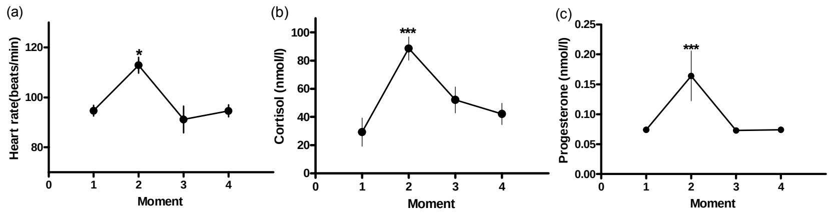

Figure 4. Means (±SE) in heart rate (a) plasma cortisol (b) and plasma progesterone (c) concentrations before, during and after isolation in six male castrated goats. The figures indicate test moments as follows: before test (1), isolation (2), 30 min after isolation (3) and 60 min after isolation (4). The heart rate, plasma cortisol and progesterone concentrations increased significantly during isolation. ⋆ and ⋆⋆⋆ indicate significant differences from sample 1 (⋆=P≤0.05 and ⋆⋆⋆=P≤0.001).

The behavioural response to isolation of adult male goats differed between individuals. The latency time to vocalise ranged from 3-443 seconds and vocalisation rate from 1-25 vocalisations/min. There were individual differences in exploring during the isolation from 31 to 61 %, mean 43±4 (% of observed time).

Comments: Since provoked fear in dogs resulted in a major physiological reaction, it was of interest to create a fear test and study the reactions in completely different species. In goats, which are highly gregarious, forced isolation can be expected to generate a strong negative reaction. Isolation has been used to study both physiological and behavioural reactions to aversive situations in goats (Carbonaro *et al.*, 1992; Kannan *et al.*, 2002; Al-Qarawi & Ali, 2005). The increase in plasma cortisol concentrations was accompanied by progesterone release during isolation which is in accordance with the results from the dogs fearful of gunshots. However, the levels were very low and in some samples the concentrations were below least detectable value. Despite the low concentrations, the suggestion of plasma progesterone as an alternative for plasma cortisol in dogs, may also be valid in male castrated goats.

General Discussion

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, 2010) and is important to take into consideration in most research fields.

When studying behavioural problems in dogs, a good collaboration with dog owners is necessary. The questionnaire for recruiting dogs to this study worked well. All dogs that were classified as fearful of gunshots in this study were described to be so by their owners, but this was not confirmed in all dogs in our study. An explanation can be the existence of numerous types of floors and that the dogs were used to different types of floors in their home environment.

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

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 fearless, indicating a massive activation of both SNS and HPA-axis. Behaviourally, the dogs used different strategies to cope with the situation. However, they could not be assigned to a distinct coping style, because the physiological reactions were to a large extent similar among the dogs.

The preliminary results from the isolation in the castrated male goats showed that both the SNS and HPA-axis were activated in the goats, as indicated by the increased heart rate, plasma cortisol and progesterone concentrations. In addition, there were large individual differences in behavioural strategies (for example in exploring) which will be further analysed. Despite the different behavioural reactions in dogs and goats, the different species showed a similar physiological stress reaction.

The difference in cortisol concentration between fearful and fearless dogs was striking – the plasma peak level was four times as high in fearful dogs as in fearless dogs. It has been suggested that a doubling of plasma cortisol concentrations indicate stress or poor welfare (Houpt, 2004). In fearful dogs, the cortisol release was accompanied by increased plasma progesterone concentrations suggesting a release from the adrenal cortex. This was further supported in the isolated male, castrated goats, where the plasma progesterone concentration increased simultaneously with an increase in plasma cortisol concentrations. The results indicate that progesterone may be an alternative to cortisol analyses for assessing stress, at least during some circumstances, but that there are species differences that are important to consider.

Both testosterone and vasopressin concentrations were elevated after gunshots compared to the sample taken before the experiment started in both fearless and fearful dogs. Both these hormones are reported to be related to physical activity (Campbell *et al.,* 1982; Wade, 1984) 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. Stimulation with ACTH has been reported to increase testosterone concentrations in female dogs but not in males (Frank et al., 2003), which may further explain the lack of differences in testosterone between fearful and fearless male dogs.

The elevated β-endorphin levels after gunshots in fearful dogs may have been caused by pain in the dogs' ears. Pain is reported to be associated with increased β-endorphin concentrations for example during labour in women (Bacigalupo *et al.*, 1990). However, it may also simply be an effect of that the fearful dogs experienced the situation as extremely demanding.

Regardless of how the fearful dogs tried to cope with the situation, fear of gunshots apparently constitutes a severe stress for the individual. It can be argued that dogs in modern society seldom are subjected to the sound of gunshots. However, fear of loud noises is reported to be prevalent among dogs and fear of gunshots was the 3rd most common fear reported (after fireworks and thunderstorms) (Blackwell, *et al.,* 2005; Sherman & Mills, 2008). The occurrence of fearful dogs is of concern for modern society from both dogs and humans point of view. For dogs, it is question of welfare. Good welfare includes freedom from fear (Fraser, 1993), and as already discussed, fear of gunshots puts a severe strain on fearful dogs. The handling of dogs fearful of loud noises places great demands on the dog owners since these dogs do not recover by themselves (Blackwell, et al., 2005). In rats, aggression toward humans is a hereditary trait that represents fear induced defence behaviour (Popova *et al.*, 1993). Fearful dogs that develop aggressive behaviour towards humans is a serious problem (King *et al.,* 2003), and 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) this should be taken into consideration in breeding.

In dogs fearful of floors, only the heart rate was elevated compared to fearless dogs during the floor test. Increased heart rate caused by emotional stimuli or stress is known to decrease rapidly (Caraffa-Braga *et al.*, 1973) but in this study, the heart rate remained at a high level during the whole floor test demonstrating an activation of the SNS for a long time period. It was probably not just an effect of physical activity since there was a difference between fearful and fearless dogs.

The separation of goat and kid did not alert the SNS or activate the HPA-axis as indicated by the absence of a physiological response. After the first hours of maternal care, newborn kids have been reported to leave their mother and hide during the first week after birth (O'Brien, 1984; Lickliter, 1984). Goat mothers do not follow their kids but rejoin the adult herd (Lickliter, 1984). However, the separation in this study caused an intensive, but short lived, vocalisation that was expressed in all goats. The goats and kids had been together for 3-4 days, which might have strengthened the maternal bonding. In cows, the vocalisation rate is higher if separation from calves is performed a few days after parturition than if they are separated immediately (Lidfors, 1996; Weary & Chua, 2000; Stěhulová *et al.*, 2008). The goats and kids could hear each other after separation which also may have influenced the vocalisation after isolation. Cows that can see and hear their calves after separation vocalise more than cows with no visual or auditory contact (Stěhulová *et al.*, 2008). Despite the intensive vocalisation, no physiological responses were found in the goats at separation.

All male castrated goats vocalised during isolation, which is in accordance with earlier studies in goats (Carbonaro *et al.*, 1992; Al-Qarawi & Ali, 2005) although there were individual variations. One of the goats vocalised only once, but most of the goats vocalised to a high extent. The physiological variables, heart rate, cortisol and progesterone increased, indicating activation both of the SNS and the HPA system. However, both the goats separated from kids and the isolated castrated male goats responded by vocalisation, but the differences between individuals were large. The diverse physiological changes during intensive vocalisation found in these studies indicate that vocalisation is not a reliable indicator of discomfort and stress in goats as earlier suggested.

In this thesis, the hormones have been measured in peripheral blood. Several hormones, for example ß-endorphin has its main function in the brain. Studies of physiological reactions in the brain or brain activity, is an interesting tool to investigate animals emotions but difficult to perform practically and hardly applicable in privately owned animals.

The physiological reactions to gunshot were strong in the dogs. This was also true for the isolated male goats although the behavioural reactions varied between individuals in both dogs and male goats. In the goats that were separated from their kids, no cardiovascular or endocrine changes occurred despite frequent vocalisation. The results indicate that the fear of gunshots in dogs and the reaction to isolation in male goats triggered the innate behavioural response to danger, which is necessary for survival (Misslin, 2003). This is an automatic reaction to dangerous stimuli. It is mediated by sensory input to the thalamus and amygdala but bypass cortex and thereby enables a fast unconscious response (LeDoux, 1996). This response differs from the slower pathway that passes the cortex and allows conscious response to stimuli (LeDoux, 1996), which could be the case in the goats separated from their kids and in the dogs during the floor test.

Behavioural signs of fear or discomfort are not always accompanied by physiological changes, as shown in this thesis. 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 animals emotions. Therefore, combining behaviour and physiology and using several variables is necessary when evaluating animal welfare.

Conclusions

The individual variations in behavioural expressions of fear were large in the studies presented in this thesis. Despite this, the physiological responses were striking and did not vary between individuals to the same extent. One exception, however, was the separation of mother and young which did not induce physiological responses but a short-lasting behavioural reaction. Taken together, this emphasises the importance of using both behavioural and physiological measurements when the aim is to understand animal reactions.

Specific conclusions:

- Dogs fearful of walking on different floors had higher heart rate than fearless dogs during the floor test.
- Despite different behavioural reactions, all dogs fearful of gunshots had high heart rate, haematocrit and plasma concentrations of cortisol, progesterone, vasopressin and β-endorphin during the gunshot test.
- Dogs fearful of gunshots showed a strong physiological response which the fearless did not. It was therefore possible to separate fearful dogs from fearless by physiological variables.
- Suckling elevated plasma cortisol and β-endorphin concentrations but not oxytocin, vasopressin or cardiovascular variables in goats.
- Permanent separation of goats and kids with established bonding was not accompanied by cardiovascular or endocrine changes despite intensive vocalisations.
- Behavioural signs of fear of discomfort are not always accompanied by corresponding changes in physiological variables.

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