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Affective-autonomic states of domestic pigs in the context of coping and animal welfare

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Gutachter:

Prof. Dr. Birger Puppe, Universität Rostock, Agrar- und Umweltwissenschaftliche Fakultät, Professur für Verhaltenskunde und Leibniz-Institut für Nutzierbiologie, Institut für Verhaltensphysiologie

Prof. Dr. Eberhard von Borell, Martin-Luther Universität Halle-Wittenberg, Naturwissenschaftliche Fakultät

Prof. Dr. Edna Hillmann, Humbold-Universität zu Berlin, Lebenswissenschaftliche Fakultät

Dr. Jan Langbein, Leibniz-Institut für Nutzierbiologie, Institut für Verhaltensphysiologie

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Chapter ONE.

General Introduction

1.1 Animal welfare

A central issue in the study of animal welfare is to answer the question of how can we objectively and scientifically assess the welfare state of an animal? The concerns about the animals' welfare are closely associated with the concept of sentience. The term "sentience" has been used to describe the capacity of an individual to experience one or more of the various states of feelings (Broom, 2016). A brief history of this concept reveals that some acceptance of sentience, at least in mammals, has been present for years. According to Preece (2002), ancient thinkers, such as Hippocrates and Pythagoras, were supporting the view that animals reside the capacity to feel pain and suffer. Although writings of Leonardo da Vinci, Erasmus and Shakespeare evidenced that animal sentience was accepted, there is a clear line of philosophic argument for non-sentience from Aristotle (Duncan, 2006). Later perspectives, such as the view from Descartes (1596-1650), who saw animals as "automata", are reminiscent of the Renaissance (Duncan, 2006). A more compassionate and reasoned understanding of the experiences of animals evolved in the 19th century, which was primarily characterised by Darwin (1872). He accepted that animals were capable of many emotions and experiences, both similar and different to humans, including self-consciousness, a trait once generally assumed to be solely human (Darwin, 1872). So, nearly 150 years ago, it was discussed by scientists that animals were sentient. However, following numerous trends and progresses, including "Behaviourism", which had an inhibiting influence on the study of sentience and feelings by scientists, there was a surge of interest in animal sentience in the last quarter of the 20th century (Duncan, 2006). It is the belief that animals possess sentience and feelings that gives rise to concerns for their welfare (Dawkins, 2006) and our understanding of animal sentience and how animals feel will have a huge effect on how we deal with animal welfare.

The number of scientific investigations into animal welfare gradually increased in the late 1960s, following the publication of Ruth Harrison's book "Animal machines" (Harrison, 1964). In her book, Ruth Harrison exposed the realities of intensive farming at the time, and the suffering of the animals within their environment. In response to this, the UK government commissioned an investigation into the welfare of intensively farmed animals. The resulting Brambell Report outlines five aspects ("five freedoms") of the welfare of farm animals (Brambell, 1965; Broom, 2011). Welfare is preserved if the animals are kept free from (i) hunger, thirst and malnutrition, (ii) thermal and physical discomfort, (iii) pain, injury and diseases, (iv) fear and chronic stress, and were (v) free to display normal, species-specific behaviour patterns. Such early approaches to the assessment of animal welfare were often based on the simple exclusion of negative attributes and states (Webster, 2006; Broom, 2011). Thus, positive welfare was defined as the absence of pain or being in some way comprised (Ohl and van der Staay, 2012). The first four of the "five freedoms" were formulated from the perspective that the absence of negative states ensures welfare; only the fifth freedom, although more indirectly, implied that positive aspects contributed to welfare. However, the Brambell Committee also realised that an understanding of sentience was an essential part of assessing welfare (Duncan, 2006) and also acknowledged the role of mental processes in reducing welfare. They stated, "*Welfare is a wide term that embraces both the physical and mental well-being of the animal. Any attempt to evaluate welfare, therefore, must take into account the scientific evidence available concerning the feelings of animals that can be derived from their structure and functions and also from their behaviour*" (Brambell, 1965). Since the end of the 1990s, farm animals have a status as "sentient beings" at European law level and therefore require special protection (Duncan, 2006, Korte et al., 2007). Since then, there have been numerous attempts to define and understand animal welfare on a scientific level based on ethical concerns over the quality of life of animals.

Scientists have proposed different concepts and research methods to assess the welfare state of an animal. All in all, three different but overlapping approaches have evolved in modern animal welfare science, depending on what is considered important for the well-being of the animal (Fraser et al., 1997; Fraser and Duncan, 1998; Appleby, 1999; Verhoog et al., 2004; Veissier and Boissy, 2007). According to Fraser (2008, 2009a), one approach refers to the naturalness of the circumstances in which animals are kept. This approach was developed in response to concerns over highly restrictive and unnatural forms of animal housing. It focuses on the extent to which the animal is free to express its natural behavioural repertoire, with the idea that an animal being able to fulfil its inherent nature will have good welfare (Marchant-Forde, 2015). Another view of animal welfare focuses on the biological functioning of the animals, assigning special importance to physical health, growth and the normal functioning of the animal's biological systems (Duncan, 2005; Fraser, 2009a). This approach emphasises the successful coping of animals during the interaction of their adaptive repertoire (that an animal possesses as result of evolutionary history) with current challenges in the environment (Broom, 1986; Fraser et al., 1997). The animal's welfare is not threatened as long as it is able to cope with environmental challenges (Broom, 1996; Korte et al., 2007). The understanding of welfare in this approach is closely related to the concept of physiological stress responses (Veissier and Boissy, 2007). Selye, who was the first in shaping the term "biological stress", defined stress as the unspecific response of the body to external challenges and called the whole range of behavioural and physiological modification the "general adaptation syndrome" (Selye, 1936, 1973) that an organism undertakes to avoid or adapt to a perceived threat affecting internal homeostasis (Moberg and Mench, 2000). Only if an individual is challenged beyond its ability for adaptation, stress and impaired welfare occur (Korte et al., 2005, 2007). A third view of animal welfare emphasises affective states, emotions and experiences of animals as sentient beings (Harrison, 1964; Dawkins, 1990; Duncan, 1993; Fraser and Duncan, 1998; Mench and Duncan, 1998; Veissier and Boissy, 2007; Broom, 2016), as it was stated by the Brambell Committee (Brambell, 1965). Besides the absence of suffering, positive and negative emotions were integrated in the concept of welfare (Duncan, 1998; Boissy et al., 2007b; Mellor and Beausoleil, 2015) and play an increasing role in how animal welfare is discussed (Duncan, 2005). These different but overlapping approaches into animal welfare are not necessarily mutually exclusive, however, they resulted in numerous attempts to define animal welfare in a scientific manner (see Blokhuis et al., 2007). One global definition of animal welfare which represents one attempt to comprise different approaches was introduced in 2012: *"Welfare is the state of physical and mental health resulting from the process of behavioural and physiological adaptation when coping with environmental challenges, and the associated subjective experiences and* ***emotional evaluation****, all in the light of* ***individual*** *and/or cognitive needs and abilities"* (Puppe et al., 2012).

In regard to my thesis I highlighted two main aspects of this definition concerning animal welfare, as they account for the key components of my work. The first aspect of this thesis provides one attempt to gain insight in the **emotional evaluation** of pigs by assessing the neurophysiological pathways underlying the animal's affective-emotional state. This represents one approach to address the initial question of how we can objectively assess an animal's welfare state. The second aspect links this context-specific emotional evaluation to **individual** differences on behavioural and physiological level. Both aspects play a significant role in measuring, understanding and improving animal husbandry and welfare.

1.2 Affective states and emotion

In recent years, animal welfare has been extended to include the animal's own perceptions and is strongly associated with the attribution of mental states to animals including the capability of affective experiences (Dawkins, 1990; Mellor and Reid, 1994; Fraser and Duncan, 1998; Désiré et al., 2002; Fraser, 2009b; Rogers, 2010; Boissy and Lee, 2014; Mellor, 2016; Gygax, 2017). The idea that affective states are an important component of welfare was given more scientific credibility by Marian Dawkins in her book "Animal suffering", published in 1980 (Dawkins, 1980). Within this framework, welfare is fulfilled when the animals do not feel long lasting negative emotions and when they can experience positive emotions. To date, there is a broad consensus that affective states play an important role in the context of welfare (Dawkins, 1990) in that they influence and partly control the behaviour of human and animal beings (Rolls, 2015). Thus, the assessment of animal welfare requires a good understanding of the affective states and experiences of animals, including their emotions (Dawkins, 2000). While physical health can often be assessed by clinical symptoms, the identification and interpretation of affective states is the most elusive aspect in animal welfare science (Paul et al., 2005) as part of the 'mental health' in the given definition of animal welfare (see above, Puppe et al., 2012).

There is abundant literature from both biology and psychology on the concept of affective (emotional) states. The terms affect and emotion are often used synonymously in literature, and I will use them in this way throughout the manuscript. However, they are sometimes given specific and distinct meanings in different approaches. For example, according to Posner et al. (2005) affect is described as the first neurophysiological reaction to an event, which is processed subcortically and is therefore unconscious. By linking with other brain systems involved, a first rapid reaction can be triggered that occurs before the typical cognitive processes considered necessary for the formation of a more complex emotion (Zajonc, 1980). This immediate, automatic reaction is thought to release autonomic, neuroendocrine, and somatomotor (facial, gastral, vocal, behavioural) responses as well as cognitive operations, such as attention, learning, memory and action planning. The interpretation with sensory inputs and further brain systems including neocortical structures results in a modulation of these reactions, and, in humans at least, in a subjective experience (Dantzer, 1988; Désiré et al., 2002; Boissy et al., 2007b). This result is accepted as emotion in the narrower sense and is described as an intense but short-lived affective response to an event including a subjective component and two concerted expressive components, one motor (behavioural) and the other physiological (Damasio et al., 2000; Désiré et al., 2002; Russell, 2003; Boissy et al., 2007a). Following this componential view, the affective response, and thus welfare of an individual, does not depend directly on the situation, but on the subjective interpretation by the individual (Duncan and Petherick, 1991; Désiré et al., 2002). To differentiate emotions from mood states, several approaches refer to their duration, whereas both conditions are combined under the term of affective states (Paul et al., 2005; Mendl et al., 2010). While short-term affective states are characterised as emotion and often only last for a few seconds to hours (Verduyn and Lavrijsen, 2015), long-term affective states can be viewed largely equivalent to mood states that last over longer periods of days or even longer (Williams et al., 2008).

A large body of experimental research on emotion has been carried out in humans, and appraisal theories have been constructed by psychologists where emotions are regarded as processes resulting from a cognitive evaluation of the eliciting situation (Veissier and Boissy, 2007). These theories state that responses of individuals to a triggering event are specific to the way this event is evaluated (Lazarus, 1993; Scherer, 2001). The evaluation of the environment is performed according to a series of criteria namely: relevance (i.e. suddenness, familiarity, pleasantness), implication (i.e. outcome probability, expectancy), coping potential (i.e. control, adaptation) and normative significance (i.e. internal and external standards), which results in an emotion and elicits specific behavioural and physiological reactions (Veissier and Boissy, 2007). There is evidence that at least some farm animal species use similar 'stimulus checks' to assess situations they face and this process is assumed to be the key component in the generation of emotions (Sambrook and Buchanan-Smith, 1997; Désiré et al., 2002, 2004, 2006; Ellsworth and Scherer, 2003; Bassett and Buchanan-Smith, 2007; Greiveldinger et al., 2007; Mendl et al., 2010).

From an evolutionary perspective, emotions are considered as elementary modes of responding to affectively-arousing events and are supposed to be shaped by adaptive processes that have appeared during evolution because they confer more fitness (Nesse and Ellsworth, 2009). They are beneficial to the animal in the sense that the modulation of autonomic and neuroendocrine responses prepares the individual to react (fight/flight/immobility); they enable communication (e.g. social bonding) and enhance the evaluation of internal and external information (e.g. reward/punishment, memory). This enables the individual to avoid situations that evoke negative emotions, for example, harm or punishment, as they trigger protective responses such as avoidance (Fraser and Duncan, 1998; Veissier and Boissy, 2007), or to specifically seek situations that generate positive emotions, such as valuable resources and reward (Panksepp, 1994; Rolls, 1998; Désiré et al., 2002; Paul et al., 2005). The ability to perceive its own emotions allows the individual to detect and assess a discrepancy between its requirements and environmental conditions (Désiré et al., 2002). These adaptive events are likely to have evolved across animal species, although there is an ongoing debate as to whether consciousness of an emotion (and the corresponding knowledge of the ability to experience emotion), is present in non-human animals, and if so in which species (Damasio et al., 2000; Berridge and Winkielman, 2003; Burgdorf and Panksepp, 2006; Boissy et al., 2007b; Dawkins, 2017). However, the non-conscious processes involved in affective states make them appropriate for animal studies, even in animals for which conscious emotional experiences cannot be proven to occur (LeDoux, 1996). This avoids a discussion about if, or if not, animals are capable of conscious experience (Paul et al., 2005; Dawkins, 2017).

1.2.1. Theories of emotion and affect

Two primary approaches have been advanced concerning the structure of emotion. According to the "discrete emotion approach", humans are evolutionarily provided with a discrete and limited set of emotions which can be divided into independent categories, for example fear, frustration and anger (Ekman, 1992; Izard, 2007; Panksepp, 2007). Specific neural structures and pathways are thought to subserve each of these "basic" emotional categories (Ekman, 1992; Panksepp, 1998; Posner et al., 2005). After performing a series of cross-cultural studies, various similarities were found in the way people across the world produce and recognize the facial expressions of at least six emotions (Ekman, 2007). This theory of basic emotions has yielded significant advances in the study of affect. Critics of this view emphasise the variability and context-sensitivity of emotions (Barrett, 2009; Colombetti, 2009). There is no consensus how many basic emotions exist and what characterises them as basic (Orthony and Turner, 1990). The discrete emotion approach also lacks a framework that can integrate the wide range of possible emotional states (Mendl et al., 2010), including mood disorders (Posner et al., 2005) and especially positive emotions remain under-studied. Investigations of the subjective component of emotion, rather than supporting a one to one correspondence between a discrete emotion and an underlying neural system, have instead suggested that emotions arise from cognitive interpretations of core physiological experiences (Cacioppo et al., 2000; Russell, 2003). In the field of dimensional approaches, several theories based on differing models have developed. Following human psychophysiology, "two-dimensional approaches" that conceptualize emotions in terms of affective characteristics, propose that all affective states arise from two fundamental neurophysiological dimensions, one related to valence and the other to arousal, or alertness, or activation (Russell, 1980; Thayer, 1989; Posner et al., 2005; Norman et al., 2014). The valence dimension describes the perception of emotions as positive or negative, rewarding or punishing, pleasant or unpleasant. The arousal dimension represents emotional experiences as a variation in the degree of excitement, ranging from low, through various stages of alertness, to high. According to Mendl et al. (2010), who proposed a framework which combines the dimensional and distinct emotions approach, each emotion within this space can be understood as a linear combination of these two dimensions, or as varying degrees of both dimensions. Therefore, emotional stimuli, processes, memories, action plans, and central and peripheral physiology are differentiable in terms of both valence and arousal. For example, the states of joy and contentment are both positively valenced, but the former involves a higher degree of arousal than the latter (Mendl et al., 2010). The combination of these two dimensions, which characterise the structure of subjective emotional experience, has been labelled "core affect" (Russell, 2003; Barrett et al., 2007; Mendl et al., 2010; Lindquist et al., 2012) and can be conceptualized as the fundamental manifestation of any emotion within core affect space.

1.2.2. Measuring affective states

Following the componential view of emotion, as mentioned above, emotional processes are multifaceted, comprising physiological, behavioural and subjective components. The subjective emotional experience can be inferred from linguistic report in humans, but is inaccessible to direct measurement in animals. As emotions emerge in the brain, LeDoux (1996) proposed to look directly at the processes in the brain to objectively measure an individual's emotional state. Several brain areas are known to be involved in emotional processing and techniques, such as lesioning, electrical stimulation, and microdialysis were developed and used to identify neurobiological mechanisms underlying emotional processes. To date, neuroimaging approaches have made fundamental progress in human brain research and methods such as positron emission tomography (PET), functional magnetic resonance imaging (fMRI) or functional near-infrared spectroscopy (fNIRS) have been used to study the cortical processing of emotions in humans and animals (Takamatsu et al., 2003; Phan et al., 2004; Barrett and Wager, 2006; Muehlemann et al., 2011; Gygax et al., 2013; Vögeli et al., 2015). However, the sensory perception of an emotional (eliciting) stimulus initiates changes in a number of different biological processes and pathways and a wide variety of methods have been employed for monitoring affective states in animals by measuring its components on behavioural or physiological level, often used in concert with one another.

The behavioural component is the most direct and easy to measure feedback of an individual in reaction to a pleasant or unpleasant stimulus (e.g. approach and avoidance behaviour) and provides insight into the functionally relevant action tendencies that accompany specific affective states (Gygax et al., 2013; Frijda, 2016). Facial expressions are largely used to provide subjective evidence of affect across species, including humans (Darwin, 1872; Ekman, 1973; Yeates and Main, 2008; Somerville et al., 2011). Species-specific facial expression scales and grimace scales for horses, sheep, rats, mice, and cats have been developed as a pain assessment tool (Sotocinal et al., 2011; Leach et al., 2012; Dalla Costa et al., 2014; Holden et al., 2014; Guesgen et al., 2016). In rats, monkeys, and human infants, tongue protrusion was shown as an indicator of liking food (Grill and Norgren, 1978; Steiner et al., 2001) and a high proportion of visible eye white was found to indicate negative emotional states (e.g. frustration) in cattle and sheep (Sandem et al., 2002; Sandem and Braastad, 2005; Reefmann et al., 2009c; Proctor and Carder, 2015). Vocal expression of valenced affective states has also been identified in several species to enable the evaluation of stress levels (Scherer et al., 2003; Manteuffel et al., 2004; Schön et al., 2004; Burman et al., 2007; Düpjan et al., 2008; de Waal, 2011), for example, purring in cats and "chirping" by rats was observed in situations one would expect to entail positive affect (Panksepp and Burgdorf, 2000; Knutson et al., 2002). Other behaviours, such as play behaviour could also be useful for the study of emotion (Held and Špinka, 2011). As play behaviour is motivated by positive affect, a high level of performance could be tentatively used as a measure of welfare (Jensen and Kyhn, 2000; Svartberg, 2005; Trone et al., 2005). However, the sole consideration of behavioural measurements conveys an incomplete picture in the context of emotion, as some behaviours are characterised by ambiguity. For example, tail wagging in dogs is thought to reflect positive affect, but has also been observed in the context of withdrawal (Quaranta et al., 2007). In sheep, similar ambiguity was found regarding ear postures (Reefmann et al., 2009a; Boissy et al., 2011).

Physiological measurements are central to emotions as they play an important role in optimising the body state for different types of action. For example, immunological parameters, such as natural killer cell activity, salivary immunoglobulin-A concentrations and tumour necrosis factor in humans which were found to decrease in individuals in positive affective states (Pressman and Cohen, 2005). Neurotransmitters, such as dopamine, noradrenaline and serotonin, showed high relevance in experiencing and processing affective information (LeDoux, 1998; Damasio et al., 2000; Leknes and Tracey, 2008; Stracke et al., 2017). From stress research it is commonly known that neuroendocrine systems - the hypothalamic pituitary adrenal (HPA) axis and sympathetic adrenal medullary system – are involved in stress responses and emotional states like fear and anxiety (Paul et al., 2005; Gygax et al., 2013). The assessment of changes in the activity of the autonomic nervous system (ANS) is one promising physiological indicator in the context of emotional processing in different species (Désiré et al., 2004; von Borell et al., 2007; Reefmann et al., 2009a; Düpjan et al., 2011; Macefield et al., 2013; Zebunke et al., 2013; Coulon et al., 2015) and constitutes a key player in demonstrating how closely behavioural and physiological components of affect are connected with one another. For example, in the context of facial expression, the proportion of eye white is physiologically regulated by the activity of sympathetic postganglionic axons innervating the muscle of the upper eyelid resulting in a higher proportion of visible eye white in affective states such as frustration or fear (Sandem et al., 2002). Different ANS-related indicators of affective states have been evaluated in humans and animals, for example, alterations in pupil diameter (Bradley et al., 2008), skin conductance (Vetrugno et al., 2003), skin temperature (Erber et al., 2012), neuroendocrine activity (Kanitz et al., 2016), respiration rate (Briefer et al., 2015b), or cardiovascular parameters, such as heart rate (HR) and blood pressure (BP). The strong relationship between emotion and the autonomic nervous system has been appreciated throughout the contemporary study of animal behaviour and will be elucidated in more detail in the following section.

1.2.3. Affective states and the autonomic nervous system

It has been assumed that the nervous system provides the functional units for the bidirectional transduction of psychological and physiological processes and thus, it is possible to link psychological to neurophysiological processes and brain structures by measurement, for example, of autonomic activity. Autonomic responding in emotion has been an active research topic since Walter Cannon (1915) first studied the physiology of emotion (Brown and Fee, 2002). The two major subsystems, the sympathetic and parasympathetic systems, respectively, control fight/flight and rest/digest functions in a reciprocal fashion. As the parasympathetic supply to the heart runs in the vagal nerves (Aubert et al., 2003), I will use the term vagus as a synonym of the parasympathetic system in the context of cardiac activity in the following sections.

As emotional perceptions of different natures have been found to induce different shifts of autonomic balance, towards either a sympathetic or parasympathetic prevalence, it is hypothesised that these indicators can be used to draw conclusions about affective states and ongoing appraisal processes. But what exactly can the changes in autonomic activity tell us about emotion? In recent years, cardiac vagal tone as reflected in heart rate variability (HRV) has received considerable attention as a psychophysiological marker of emotion regulation (Porges, 1995a; Beauchaine, 2001; Thayer and Sternberg, 2006; Boissy et al., 2007b). According to Porges' concept of stress (1995a), the regulation of cardiac activity via the vagus tone is proposed as an index of homeostasis. Thus, conditions that disrupt homeostatic processes (i.e. stress) would result in depressed vagal tone. A high vagal tone is linked to a more efficient autonomic control, which ensures an increased sensitivity and reactivity of the organism to changes in the environment (Porges et al., 1996; Thayer et al., 1997; Friedman and Thayer, 1998). Animal and human psychology study of well-being has long been dominated by stress studies, focussing on emotional responses to negative stimuli. For example, in the context of fear, a negative stimulus (electric shock) was associated with tachycardia accompanied by vagal withdrawal in rats and mice (Randall et al., 1975; Stiedl et al., 2004). This physiological reaction was also reported in different species in reaction to several non-social stressors (Korte et al., 1998; Mohr et al., 2002; Visser et al., 2002; Langbein et al., 2004; Rietmann et al., 2004; Valance et al., 2007; Reefmann et al., 2009c) and also in social contexts, for example, during food competition or social isolation in pigs and social defeat in rats (Sgoifo et al., 1997; de Jong et al., 2000; Reimert et al., 2014). These situations are thought to be characterised by a rather negative valence. However, Boissy et al. (2007b) suggested that the cardiac vagal tone is also a potential indicator for positive emotions. This assumption is supported by studies demonstrating vagal activation in positive contexts, for example in humans watching an emotionally positive film (Matsunaga et al., 2009) or listening to pleasant music (Sokhadze, 2007) or experiencing self-induced appreciation (McCraty et al., 1995). Similar reactions were shown in farm animals, for example in sheep being voluntarily groomed (Reefmann et al., 2009c). Pigs were shown to exhibit a state of positive arousal associated with vagal activation when they were individually called to feeding in an operant conditioning paradigm (Zebunke et al., 2011). A consistent finding across these studies is that positive emotions significantly increase vagal tone, whereas the opposite occurs with negative emotions. Therefore, vagal tone is assumed to reflect the valence dimension of affect.

On the other hand, sympathetic activation has been found to play a major role in both physical (body movements) as well as psychological (e.g., mental load in solving mathematical tasks) challenges (Malliani et al., 1991; Porges, 1995b). A huge body of evidence in rats and mice describes reactions to negative cues with pronounced sympathetic activation and tachycardia (Stiedl et al., 2004). Increased sympathetic activation in humans is widely known to arise during “white-coat hypertension”, a phenomenon which appears in patients who are normotensive during everyday life but respond to the stress of a clinic visit with a substantial increase in their BP, thus making them appear hypertensive (Mancia et al., 1983b; Siegel et al., 1990; Parati and Mancia, 2006; Stergiou et al., 2014). Stressful mental situations, such as cognitive challenges were also shown to result in sympathetic activation (Kanemaru et al., 2001). In contrast, sympathetic activation has also been shown in positive contexts in dogs (Randall et al., 1985), non-human primates (Randall et al., 1975; Braesicke et al., 2005) and humans (Cacioppo et al., 1992; Warner and Strowman, 1995; Neumann and Waldstein, 2001). For example, the sight of highly motivating stimuli increased the BP response in marmosets depending on the integrity of the amygdala providing a convergence of behavioural, autonomic, and neural analyses of positive emotion in the primate (Braesicke et al., 2005). According to Tranel and Damasio (1994), increased activity in brain regions involved in emotional processing link pleasant and unpleasant stimuli to sympathetic activation. Lane et al. (1997) noted that both pleasant and unpleasant picture presentation in humans was associated to increased sympathetic activation, as indicated by a greater skin conductance response. Finally, neuroimaging evidence reveals a co-occurrence of activation in cortical and subcortical structures and increased sympathetic activation during pleasant and unpleasant emotional states (Damasio et al., 2000). Owing to these valence-independent effects, the view that changes of the sympathetic activity may reflect arousal (or intensity of affective stimulation) is emphasised.

Affective states, at least within normal ranges, are natural responses that develop during the course of life. These reactions vary with the individual’s personality, experiences and genetic background (Feldman, 1995; Schweiger et al., 1998; Russell, 2003; Hariri and Holmes, 2006; Thayer and Lane, 2009). Therefore, affective states may underlie influencing factors, for example, the specific way how an organism expresses its emotions can be different depending on the individual perception of personally relevant interplay with the environment including not only challenges and threats but also their ability to respond to or cope with them (Thayer and Lane, 2000; Vigil, 2009). As such, affective states serve as an integrative index of an individual’s adjustment to the constantly changing environmental demands he/she/it faces. Among humans and animals there has been evidence that the individual capacity to cope with challenges is characterised by specific behavioural and physiological responses to external stimuli (for review see Koolhaas et al., 1999), which is directly related to individual fitness/survival in natural populations and to animal welfare in production animals. This individual variation in behaviour and physiology may, in turn, be influenced by ongoing appraisal processes. In the following section I will refer to these individual reaction patterns and how they relate to behavioural, physiological and affective differences.

1.2.4. *Affective states and coping styles*

During the last decades a wide variety of scientific disciplines has shifted its interest towards the causes and consequences of individual variation. Ecologists and evolutionary biologists aim at understanding the ecological function of individual variation in behaviour and its consequences for evolutionary fitness (Réale et al., 2007; Wolf et al., 2008). In studies of humans and animals, individual traits, which may be categorized in distinct phenotypes based on consistency over time and/or situations, are used to describe personality (see Finkemeier et al., 2018 for review, Réale et al., 2007). Several terms are used for this phenomenon, for example, behavioural syndrome (Sih et al., 2004; Dingemanse et al., 2012), behavioural profile (Groothuis and Carere, 2005) or temperament (Rothbart et al., 2000; Graunke et al., 2013; Brand et al., 2015).

A special approach in the personality concept that plays an essential role in the context of animal welfare is 'coping' (Koolhaas et al., 1999), which is based on the animal's reaction to its environment with respect to reducing the effect of aversive stimuli (Cannon, 1929; Korte et al., 2005). From human research, Lazarus (1993) has emphasised coping as a key concept for research on adaptation and health. Because coping is a behavioural reaction to aversive situations accompanied by several physiological stress reactions (Wechsler, 1995), individual ways of dealing with stress have an enormous impact on health and thus, welfare. Like in humans, animals show individual differences in coping with different environmental changes, confirming that personality and coping style are closely linked (Koolhaas et al., 1999; Korte et al., 2005). From stress research in humans and animals, Henry and Stephens (1977) distinguish generally two fundamental coping patterns (styles): a proactive (or active) pattern and a reactive (or passive) pattern. On the level of behaviour, the proactive reaction pattern is characterised by a typical active fight-flight response (Cannon, 1915) and associated with territorial control, higher aggression towards conspecifics, boldness, and risk-taking behaviour. Proactive individuals adapt more active behaviours to escape or eliminate stressors, act on the basis of previous experience and tend to develop routines (Koolhaas et al., 1999). Conversely, the reactive coping individual shows a conservation withdrawal response in aversive situations (Engel and Schmale, 1972) which is characterised by immobility, low levels of aggression, and shyness. Reactive individuals display more passive and cautious behaviours when faced with stressors and act on the basis of actual environmental information (Verbeek et al., 1996; Carere et al., 2010; Coppens et al., 2010; Koolhaas et al., 2010).

Further studies supported the hypothesis that the two types of behaviour patterns can be considered to represent distinct coping styles as they both aim at environmental control contributing to the individual fitness (for review see Benus et al., 1991; Koolhaas et al., 1999). Both styles have evolutionary advantages, especially in the context of different environmental conditions. In a stable, predictable environment, the proactive style is more beneficial as proactive individuals easily develop routines, show decreased flexibility and risk-taking behaviour results in success (Van Oortmerssen, 1989). Furthermore, a strong relationship to aggressive behaviour was found in some species, especially rats (Benus et al., 1990). Reactive copers are more adapted to changing environmental conditions, as they are characterised by increased flexibility, high attention towards external cues and avoidance of any risks (Bohus et al., 1987; Sluyter et al., 1996). In addition, they act more pro-social which facilitates the connection to a new group (Schürch et al., 2010; Aplin et al., 2013; Finkemeier et al., 2018). These findings also play a significant role for livestock farming, and applied ethologists have found interest in the concept of these individual reaction patterns (Boissy, 1995; Forkman et al., 1995), especially in their practical implementations in the context of animal housing, management and welfare.

Besides behavioural implications, external stimuli can also affect the organism on a physiological, neuroendocrine and psychological level. According to Koolhaas et al. (1999), proactively coping rodents have been characterised by low HPA-axis reactivity (low plasma corticosterone response) in aversive situations, high sympathetic reactivity in terms of high levels of catecholamines, increased cardiovascular activity (HR and BP) and low parasympathetic reactivity measured by decreased HRV (Sgoifo et al., 1997, 2005; Koolhaas et al., 2007). In contrast, reactive coping rodents show higher HPA-axis reactivity and higher parasympathetic reactivity (for review see Koolhaas et al., 1999). Also in pigs, this differential autonomic response of the two coping styles in different social and non-social situations was found to be correlated to the behaviour in the so-called backtest (Hessing et al., 1994b, 1994a; Ruis et al., 2000), a method that was developed to detect different coping styles in domestic pigs at young age. The mechanisms underlying these findings are not completely understood yet, but in several species the differences between the two coping styles in autonomic balance were supported. Since both subsystems of the ANS affect an organism's reactions to external challenges differently it seems reasonable to assume that shifts in ANS activity are related to individual coping characteristics. According to Porges et al. (1994) different behavioural reactivity patterns in infants and children are mediated by differences in vagal activity. Because of the role of the parasympathetic and sympathetic regulation of cardiovascular control in the context of affective states (see Chapter 1.2.3), one may expect a differential situational appraisal as well, which may underlie the individual behavioural response pattern to external stressors. According to the approach of Koolhaas and van Reenen (2016), the relationship between coping characteristics/personality and welfare can be described by a three-dimensional model, including coping style, emotionality and sociability as independent factors which are defined as being stable over time and across contexts within the individual. Emotionality in this context is strongly associated with fearfulness in the way that fearful animals are highly emotionally aroused by a challenging situation and non-fearful animals do not show any enhanced biological responses indicating that they do not perceive the same situation as stressful or alarming (Koolhaas and Van Reenen, 2016). The coping style seems to reflect the type of response an animal makes (i.e., how an animal reacts) and emotionality indicates the level of responsiveness to challenge (i.e., how strongly an individual reacts).

Affective states and coping are both significant components of animal welfare (Broom and Johnson, 1993) and it is important to understand the mechanisms and factors underlying the individual's capacity to cope with environmental challenges. To date, it is unknown to what extent coping styles modify the individual's autonomic balance and if the different coping styles also differ in their situational appraisal, contributing to their general affective state and thus, welfare.

1.3 Neurophysiological processing of affect

The diversity of approaches and contributing factors to the subject of emotions shows at the same time its complexity and the need for a holistic view. In this thesis, a neurophysiological approach was used to draw conclusions about affective states based on the intimate connection between the brain and the heart which was enunciated by Claude Bernard over 150 years ago (Bernard, 1867). There has been evidence that emotions can induce shifts of autonomic balance, towards either a sympathetic, or parasympathetic prevalence that modulate cardiovascular activity providing information about ongoing emotional states and appraisal processes (Hagemann et al., 2003; Boissy et al., 2007b; Thayer and Lane, 2009; Kreibig, 2010; Lackner et al., 2014). In the next sections, I want to give an overview of the neurophysiological processes underlying affective states on a more proximate, mechanistic level, describing the cortical control of autonomic function which is influencing cardiovascular regulation.

1.3.1. Cortical control of autonomic function

At a neurophysiological level of analysis of emotions, much current research seeks to elucidate the neural networks that underlie emotion (Hagemann et al., 2003). Pioneering work by Walter Cannon (1927), James Papez (1937), and Paul D. MacLean (1952) identified brain regions that are involved in emotional processing, for example, the limbic system, a collection of cortical and subcortical structures, which include the amygdala, hypothalamus, cingulate cortex, hippocampus and other structures (Damasio, 1994, 1999; Dalgleish, 2004; Lindquist et al., 2016). Results of numerous investigations suggest the involvement of both limbic structures and non-limbic regions, such as the ventral striatum (especially nucleus accumbens), basal ganglia, orbitofrontal cortex, and the ventromedial prefrontal cortex across a variety of positive and negative emotions (Borod, 2000; Lane and Nadel, 2000; Thayer et al., 2009; Nikolin et al., 2017).

In humans, psychophysiological research has identified patterns of autonomic nervous system correlates of emotion (Ekman et al., 1983; Sinha et al., 1992; Kreibig, 2010; Norman et al., 2014). Complex models of association between emotion-related central and autonomic response patterns have been proposed and many of these models focus on cortical and subcortical interconnections. A broad review of the literature gives an impression of the multi-modal and reciprocal connections between emotion-related brain structures and the complexity of neural processes during emotion-mediated ANS response (for a review see Hagemann et al., 2003). A number of researchers have identified functional units within the central nervous system through which the brain controls visceromotor, neuroendocrine, and behavioural responses. According to the neurovisceral integration model a network of neural structures generate, receive, and integrate internal and external information in the service of goal-directed behaviour and organism adaptability (Thayer and Lane, 2000; Thayer and Sternberg, 2006). One important functional unit is the central autonomic network (CAN), a network of interacting cortical, subcortical, and brainstem structures that integrates autonomic cardiovascular responses with changing external demands (Cechetto and Saper, 1990; Benarroch, 1997; Saper, 2002; Cersosimo and Benarroch, 2013; Harrison et al., 2013; Cechetto, 2014). Structurally, the CAN includes the anterior cingulate, insular and ventromedial prefrontal cortices, the central nucleus of the amygdala, several nuclei of the hypothalamus, the periaquaductal gray matter, and several other brain structures (for details about the CAN, see Benarroch, 1997; Thayer and Lane, 2000).

Sensory stimuli are relayed via the thalamus to the orbitofrontal cortex which is a part of the prefrontal cortex and involved in cognitive processing of decision-making. It receives information from all the sensory modalities, including gustatory, olfactory, auditory, visual, somatosensory, and also visceral information (Rolls, 1998, 1999). Strong reciprocal connections between the amygdala and the orbitofrontal cortex, which is necessary for a flexible, experience- or context-dependent representation of a stimulus' value, form a functional circuit by linking sensory information to a representation of how the stimulus affects the animal's somatovisceral state in order to establish the value of the eliciting stimulus (Barbas et al., 2003; Kringelbach and Rolls, 2004; Barrett et al., 2007). Several investigations have suggested that the amygdala is involved in estimating the biological relevance of the stimulus or situation as one of the first steps in subcortical stimulus evaluation in humans and animals (LeDoux, 1996; Sander et al., 2003; Phelps and LeDoux, 2005; Posner et al., 2005). Reciprocal connections between the prefrontal cortex, the anterior cingulate cortex, which appears to play a role in a wider variety of autonomic function, but is also involved in certain higher-level functions that are correlated with conscious experience such as attention, reward anticipation, impulse control, and emotion (Lane et al., 1998; Jackson et al., 2006) and the amygdala, which plays a primary role in the formation of memories associated with emotional events (fear conditioning, appetitive conditioning) modulate the visceromotor (autonomic, chemical, and behavioural) responses. This circuity projects directly and indirectly (via ventral striatum) to hypothalamus and brainstem nuclei, in particular the formation reticularis of the medulla oblongata (medullar circulation centre), which ultimately regulate sympathetic and parasympathetic outflow to the body (Barrett et al., 2007). The resulting changes in the animal's somatovisceral state translate information about the external world into an internal affective code (Damasio, 1994, 1999). So, the relative activity of these two subsystems of the ANS is an indicator of an organisms' general physiological state (action or digestion), which depends on homeostasis, but is also determined to a great degree by physical, environmental and mental factors, such as current emotions.

1.3.2. Autonomic regulation of cardiovascular function

The ANS represents the principal regulatory route of the internal environment of the body to maintain homeostasis (Porges, 1995a; Critchley, 2005). The two subsystems, the parasympathetic and sympathetic system, represent neural control systems that originate in the brainstem and contribute to the regulation of a variety of target organs. Their interaction enables dynamic modification of bodily state in response to environmental challenges. The parasympathetic system promotes anabolic activities concerned with the restoration and conservation of bodily energy, whereas the sympathetic nervous system prepares the individual for action and increases metabolic output (Porges, 1995a). Both systems are reciprocally activated (an increase in one system results in a decrease in the other) and the effects are antagonistic. Although this reciprocal model may represent one common mode of ANS regulation, the empirical literature has shown that other modes of collaboration exist between the two ANS systems. Berntson et al. (1991, 1993) introduced a two-dimensional model with orthogonal sympathetic and parasympathetic axes. Autonomic responses may be either coupled or uncoupled. Moreover, coupled responses may be reciprocal or nonreciprocal, the latter entailing concurrent increases (coactivation, e.g. sexual arousal) or decreases (coinhibition, e.g. anesthesia). In other words, the two subsystems can either act synchronously or independently in autonomic control over dually innervated target organs (Berntson et al., 1994; Schweiger et al., 1998).

Heart

The output of the CAN has connections to the sinoatrial node of the heart via the stellate ganglia and the vagus nerve (Benarroch, 1997). Both left and right vagus nerves stimulate the sinoatrial node, the atrioventricular node, as well as the atrium muscle and ventricle muscle. Sympathetic fibres innervate almost all centres of the heart including the atrioventricular node, heterotrophic centres, atrium, and ventricle myocardium (von Borell et al., 2007). In healthy systems both branches of the autonomic nervous system are tonically active when regulating cardiac activity. Parasympathetic tone decreases HR and cardiac contractility, whereas activity of the sympathetic branch opposes these effects at the heart level and also regulates peripheral vasoconstriction (Thayer and Lane, 2009; Robles-Cabrera et al., 2014).

Average HR is often used as an index of emotional reactivity in many vertebrate species including humans (Baldock and Sibly, 1990; Marchant et al., 1995; Cabanac and Cabanac, 2000; Cabanac and Guillemette, 2001; Loijens et al., 2002; Brosschot and Thayer, 2003). The complex interaction between the two parts of the ANS on cardiac function produces irregular time intervals between consecutive heart beats (Cerutti, 1995; Boissy et al., 2007b; von Borell et al., 2007) that function to maintain cardiovascular homeostasis within a defined range (Akselrod, 1995) and to arrange responses to internal and external challenges (Cerutti, 1995). In a healthy individual the role of the ANS in the beat-to-beat adjustment of hemodynamic parameters is essential to adequate cardiovascular functioning. Rhythmic oscillations originating from the parasympathetic part exhibit higher frequency than those of the sympathetic part. Rapid changes in HR are caused by decrease in vagal tone (Eckberg, 1991; Hainsworth, 1995), as the sinoatrial node responds to stimulation of the vagus almost immediately within one to two heartbeats. Upon completion of the vagal stimulation, the HR returns to its original value in less than five seconds (Hainsworth, 1995). On the other hand, sympathetic stimulation evokes a much slower cardiac reaction, with a delay of up to five seconds before the heart is affected and the maximal progressive response after 20 to 30 seconds (Hainsworth, 1995; Malliani, 1995). Mean HR, at any point in time in healthy individuals, represents the complex net interactions between vagal and sympathetic regulation. On account of this complexity, mean HR is of limited use for accurately assessing direct sympathovagal regulation. Indeed, the variability of the time intervals between consecutive heart beats (HRV) is a widely accepted means of autonomic assessment of a dual-innervated organ, providing better information on autonomic modulation of the sinoatrial node (Malik et al., 1996; Friedman and Thayer, 1998) due to the non-additive activity originating from the individual branches of the ANS. The amount of variability is representative of the type of autonomic control which is dominating cardiac activity; for instance, the greater the variability the higher the vagal control of cardiac function (for review see von Borell et al., 2007). Hence, it is important to emphasise that some parameters of HRV provide information about the combined activity of both branches of the ANS or about vagal activation alone, but there does not appear to be any valid HRV index that adequately reflects sympathetic modulation or activity (Michael et al., 2017), although this finding is not without debate (see section 1.3.3.).

Vasculature

Given that systemic vasculature receives no direct parasympathetic innervation, unlike the heart, an assessment of BP allows to examine (presumably) sympathetic function exclusively (Brown et al., 2012; Delgado et al., 2014). This is one possible approach to augment information about autonomic regulation in the context of affective states. With increased sympathetic activity, norepinephrine is released from sympathetic postganglionic terminals (increasing HR and cardiac contractility, as mentioned above) and acts on the smooth muscles of the arterioles to increase the tone of the peripheral vessels (Purves et al., 2001). In this way, the sympathetic nervous system is able to regulate vasomotor tone to direct blood flow in the vessels. Blood is drained from the intestinal reservoir to those organs where oxygen and metabolites are urgently needed to maintain function if challenged, for example, the brain, lungs, heart, and skeletal muscles (Porges, 1995b). Similar to HR, BP is not constant, but fluctuates around mean values (de Boer et al., 1985). The occurrence of these fluctuations, expressed as blood pressure variability (BPV) over time, represent a rich source of information that can provide considerable insight into the mechanisms of cardiovascular control and emerge as useful index of autonomic modulation of cardiovascular function (Akselrod et al., 1981; Appel et al., 1989; Parati et al., 1992, 1995; Julien et al., 2001; Langager et al., 2007; Joyner et al., 2008; Stevens et al., 2016). In animal studies, BPV has received far less attention than HRV. Studies with human participants or laboratory animals in medical research focus mainly on the relationship between autonomic functioning and diseases such as Parkinson's disease (Brown et al., 2012), schizophrenia (Bär et al., 2006), impaired renal function (Diaz et al., 2012), hypertension (Laughlin et al., 1980; Mussalo et al., 2003), congestive heart failure (Radaelli et al., 1999), target organ damage (Parati and Lantelme, 2002), or mental stress (Hjortskov et al., 2004). BP fluctuations were demonstrated to deliver information about sympathetic control, as Hedman and colleagues (1992) found that blocking sympathetic nerve transmission in dogs decreased all components of BPV, whereas vagotomy decreased HRV but did not influence BPV. This finding was supported by studies showing a close connection between changes in short-term BPV, mean pressure levels, and sympathetic activation (Laitinen et al., 1999; Janssen et al., 2000; Van Vliet and Chafe, 2000).

1.3.3. Analysis of cardiovascular parameters

A suitable tool for the analysis of cardiac activity is the use of an electrocardiogram (ECG), which shows the plot of the bio-potential generated by the activity of the heart and is primarily used by physicians to predict and treat various cardiovascular diseases. Starting with pacemaker cells in the sinoatrial node, a progression of depolarization spreads out to the different parts of the heart, through atrium, atrioventricular node down to the bundle of His and into the Purkinje fibres and through the ventricles. The electrical changes that arise from the heart muscle's electrophysiological pattern of depolarizing and repolarizing during each heartbeat are measured as dipoles (potential differences) and mapped as the sum of all individual vectors in a curve (Harmeyer, 2010). Hence, the different waves in the ECG represent certain phases of the heart muscle's electrophysiological pattern during each heartbeat. It is widely accepted in the literature (Katz, 2010; Lilly, 2012; Subramanian, 2017), that one cardiac cycle is characterised by three main components: the P-wave (reflecting depolarization of the atria), the QRS-complex (reflecting ventricular depolarization and contraction) and the T-wave (reflecting rapid repolarization of the ventricles), which are exemplarily illustrated in Figure 1. Mean HR is measured by the number of contractions (beats) of the heart per minute (bpm). Concerning HRV analysis, cardiac interbeat interval (IBI) data (the time between two successive intervals) play the major role and can be derived from RR intervals (time between two successive R-peaks) in an ECG. Time and frequency domain (spectral) analyses are two approaches that can be used to determine overall autonomic activity from cardiac IBI data (for review see Malik et al., 1996, but also

Kleiger et al., 1992, 2005; Salo et al., 2001; von Borell et al., 2007; Goldstein et al., 2011; Shaffer et al., 2014). The time domain measures represent statistical calculations of RR intervals, or numerical estimation of the geometrical shape of the distribution of RR intervals. The SDNN (the standard deviation of all RR intervals of the data set) reflects all the cyclic components responsible for variability in the period of recording and provides information about the integrated contribution of the parasympathetic and the sympathetic branches of the autonomic nervous system to the control of the heart (Kautzner et al., 1995; Malik et al., 1996). The RMSSD (the square root of the mean of the sum of the squares of differences between successive RR intervals) is used to estimate the short-term components of the heart; thus, it equates high frequency beat-to-beat variations. The parasympathetic branch of the ANS is the major input to this high-frequency component (HF). RMSSD therefore is regarded as an indicator for cardiac vagal control (Hainsworth, 1995; Sgoifo et al., 1997; Aubert et al., 2003; von Borell et al., 2007; DeGiorgio et al., 2010; Bär et al., 2016; Krejčí et al., 2018). The ratio of these two measures (RMSSD/SDNN) is thought to reflect general changes of the vago-sympathetic balance of the organism (Langbein et al., 2004; Zebunke et al., 2011, 2013). Spectral analysis by Fast Fourier transformation (FFT) can be used to 'decompose' a waveform into its sine and cosine constituents to assign the power in different bands to different underlying physiological functions. The spectrum is usually divided into species-specific regions of power: high frequency (HF) band, centred at the respiratory frequency (0.15–0.4 Hz in humans), the low frequency (LF) band, related to the baroreflex (0.04–0.15 Hz) and the very low frequency (VLF) band mainly associated with thermoregulation (≤0.04 Hz)(von Borell et al., 2007). The HF component of the spectral analyses, which is reflected in respiratory sinus arrythmia, indicates the vagal input of the heart and correlates strongly with RMSSD (Akselrod, 1995; Malik et al., 1996; Sevcencu and Struijk, 2010; Uusitalo et al., 2011; Esco and Flatt, 2014; Krejčí et al., 2018). The LF component is associated with vagal and sympathetic contributions and the ratio of LF:HF is generally used as a measure of sympathovagal balance. The relationship between HRV parameters and the autonomic input to the heart was validated previously (Hull et al., 1990; Kleiger et al., 1991; Stein et al., 1994; Després et al., 2002), but there is no doubt that HRV has been the subject of much interpretation and debate. Recently, the SDNN:RMSSD ratio has been suggested as an appropriate surrogate to the spectral LF:HF ratio in representing sympathovagal balance (Sollers et al., 2007; Wang and Huang, 2012; Esco et al., 2018; Krejčí et al., 2018) although controversy exists regarding the underlying physiological mechanisms of LF:HF (Billman, 2013; Medeiros et al., 2018). Especially the quantification of the sympathetic contribution to HRV remains controversial (Malik et al., 1996). Although some authors favour the LF component in the power spectrum and the SDNN from time domain analysis as useful parameters for sympathetic activity (Malliani et al., 1991, 1994; Kamath and Fallen, 1995; Malliani, 1995), other authors emphasise that these parameters are also affected by vagal tone (Després et al., 2002; Brown et al., 2012; Billman, 2013; Esco et al., 2018), whereas it has also been suggested that LF power should be seen as an index of baroreflex function (Goldstein et al., 2011).

Vascular fluctuations can be studied through beat-to-beat BP monitoring and calculation of the variance or standard deviation of their average value. In several studies, the standard deviations of systolic (SDS), diastolic (SDD) and mean arterial pressure (SDM) are used to quantify the amplitude of fluctuations (Cowley et al., 1973; Littler et al., 1978; Mancia et al., 1986; Parati et al., 1995; Van Vliet et al., 2000; Rivera et al., 2016). Similar to HRV, frequency domain analysis has also been used to subdivide the variability of BP into different frequency components and to quantify the variance or "power" at each specific frequency.

A wide variety of algorithms and models have been proposed in this context to study spontaneous cardiovascular variability in humans (de Boer et al., 1985; Pagani et al., 1986; Delgado et al., 2014), dogs (Pagani et al., 1986), and rats (Rubini et al., 1993; Mager et al., 2006). However, the optimal methods for extracting such information and the most appropriate interpretations of the results obtained are matters of considerable debate (Omboni et al., 1996). For example, several authors emphasise that low frequency BP fluctuations, the so-called Mayer waves, are associated with sympathetic modulation of vascular tone (Cerutti et al., 1991; Julien et al., 2001; Mager et al., 2006), whereas others found that the LF component of BP does not appear to be a suitable marker for the prevailing sympathetic nerve activity (Arai et al., 1989; Stauss et al., 1995).

Despite this sometimes controversial interpretation of BP and HR spectra, the analysis of HRV and BPV by the time domain technique and the spectral approach may represent a useful tool for the study of the mechanisms involved in cardiovascular regulation in the context of ANS activity. In the next section, I will refer to different appropriate methodologies for the assessment of cardiovascular parameters which find application in free-moving animals including strengths and weaknesses of the different systems.

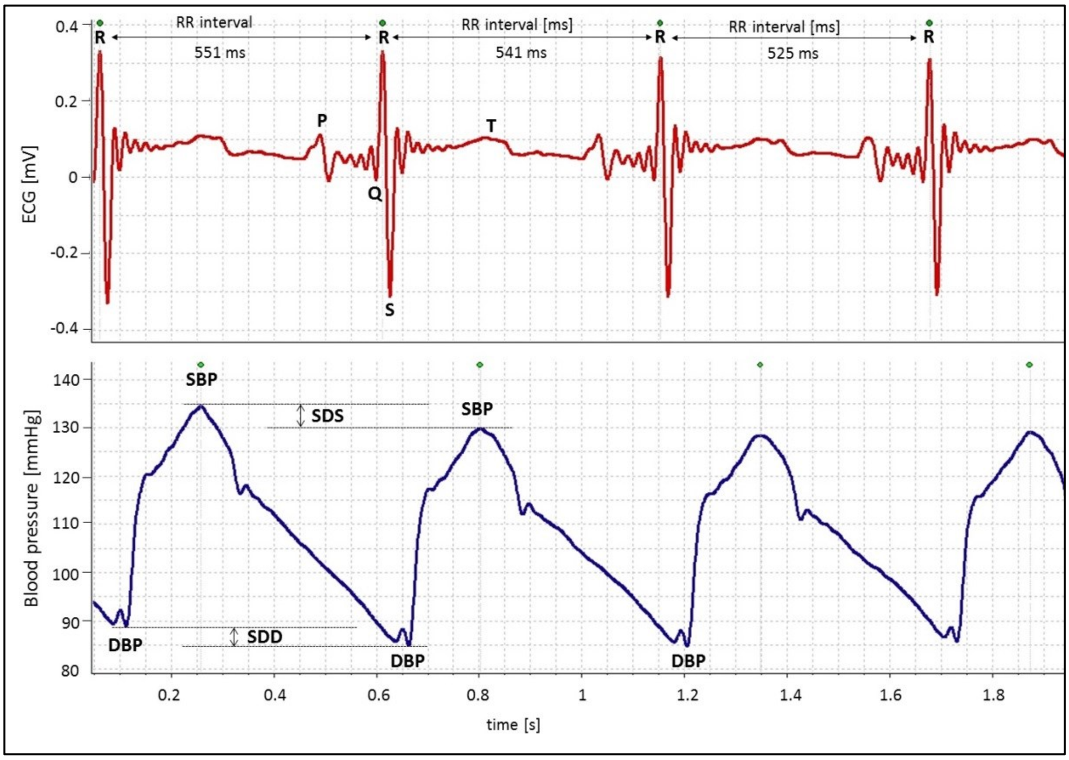

Figure 1. Exemplary electrocardiogram (ECG in mV) and arterial blood pressure curve (in mmHg) of a domestic pig during idling behaviour in the home pen (data assessed in the context of study 3). Abbreviations: systolic blood pressure (SBP), diastolic blood pressure (DBP), standard deviation of systolic (SDS) and diastolic (SDD) blood pressure. P-wave, QRS-complex and T-wave were identified according to the software (LabChart, ADInstruments). Green points indicate correctly triggered R-peaks (ECG) and systolic pressure maxima (blood pressure).

1.4 Assessing autonomic activity

1.4.1. Non-invasive methods for measuring autonomic activity

One of the most promising non-invasive diagnostic methods increasingly used in humans to assess cardiac activity in the context of physical and mental stress or cardiovascular diseases is the analysis of HRV (Malik and Camm, 1990; Acharya et al., 2007; Ernst, 2014). In animals, HRV has been studied to investigate changes in sympathovagal balance related to several aspects, for example pathological conditions (Little et al., 1996; Pomfrett et al., 2004), training regimes (Thayer et al., 1997), behavioural disorders (Bachmann et al., 2003), management practices (Langbein et al., 2004; Hagen et al., 2005), stressors (de Jong et al., 2000; Mohr et al., 2002), and also cognitive appraisal and emotions (Reefmann et al., 2009c; Zebunke et al., 2011) and is progressively emerging as a suitable indicator of welfare states in farm animal research including studies investigating affective states in animals. Different species such as cats (Raetz et al., 1991), dogs (Pagani et al., 1986; Jonckheer-Sheehy et al., 2012), sheep (Zugaib et al., 1980; Reefmann et al., 2009b, 2009c), dwarf goats (Langbein et al., 2004; Gygax et al., 2013), pigs (Zwiener et al., 1996a; de Jong et al., 2000; Geverink et al., 2002; Marchant-Forde et al., 2004; Zebunke et al., 2011; Tallet et al., 2014), horses (Kuwahara et al., 1996; Visser et al., 2002; Erber et al., 2012; von Lewinski et al., 2013), rats (Troncoso et al., 1995), rabbits (Zwiener et al., 1996b), and even crabs (Gribble and Broom, 1996) have been investigated. The most commonly chosen approach to measure cardiac activity is the use of non-invasive portable equipment for storing ECGs provided with specific algorithms for the detection of IBIs and analysis of HRV. In farm animals, a digital human heart rate monitor (Polar® Electro Öy, Finland) that records cardiac activity and detects IBIs at a sampling rate of 1000 Hz has been widely applied in veterinary and behavioural research and has been validated for the use in cows (Hopster and Blokhuis, 1994) and pigs (Marchant-Forde et al., 2004). This device uses a belt containing two coated electrodes and fits around the thorax of the animal. IBI data are detected during recording, transmitted wirelessly and stored in an external data logger. A general concern with cardiac monitors, such as Polar, that only record IBI data is identifying true errors in the data. As the ECG itself is not recorded, there is no possibility to control for errors and artefacts or to identify ectopic beats beyond the application of the company's correction algorithms (Marchant-Forde et al., 2004; Norman et al., 2005; von Borell et al., 2007).

Besides the Polar system, various mobile systems have also been developed to record not only IBI data but also ECG in unrestrained animals using external electrodes that detect electrical changes at the skin reflecting the characteristic ECG tracing. Recently, a new biological data logger and telemetry system, capable of assessing this continuous ECG trace from electrical differences on the body surface found application in farm animal science, at least in goats (Briefer et al., 2015a, 2015b; Baciadonna et al., 2016: BioHarness™ Telemetry System, Zephyr Technology Corporation, Annapolis, MD, U.S.A.). Other examples are Televet 100 (Engel Engineering Services GmbH, Offenbach am Main, Germany, Jonckheer-Sheehy et al., 2012) and Holter monitors (DelMar Reynolds GmbH, Alpnach Dorf, Switzerland and Spacelabs Healthcare, Snoqualmie, Washington). These systems found broad interest in veterinary and experimental practice in different species (for overview see Scheer et al., 2010, dogs: Kusakabe et al., 1990, monkeys: Vogel et al., 1991, miniature swine: Suzuki et al., 1998, sheep: Reefmann et al., 2009b, cow: Hagen et al., 2005). The recorder is attached to the animal using external jackets and transmits the data via radio telemetry or Bluetooth to a receiver unit. The jackets also serve as protection of the cables in different free-ranging animals (Kusakabe et al., 1990; Suzuki et al., 1998; Kuwahara et al., 1999; Hassimoto and Harada, 2003). Different types of clips (Eckenfels and Schuler, 1988), self-adhesive electrodes (Suzuki et al., 1998), or subcutaneous needles (Nahas et al., 2002) were used to derive the ECG from the body surface. Limitations of these measurements comprise the external equipment that requires handling prior to each data scoring and the limited use in complex experimental designs, for example, during social interactions. The external cables and restraining belts or jackets may lead to stress-induced tachycardia resulting in biased results. However, these systems offer a solution for non-invasive ECG recordings to calculate HR and HRV for evaluating vagal activity, but they are not suitable for allowing unambiguous statements about sympathetic regulation as mentioned above (section 1.3.3).

For the measurement of the arterial BP, indirect (Doppler flow detection, oscillometry, photo-plethysmography), and direct (pressure catheter in the vessel) methods are available (Binns et al., 1995; Kramer et al., 2000; France et al., 2018). For most indirect methods, the animals are equipped with a cuff (upper arm, forearm or tail root) which initially prevents blood flow in the underlying vessel. Subsequently, the arterial BP is calculated from the resuming blood flow with decreasing pressure of the cuff (via ultrasound frequency or oscillating vessel wall). In direct measurements, catheters are inserted into the vessel and connected to a device outside the body. For chronic experiments, this measurement device is placed outside the cage/home pen which allows the animals only to move in a restricted place. The emitted cables may become a major cause of stress and portal of entry for infections. As another option, it is possible to connect the catheter to a telemetric transmitter unit which is attached to the back of the animal transmitting the signals to a remote receiver (Bulpitt et al., 1970; Vatner and Patrick, 1974; Armentano et al., 1990) However, weaknesses of this approach comprise potential risk for infection by leaking cables and additional stress by carrying the device on the back which reduces mobility of the experimental animals. One way to avoid these limitations is the use of a catheter that is connected to a chronically (subcutaneously, intramuscularly or intraperitoneally) implanted telemetric device transmitting the recorded intra-arterial BP data wirelessly to an external receiver unit.

1.4.2. Implantable methods for measuring autonomic activity

Advances in technology have resulted in the introduction of implantable, telemetric systems capable of the assessment of autonomic activity via the simultaneous recording of ECG and intra-arterial BP data in free-moving animals. Technologies from different manufacturers are commercially available, for example Telemetry Research (Millar Instruments Inc. (Houston, Texas, USA) and Telemetry Research Ltd. (Auckland, New Zealand) Merge), Data Science International (DSI: St. Paul, MN, USA) and Konigsberg Instruments Inc. (Pasadena, CA, USA). In the last two decades a number of studies have used telemetric systems in the context of pharmacological methods and cardiovascular diseases (Gelzer and Ball, 1997; Gross et al., 2002), circadian rhythms (Li et al., 1999; Janssen et al., 2000; Van Vliet et al., 2003), and stress (Maslova et al., 2002; Farah et al., 2004) in various species, such as mice (Van Vliet et al., 2000; Butz and Davisson, 2001), rats (Sgoifo et al., 1997; Beig et al., 2007), monkeys (Hassimoto and Harada, 2003; Chaves et al., 2006), dogs (Soloviev et al., 2006; Ollerstam et al., 2007; Cools et al., 2011), poultry (Korte et al., 1998), guinea pigs (Akita et al., 2002), goats (Hydbring et al., 1999), but also in rabbits (van den Buuse and Malpas, 1997), miniature pigs (Kuwahara et al., 1999; Stubhan et al., 2008), and domestic pigs (de Jong et al., 1998; Poletto et al., 2011).

The telemetric system used in my studies (Telemetry Research and AD Instruments) basically consisted of following components, which are exemplarily shown in Figure 2: (1) an implantable telemetric device, including sensors for measuring cardiovascular parameters (two biopotential ECG leads and a pressure catheter, temperature sensor), (2) individual receivers, (3) data acquisition devices (Power Lab), and (4) analysis software (LabChart). The implanted device transmitted digital signals via telemetry from within the animal to a receiver unit using an individual frequency. A switch at the receiver enabled the transmitter to be turned on and off in vivo without affecting the animal. The receivers reconstructed an analogue signal and were wire connected to two digital data acquisition systems (PowerLab 16/35 and 4/35, AD Instruments) that allowed the simultaneous real-time recording of up to six pigs at a sampling rate of 2 kHz. Animals can be co-housed with simultaneous monitoring in each animal with a signal transmission range of 5 m.

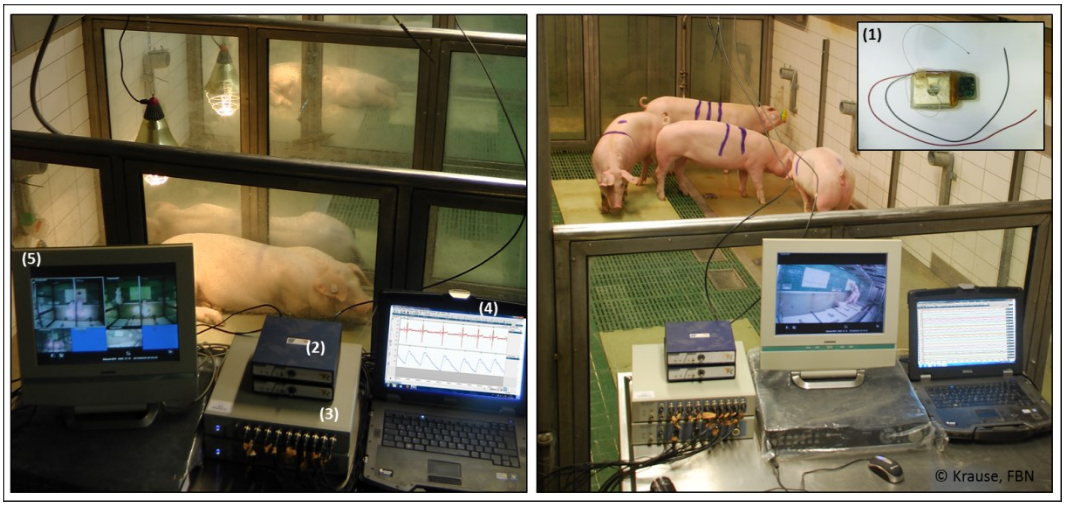

Figure 2. Exemplary setup of the telemetric system (Telemetry Research and AD Instruments) with the according components: (1) implantable telemetric device; (2) individual receivers; (3) data acquisition devices; (4) analysis software. (5) Monitor with video observations using Everfocus. Animals can be housed individually (left side) or in groups (right side). The photographs originate from animals used in study 3.

Electrocardiogram setup

As described above, numerous non-invasive approaches were used to measure the electrical activity deriving from the body surface, however, pigs were found to show highly variable ECG pattern with a strong inter- and intra-individual deviation in shape and polarity (Dukes and Szabuniewicz, 1969). Poletto and colleagues (2011) implanted ECG leads directly into the myocardium of domestic pigs in the context of pharmacological blockade using a DSI telemetric system. De Jong and colleagues (1998) positioned ECG electrodes caudal and cranial to the thorax, whereas Kano et al. (2005) implanted the negative electrode subcutaneously at a right outer site covering the second and third intercostal region and the positive electrode at a site above the *manubrium sterni* in minipigs. Both latter invasive setups resemble the M-X lead in external ECG measurements in minipigs (Suzuki et al., 1998) and in dogs (Kusakabe et al., 1990). Suzuki et al. (1998) tested another external electrode configuration by attaching the negative electrode on *processus spinosus* of the a*xis vertebrata*, and the positive electrode on the *processus xiphoideus* (A-B). This setup verifies the line extending from the long axis of the heart passing through both the apex and the base. According to Suzuki et al. (1998) this electrode positioning shows larger and more obvious QRS-complexes and less baseline drift compared to other tested lead positions. To date, this approach has not been transferred to invasive ECG measurements in free-moving domestic pigs.

Blood pressure setup

Telemetric methods for determining arterial BP have been evaluated in rodents (Bazil et al., 1993; Brockway and Hassler, 1993; Van Vliet et al., 2000) and dogs (Armentano et al., 1990; Truett and West, 1995) presenting an accurate, reliable method of measurement. The most common implantation method for BP recordings involves the use of the abdominal aorta for catheter placement by attaching the transmitter unit to abdominal muscles in the peritoneal cavity (for review see Van Vliet et al., 2000, guinea pigs: Depasquale et al., 1994, mouse: Janssen et al., 2000). Several studies have also reported other access sites, such as the left ventricle (rat: Sato et al., 1995), pulmonary artery (rats: Hess et al., 1996), or carotid artery (mice: Carlson and Wyss, 2000; Farah et al., 2004). In pigs, only few data are available on arterial BP measurements with chronically implanted, telemetric transmitters. Two studies in (micro-) minipigs used pressure catheters in the descending thoracic aorta connected to a telemetric unit that was implanted into a pocket of abdominal muscles (Fossum et al., 2003; Stubhan et al., 2008). Also in minipigs, Kano et al. (2005) implanted the transmitter into the left ventral part of the animal. The sensor for measuring BP was inserted into the femoral artery and advanced to the abdominal aorta. The only study in domestic pigs (Poletto et al., 2011) used the external carotid artery for placement of the pressure catheter which was inserted and advanced to a point just proximal to the aortic arch. For placement of the telemetric unit, a subcutaneous pocket was formed on the left side of the neck.

To summarize, a potentially suitable approach for surgical implantation of a telemetric device in domestic pigs for the continuous measurement of ECG and BP in free-moving animals involves (i) ECG electrode placement in muscle tissue with the negative electrode close to the sternum and the positive electrode close to the scapula and (ii) BP catheter placement in the external carotid artery advancing to a point proximal to the aortic arch and (iii) subcutaneous placement of the telemetric device at the left side of the neck. To date, such a setup for the positioning of the individual components of an implantable telemetric system inside the animal has not been conducted in domestic pigs.

1.4.3. Strengths and weaknesses of implantable telemetric techniques

Generally, when recording cardiac parameters, biological and technical restrictions that may generate or perpetuate the occurrence of unusual beats or artefacts, have to be taken into account. Numerous intrinsic and extrinsic sources, such as abnormal physiological function, stress-induced arrhythmias, impaired conduction, inaccurate location of the measuring devices, movement of electrical leads, environmental electromagnetic interference, and equipment malfunction may generate extra electrical wavelets, nonsinus or spurious beats, or the merging of multiple beats into one (Lown and DeSilva, 1978; Kamath and Fallen, 1995; Salo et al., 2001; Storck et al., 2001; von Borell et al., 2007).

Furthermore, as changes in ANS activity are strongly influenced by physical activity (Bernardi et al., 1996; Hopster et al., 1998; Visser et al., 2002; Voss et al., 2002), noises and artefacts due to muscle action potentials are common, as monitoring usually occurs in non-stationary subjects. At rest, vagal regulation dominates whereas increasing physical activity is characterised by decreasing vagal and increasing sympathetic influences. This strong relationship between physical components in the context of behaviour and cardiovascular function has to be taken into account when comparing cardiovascular activity in experimental setups (Hansen, 2000; von Borell et al., 2007). Otherwise, this will provoke difficulties in welfare research since behavioural reactions that are in the main focus in a specific experimental paradigm are not seen under normal control conditions. In pigs, Marchant-Forde et al. (2004) determined five distinct types of errors in IBI data deriving from the Polar system in comparison to conventional ECG measurement. They reported that even the presence of a small percentage of error in IBI data is enough to substantially bias the outcome of HRV analysis. The importance of identifying and correcting such errors, artefacts and ectopic beats in cardiac data is well documented in the human literature (Berntson and Stowell, 1998) and postrecording editing of the data as one way to limit the impact of errors on indices of HRV has been strongly recommended for the assessment of reliable data (Kleiger et al., 1992; Kamath and Fallen, 1995; Marchant-Forde et al., 2004). To a certain extent, these challenges can be tackled by taking several details into account, for example, an adequate implantation surgery procedure, adequate recovery time after surgical intervention, the proper technical arrangement of telemetry receivers, antennas, data acquisition systems, configurators and telemeters, and the functional assessment and validation of the recorded parameters (Brockway and Hassler, 1993; Van Vliet et al., 2000; Stubhan et al., 2008). Addressing these requirements provides the basis for realizing the full potential of the telemetry method. A more general issue in studies using implants is the intensity of the intervention. The animals have to undergo a surgical procedure including complete anaesthesia and postsurgical care and medication. Of course, as with any invasive procedure, complications can emerge. According to the ethical principle of "3R" (replace, reduce, refine), which emphasises to limit the number of experiments and reduce the suffering of the animals used to an indispensable level, detailed and professional health checks as well as intensive care and medical treatment are essential to ethically support such interventions.

On the other hand, implantable telemetric systems suitable for use in large animals, such as the domestic pig, provide several advantages over non-invasive methods of cardiovascular measurement. These main advantages include the ability to obtain: (1) ECG and BP recordings in several animals simultaneously; (2) continuous recordings, 24 h/day; (3) high-fidelity recordings with frequency of up to 2000 Hz, exceeding that of external systems; (4) long-term recordings due to the fidelity of the catheter, which may be used for many weeks or months without loss of the signal; (5) parameters in unrestraint, conscious animals and (6) experimenter-independent measurements, as no external hardware must be fit to the subject.

Especially in the context of BP, non-invasive measurements were found to result in more inaccurate values compared to invasive measurements of arterial BP due to movement artefacts (Hassler et al., 1979), fixation stress (Brockway and Hassler, 1993), and user-related measurement errors (Brockway and Hassler, 1993; Erhardt et al., 2007). Various studies examined the agreement between systolic, diastolic, and mean arterial BP according to direct and indirect measurement techniques in different animal species and documented strong deviations (Cimini and Zambraski, 1985; Bazil et al., 1993; Gains et al., 1995; Van Vliet et al., 2000). A linear relationship between the results was shown in dogs, but the absolute values differed (Pettersen et al., 1988). The use of a tail cuff, as non-invasive tool to assess BP, requires immobilization, and, in some cases, warming of the animal, which may lead to stress-induced hypertension (Van Vliet et al., 2000). For example, Bazil et al. (1993) found an acceptable correlation between BP values derived by telemetric implants and external catheters (both direct BP measurements), but non-invasive (indirect) measurement via tail-cuff resulted in higher systolic BP levels and increased HR that was interpreted as elevated stress level of the animals with tail cuff. Inappropriate cuff size and limb movement were discussed as the major sources of error when using indirect BP monitors (Gains et al., 1995). Telemetric systems with fully implantable devices eliminate artefacts which may occur due to fixation, additional handling or disruptive cable systems, providing the ability to continuously measure over long periods of time (hours to days, weeks, months). Furthermore, the conventional methods for direct BP measurement usually involve exteriorized arterial catheters that require regular flushing to ensure patency. This maintenance procedure introduces the possibility of infection and thrombi (Depasquale et al., 1994). By using fully implantable units the risk of infection decreases due to the absence of exiting cables from the body.

Summing up the advantages, implantable telemetric systems enable the collection of reliable and continuous data for the evaluation of cardiovascular variables in complex experimental designs in which animals are able to range freely in groups without restraint, disturbing belts or stress-inducing handling by an experimenter. The application of such telemetric systems in experimental contexts is opening up new horizons concerning the objective investigation of affective-autonomic states in free-moving animals and provides the basis for an important step towards the understanding of animal welfare.

1.5 Key principles, aims, and hypotheses

The main focus of this thesis is the investigation of affective-autonomic states in domestic pigs by using a new telemetric technology in different behavioural situations in the context of coping and animal welfare.
The study of affective states is increasingly important in terms of improving animal welfare as they influence the behavioural reactions of animals in response to environmental stimuli. As the welfare of the animal results from an individual interacting with its environment, and the subsequent affective evaluation of this interaction, one key element in this context is the better understanding of the affective character of a situation. Evaluating autonomic responses is one promising approach to gain insight in the affective states of animals by demonstrating objectively what happens inside the animal. Due to advances in technology it is now possible to detect both branches of the autonomic nervous system, which are thought to reflect the valence and arousal dimensions of affect. Affective states may also vary between individuals based on the individual perception of personally relevant interactions with the environment. Individual coping characteristics differ in their general adaptive response patterns in reaction to challenges. And, even more important in this context: the situational appraisal depends to a large extent on the individuals' ability to cope with external and internal stimuli (Thayer and Lane, 2000; Vigil, 2009). These individual coping characteristics have been shown to correlate with several physiological responses. However, only little is known about the specific role of affective states in relation to coping characteristics. Because the activity of the two branches of the ANS is a key component in the processing of affective states, one may expect differential affective appraisal as well, which may underlie the individual behavioural and physiological response patterns to external stressors. To date, it is not well understood how and to what extent affective states are associated to individual coping characteristics and if the different coping styles also differ in their situational appraisal, contributing to their general affective states and thus, welfare. This work emphasises the importance of two processes, affective reactions and individual coping styles as mediators of the ongoing relationship between the animal and its environment. By focussing on the individual's affective state and coping style and how it responds behaviourally and physiologically to its environment will significantly increase our understanding of farm animal welfare. This will direct our perception of farm animals from being a production species more to being complex individuals with each having their own individual personality, emotions, and needs.

In order to investigate affective-autonomic states in free-moving domestic pigs in the context of coping and animal welfare, two major aims were addressed in this thesis:

(1) The establishment of a telemetric method for measuring both branches of the ANS in order to provide a valid tool for the objective evaluation of affective-autonomic states in free-moving pigs. This included (a) the development of a reliable surgical procedure for the implantation of a telemetric device for the continuous recording of ECG and BP (study 1) and (b) the functional assessment of recorded parameters to ensure reliability of the acquired data (study 1+2).

(2) The assessment of affective-autonomic responses of pigs in different housing-relevant situations and the relationship to their individual coping characteristics within the two-dimensional model of affective states (study 3).

Study 1

Assessing affective states in animals by measuring autonomic activity offers a great potential for evaluating farm animal stress and welfare. The perception of emotionally relevant stimuli is mediated via direct innervations of the sinoatrial node of the heart by sympathetic and parasympathetic fibres (Boissy et al., 2007b) resulting in a constant shifting of successive heart beats which reflects the animal's changing psychophysiological states. Most studies on the autonomic modulation of heart activity in farm animals make use of non-invasive systems, capable of assessing HR and HRV (as described in section 1.4.1). This allows drawing conclusions about vagal activity or the combined effects of sympathetic and vagal activity, whereas information about sympathetic activity is missing. Generally, besides regulating heart activity, the sympathetic system also regulates peripheral vasoconstriction. Therefore, BP measurement is particularly useful in that its variability allows conclusions about sympathetic activity. Recent developments in techniques for continuous recording (in conscious animals) of arterial BP and ECG in association with powerful computerized methods for signal analysis have permitted me to address this issue. In animal research, there are few methods available for continuously measuring ECG and intra-arterial BP, for example, invasive telemetric systems, which mostly find application in pharmacological contexts. At present, only one study in domestic pigs using invasive telemetric devices investigated changes in HRV and BPV using pharmacological autonomic blockade (Poletto et al., 2011). No such system has been evaluated in free-moving domestic pigs, investigating autonomic activity in different behavioural situations in the context of affective states. The successful surgical implantation of a telemetric device with two electrodes and an intra-arterial catheter is the key element for the assessment of reliable ECG and BP data, as failure to accurately locate the position of individual components can result in the generation of anomalous ECG and BP waveforms which exacerbates correct identification of IBIs by the software. As single disturbances and occurrences of artefacts in cardiovascular signals will interrupt normal interbeat variability and hence, would bias HRV and BPV measurements (Hopster and Blokhuis, 1994; Kamath and Fallen, 1995; Kingsley et al., 2005; von Borell et al., 2007; Liu et al., 2013; Hernando et al., 2018), the functional assessment of recorded parameters is an important step to enhance reliability of the acquired data.

To conclude, the main aspects of study 1 are first, to establish a stringent surgical procedure for the implantation of a telemetric device for the continuous measurement of ECG and BP in free-moving pigs, capable of delivering reliable signals also during behaviours with elevated activity level and second, the functional assessment of the parameters obtained by (a) processing the data (data correction) (b) verifying detection performance (artefact susceptibility) and (c) controlling the reliability of obtained parameters for application in two different behavioural situations with different physiological demands.

Study 2

Based on the findings from study 1, study 2 addresses a rather mathematical-statistical approach concerning HR and HRV indices. HRV parameters are based on calculations of variable distances between successive IBIs from instantaneous HR which are usually obtained using ECG describing the time in milliseconds between two consecutive R-peaks. As HRV may easily be biased by measurement errors in IBIs for different reasons, preferably only segments of data that are free from anomalous beats should be included in the analysis. However, changes in cardiac activity are strongly influenced by behaviour, especially those with high physical activity (Voss et al., 2002). In an experimental setup, usually these behavioural reactions that are at the centre of interest then can be impaired by the possible occurrence of high numbers of artefacts or ectopic beats. This highlights a potential methodological difficulty in ECG measurements. As shown in study 1, the telemetric measurement of ECG in free-moving domestic pigs is likely to result in the generation of anomalous waveforms that impede the correct identification of QRS-complexes by the software, especially during behaviour with elevated activity level, whereas the BP signal is more stable and less susceptible to movement artefacts. The question arose if BP is useable for the calculation of HR and HRV parameters in comparison to ECG. The motivation of this study was based on the idea of substituting HR and HRV detected from ECG signal with the one provided by intra-arterial BP signal in experimental settings, especially in situations with elevated behavioural activity (e.g. startle, flight).

The telemetric system used in study 1 allows the assessment of IBIs also from BP signal reflecting the time between two consecutive systolic pressure peaks in arterial BP (systolic IBIs). Manufacturers of telemetric systems promote this approach of HRV calculation in case of ECG malfunction and some studies make use of the calculation of HR and/or HRV parameters based on beat-to-beat recordings of invasive BP tracings (Mancia et al., 1986; Rimoldi et al., 1990b; Cohen et al., 1998). On the other hand, there has been evidence, that sympathetic influences may affect systolic IBIs by controlling cardiac contractility (Cacioppo et al., 1994; Michael et al., 2017). This may lead to differences in the sequential phases of cardiac cycle representing the duration of electro-mechanical systole with its two major components, the pre-ejection and the ejection phases. Consequently, HRV determination based on systolic IBIs would result in different values compared to HRV from ECG signal, due to sympathetic contribution to the ventricular myocardium. Unacceptable agreement in terms of HR and HRV by comparing an indirect measure of pulse pressure, such as the finapres monitor, with gold standard ECG was reported in humans (Carrasco et al., 1998). To my knowledge, no such comparisons have been made for invasive BP recordings. If BP signals were valid and reliable in measuring IBIs for the calculation of HRV in pigs, then there would be obvious potential benefits in using BP instead of ECG. The accuracy of BP in terms of HR and HRV measurements remains to be determined prior to considering any further application in the context of HRV analysis.

The main aspect of study 2 was the evaluation of the reliability of HR and HRV calculation from BP signal by assessing the relationship, agreement, and interchangeability between ECG and BP signal in terms of HR and HRV in pigs during different behavioural situations. Several statistical and mathematical methods were applied with different levels of explanatory power in order to give a broad and secure statement. This approach not only clarifies the possibility of using data derived from BP signals in the event of data loss or a high proportion of erroneous beats in the ECG signal (or vice versa), but contributes to the enhancement of HRV validation and its application in experimental setups.

Study 3

By using the established surgical procedure and the validated parameters for HRV and BPV, study 3 transfers the methodological findings to the application in an experimental context investigating the general and affective-autonomic states in domestic pigs with different coping characteristics in different situations. According to the concept of coping, animals, including humans, react with distinct behavioural styles to different sets of environmental demands. There is evidence, that different coping styles are also accompanied by differential autonomic response, in the way that proactively coping animals predominantly react with a sympathetic stress response, whereas a predominant vagal reactivity has been ascribed to the more reactive type of animal (Koolhaas et al., 1999). This seems beneficial in that proactive coping requires increased sympathetic cardiac and vasoconstrictive tone to favour redistribution of blood flow to specific vascular areas with high metabolic demands preparing the body in a readiness for action (fight or flight). Predominant vagal activity, on the other hand, deals with anabolic activities concerned with the conservation of bodily energy (conservation-withdrawal). The ANS deals with addressing the needs of the internal viscera and with responding to external challenges. Originating from the brain, emotional perceptions and assumed threats to survival, independent of the actual physical characteristics of the stimulation, may induce shifts in autonomic activity towards either sympathetic or vagal prevalence, resulting in a perceived affective state. Based on individual coping styles, animals may react differently to environmental challenges according to their emotional perception. Because of the role of the two subsystems of the autonomic nervous system in cardiovascular control in the context of affective states, one may expect a differential situational appraisal according to the respective coping style, which may underlie the individual behavioural response pattern to external stressors. Affective states play an important role in how individuals perceive their environment and therefore, how they appraise specific stimuli and situations. The extent to which individual coping pattern are based on different situational appraisal in the context of affect is not clearly understood yet.

The main aspects of study 3 were the investigation of the relationship between individual coping styles and their underlying general autonomic response in different housing-relevant situations and the assessment of the context-specific affective appraisal in pigs with different coping styles.

Hypotheses

The methodological approach of the surgical implantation of a telemetric device for the continuous recording of ECG and BP combined with the according functional assessment of cardiovascular parameters were expected to provide the basis for obtaining reliable indices of vagal and sympathetic activity enabling the objective evaluation of autonomic activity in free-moving pigs (study 1). Furthermore, it was hypothesised that IBIs deriving from ECG and BP may only be used interchangeable in the determination of HR, whereas interchangeable use may not be possible for HRV parameters due to its sensitivity to slight deviations (study 2). By investigating the relationship between individual coping characteristics and their underlying ANS response, I expected context-related differences between pigs with different coping styles in their general autonomic state as well as in their situational appraisal based on differential affective-autonomic reactions (study 3).

Chapter TWO.

Experimental Studies

2.1 Study 1: Surgical implantation and functional assessment of an invasive telemetric system to measure autonomic responses in domestic pigs

This section includes the accepted manuscript of the publication: Krause[1], A., Zebunke[1], M., Bellmann, O., Mohr, E., Langbein*, J., Puppe*, B. (2016) Surgical implantation and functional assessment of an invasive telemetric system to measure autonomic responses in domestic pigs, *The Veterinary Journal* 207, 140-146, doi: 10.1016/j.tvjl.2015.10.050

[1] these authors contributed equally

*Corresponding authors

Annika Krause designed and performed the experiment, analysed the data and wrote the manuscript with the support of and in agreement with the co-authors of this manuscript.

Abstract

The first aim of this study was to establish a surgical procedure to implant a new telemetric device for the continuous recording of electrocardiogram (ECG) and blood pressure (BP) in freely moving pigs. A second aim was the functional assessment of cardiovascular parameters, including heart rate variability (HRV) and blood pressure variability (BPV), so that these data could be used as the basis for the objective evaluation of autonomic activity and balance in different behavioural contexts. Eleven domestic pigs (German Landrace) underwent surgery for the placement of a telemetric device. At day 15 after surgery, 512 consecutive inter-beat intervals and pressure waves were analysed using different detection methods (automatic and manually corrected) while the animals were resting or feeding, respectively. HRV and BPV were calculated.

Incomplete datasets were found in four pigs due to missing ECG or BP signals. Technical and surgical issues concerning catheterisation and detachment of the negative ECG lead were continuously improved. In the remaining pigs, excellent signal quality (manually corrected data of 1%) was obtained during resting and acceptable signal quality (<10%) was obtained during feeding. Automatic triggering was sufficiently reliable to eliminate errors in BP recordings during active behaviour, but this was not the case for ECG recordings. Sympathetic arousal with accompanying vagal withdrawal during feeding was documented. The established surgical implantation and functional assessment of the telemetric system with the reliable registration of cardiovascular parameters in freely moving pigs could serve as a basis for future studies of autonomic regulation in context of stress and animal welfare.

Keywords: Autonomous nervous system; Cardiovascular parameters; Implantation surgery; Pig; Telemetry

Introduction

Advances in technology allow the use of mobile, invasive telemetric systems that are able to verify a number of cardiac parameters. Usually, telemetric systems find application in the context of pharmacological methods and cardiovascular diseases (Gelzer and Ball, 1997; Gross et al., 2002), and they have been used in animals such as rabbits (Van den Buuse and Malpas, 1997), monkeys (Chaves et al., 2006), rats (Beig et al., 2007), dogs (Ollerstam et al., 2007), miniature pigs (Stubhan et al., 2008) and domestic pigs (Poletto et al., 2011). Analysis of cardiac activity has also been used to investigate changes in sympathovagal balance related to emotional states (Désiré et al., 2004; Düpjan et al., 2011; Zebunke et al., 2011, 2013). Most studies that incorporate cardiac activity to evaluate subjective states in farm animals investigate the assessment of heart rate (HR) and its variability (HRV) by the use of noninvasive systems. Nevertheless, information about autonomic regulation obtained by these parameters is limited, as they provide information about the combined activity of both branches of the autonomic nervous system or about parasympathetic activation alone (von Borell et al., 2007). The assessment of blood pressure (BP) and its variability (BPV) is one possible approach to augment information about autonomic regulation, as the fluctuations in BP are indicative of sympathetic control (Hedman et al., 1992). Fully implantable telemetric techniques enable the measurement of electrocardiogram (ECG) and BP simultaneously while physiological variables are precisely assessed with minimal disturbance to the animal. Measurements can be realised in complex experimental designs, as animals can range freely in groups without restraint or contact by a handler. The first aim of this study was the establishment of a surgical implantation method in freely moving pigs for a telemetric device used for the continuous recording of ECG and BP. The second aim was the functional assessment of detected parameters to calculate HRV and BPV in order to enable objective evaluations about autonomic responses in different behavioural contexts. This may serve as a basis for future studies concerning autonomic control during situational appraisal in the context of stress and animal welfare.

Animals, materials and methods

Ethical statement

All procedures involving animal handling and treatment were approved by the Committee for Animal Use and Care of the Ministry of Agriculture of Mecklenburg-Vorpommern, Germany (Ref. Nr. 7221.3-1.1-037/12).

Animals

Eleven female domestic pigs (*Sus scrofa*, German Landrace) obtained from the Leibniz Institute for Farm Animal Biology in Dummerstorf (FBN) were used. At the beginning of their 10th week of life, piglets were transferred to an experimental barn and kept in individual pens (2.67 m^2–3.31 m^2). They had free access to water and were fed twice a day. After a 1-week acclimatisation period, the pigs were weighed and they underwent a physical examination. Once bodyweight exceeded 30 kg they became suitable for surgery, as arterial diameter had to be sufficient to insert and advance the catheter inside the vessel without resistance.

Telemetry equipment and signal monitoring

The implantable transmitter unit (Telemeter model TRM84PB, Telemetry Research) weighed 64 g and measured 90 × 45 × 10.5 mm. Two flexible bio-potential leads (30 cm in length) and a fluid-filled catheter (1 mm OD [3 Fr] Catheter, Millar Instruments) extended from the body of the transmitter. Using an individual frequency, each implanted transmitter relayed digital signals to a receiver unit attached at a 2.42 m height in front of each single housing pen. A switch at the receiver enabled the transmitter to be turned on and off in vivo without affecting the animal. The receivers were wire connected to two digital data acquisition systems (PowerLab 16/35 and 4/35, ADInstruments) that allowed simultaneous real-time recording of up to six pigs at a sampling rate of 2 kHz. The sampled data were stored and displayed on a computer and analysed using LabChart Pro (Version 7.0, ADInstruments).

Surgical procedure for transmitter implantation

Pigs were fasted 12 h prior to surgery but allowed access to water. They were anaesthetised IMin their home pen with a combination of xylazine 2 mg/kg (Xylariem, Riemser Arzneimittel) and ketamine 20 mg/kg (Ursotamin, Serumwerk). For local anaesthesia, procaine 4mg/kg (Isocain, Selectavet, Dr. Otto-Fischer) was injected SC according to the planned incision lines. During surgery, general anaesthesiawas maintained using IV ketamine 4 mg/kg/h (Ursotamin, Serumwerk) and diazepam 0.4 mg/kg/h (Faustan, AWD-pharma) in a 5% glucose solution. The implant was placed into a SC pouch formed on the left side of the neck. The catheter and the negative electrode were tunnelled SC to the ventral aspect of the neck using a hollow trocar. The left carotid artery was occluded with an arterial clamp, and a small incision was made to carefully insert the tip of the catheter into the vessel. Depending on the size of the pig, the catheter was advanced stepwise 7–10 cm to a point proximal to the aortic arch until a strong BP signal with characteristic slope was observed using the software. Five non-absorbable sutures (Dermafil, green polyester, USP 2/0 EP3)were placed to secure the catheter at the arteriotomy site. The negative electrode was tunnelled caudal to a small incision (1 cm) lateral to the sternum. The positive electrode was tunnelled caudal from the SC pouch to a small incision approximately 2 cm lateral to the left scapula. The tips of both electrodes were enclosed by muscle tissue and secured with circumferential ligatures of non-absorbable suture

(Dermafil, green polyester, USP 2/0 EP3). Adequate ECG signalswere verified. The transmitter body enclosed with surgical mesh was fixed to the surrounding tissue (Vitafil white, USP 2/0 EP3). To suture the dermis (subcutis), absorbable material was used (Ethicon, Vicryl plus 2-0, violet). Incisions in the epidermis were closed with a non-absorbable suture (Vitafil white USP 3 and 4 EP6). The locations of the components of the telemetric device inside the animal are shown schematically in Fig. 1.

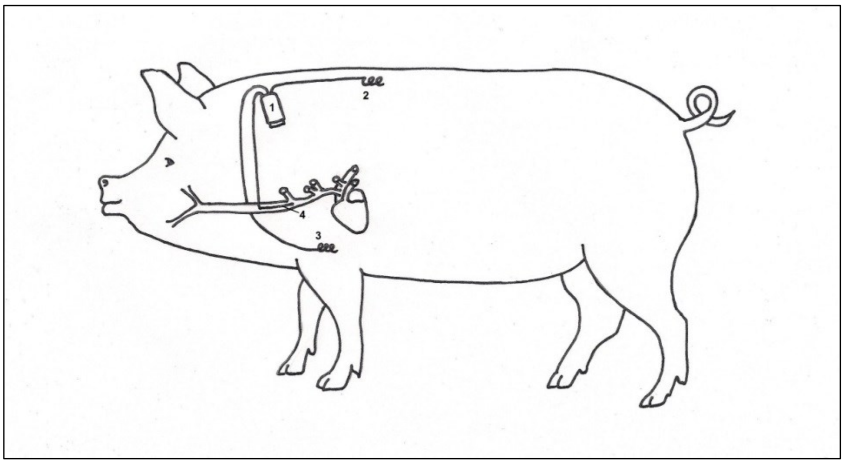

Figure 1. The location of the components of the telemetric device inside the animal: (1) the transmitter body at the left side of the neck; (2) the positive electrode on the dorsal side lateral to the left scapula; (3) the negative electrode on the ventral side lateral to the sternum; and (4) the pressure catheter in the carotid artery.

Pigs were administered a peri-operative dose of metamizole 50 mg/kg (Metapyrin, Serumwerk Bernburg) and a combination of sulfadimidine 20 mg/kg and trimethoprim 4 mg/kg (Trimethosel, Selectavet, Otto-Fischer). This regimen was repeated every 24 h over 5 days post-surgery. Pigs were continuously observed in their home pens, and ECG and BP were monitored until the pigs exhibited complete recovery from anaesthesia. Surgical sites were examined, cleaned and treated with skin protection lotion (ImmuStim, almapharm) four times daily until the incisions were fully healed. Skin sutures were removed on postoperative days 10 and 11. Depending on battery life, transmitters were explanted between 30 and 60 days after surgical implantation. Pigs were sedated with a combination of xylazine 2 mg/kg (Xylariem) and ketamine 20 mg/kg (Ursotamin) prior to being euthanased by IV injection of tetracaine hydrochloride 0.5 mg/kg, mebezonium iodide 5 mg/kg and embutramide 20 mg/kg (T61, Intervet International). During post-mortem examination, transmitter body, ECG leads and BP catheter were verified to have remained in place before transmitters were explanted.

Data acquisition and analysis

Behaviour, ECG and BP were monitored for 30 days. For the present study, data acquisition was performed on day 15 after surgery, which provided sufficient time for recovery from surgery, wound healing and examination of the compatibility of the device in the animal before data collection commenced. A familiar person entered the experimental room at 08.15 a.m. daily to feed the animals. Both behaviour (resting, feeding) and physiological responses (ECG and BP) were continuously recorded for 2 h pre-feeding and during food intake. The behaviour was videotaped. Cardiovascular data from the pre-feeding period when the pigs were lying inactive (resting) and during the consumption of food (feeding) were chosen. Segments of ECG and BP data, 512 beats in length (Poletto et al., 2011), were selected for each pig during resting and feeding. Analysis of ECG and BP data was performed using two detection methods: (1) automatic detection of valid QRS complexes and systolic pressure waves using standard software settings after applying a 50 Hz low-pass filter (treatment group: AUTO), and (2) automatic triggered points were manually checked for correct marking. If markings of a valid QRS complex or systolic pressure wave were missing or incorrectly set, they were manually adjusted. If a QRS complex or systolic pressure wave was completely missing, it was corrected by interpolation calculated from the mean of three previous and three subsequent values (treatment group: CORR). HRV was calculated on the basis of interbeat intervals (IBIs) derived from the ECG signal. For the time domain, mean heart rate (HR), the standard deviation of IBIs (SDNN, an indicator of sympathetic and parasympathetic activation), the root mean of the squared distances of subsequent IBIs (RMSSD, an indicator of parasympathetic activation), and the ratio of these two (RMSSD/SDNN, reflecting the balance of the autonomous nervous system) were computed. Fast Fourier Transformation (FFT) was performed to obtain the power spectrum of the HRV data segments (LabChart). According to Poletto et al. (2011), a Hanning window was applied to each data set (FFT size, 512) to calculate the power in the low frequency band (LF, 0.0–0.09 Hz) and the high frequency band (HF, 0.09–2.0 Hz) in absolute units (ms2), and the LF:HF power content ratio from the recordings. These parameters were assumed to be highly correlated with those of the time domain. The LF indicates sympathetic and vagal activation (analogically to SDNN), while the HF component correlates with RMSSD and is considered as a marker of vagal activation (Akselrod et al., 1981). Using the BP signal, systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP = DBP + 1/3 × (SBP – DBP)), and their standard deviations were assessed (standard deviation of systolic blood pressure [SDS], of diastolic [SDD] and of mean arterial pressure [SDM]). Analysis of BPV in the frequency domain was not performed in this case, as power spectral analysis in LabChart was not designed for BPV analyses.

Statistical analyses

The normality of distribution of each parameter was assessed using Kolmogorov–Smirnov tests. Where normality assumptions were notmet, data were logarithmically transformed. All statistical analyses were conducted using SAS (version 9.3, 2009, SAS Institute). The differences in the number of corrected values between behavioural categories (resting, feeding) were determined. One-way analysis of variance (ANOVA, Glimmix procedure) was performed for IBI, SBP and DBP, with behaviour as a fixed factor. Additionally, 512-beat intervals with continuous data series of IBI, SBP and DBP were analysed using a two-way analysis of variance (ANOVA, Glimmix). The statistical model consisted of fixed effects of the treatment group (AUTO, CORR), the behaviour category (resting, feeding) and their interaction. HRV and BPV were calculated for every 512-beat interval separately (CORR), and one-way ANOVA was performed to test differences between the behaviour categories. Mean differences with a $P < 0.05$ were considered significantly different. All analyses included the animal as a repeated factor.

Results

The pigs showed an uncomplicated healing process, as they appeared in good health, with normal grooming and normal body movements.

Method validation

Data measured during resting and feeding were analysed using two treatment groups (AUTO, CORR) and are shown in Fig. 2. During resting, 1% of IBIs and < 1% of SBP and DBP needed manual adjustment. During feeding, the number of manually corrected IBIs increased to 10% in two transmitters. However, from these, only 1% had to be replaced by interpolated values. The number of BP waves that needed manual correction increased to 4% (systolic) and 3% (diastolic) during feeding. The percentage of interpolated SBP and DBP values was < 1%.

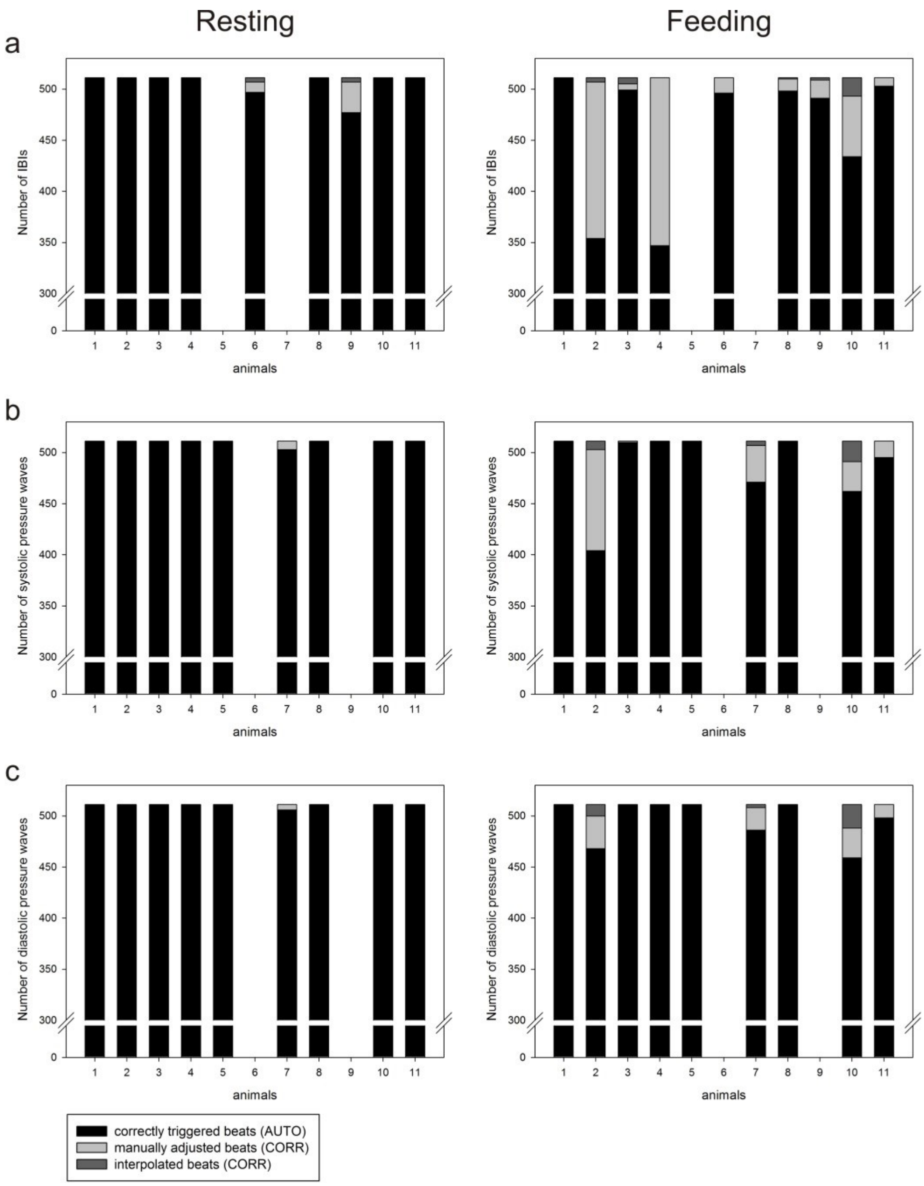

Figure 2. Number of correctly triggered (group AUTO) and manually corrected (group CORR; adjustment and interpolation shown separately) interbeat intervals (a), systolic (b), and diastolic (c) pressure waves assigned by implanted transmitters in 11 pigs at day 15 after surgery during resting and feeding. Interbeat intervals were identified using ECG signals; systolic and diastolic pressures were assigned using blood pressure signals.

The ANOVA results indicated significant differences in the number of manually corrected IBI, SBP and DBP values between resting and feeding (IBI, $F_{1,8} = 5.9$, $P<0.05$; SBP, $F_{1,8} = 10.1$, $P<0.05$; DBP, $F_{1,8} = 10.3$, $P<0.05$). The ECG measured by two transmitters and the BP measured by two transmitters did not send any data at day 15. At post-mortem we found the tip of the negative electrode separated from muscle tissue, which caused data loss at day 11 (pig 5) and day 12 (pig 7). In one animal (pig 6), BP signal developed unusual waveforms at day 3 with an increasing number of artefacts. From day 13, the catheter was still registering values, but they were not analysable. BP signal in pig 9 was missing from the first day after surgery with all values remaining at the zero line. During subsequent investigation, the catheter was verified to be in optimal position in the artery.

Detection performance

Datasets from four pigs were removed from the overall dataset due to missing ECG or BP signals; therefore, further results reflect data from a total of seven pigs. To visually describe the detection performance each during resting and feeding, plots of IBIs, SBP and DBP for each treatment group (AUTO, CORR) and behaviour category (resting, feeding), respectively, were produced (Figure 3).

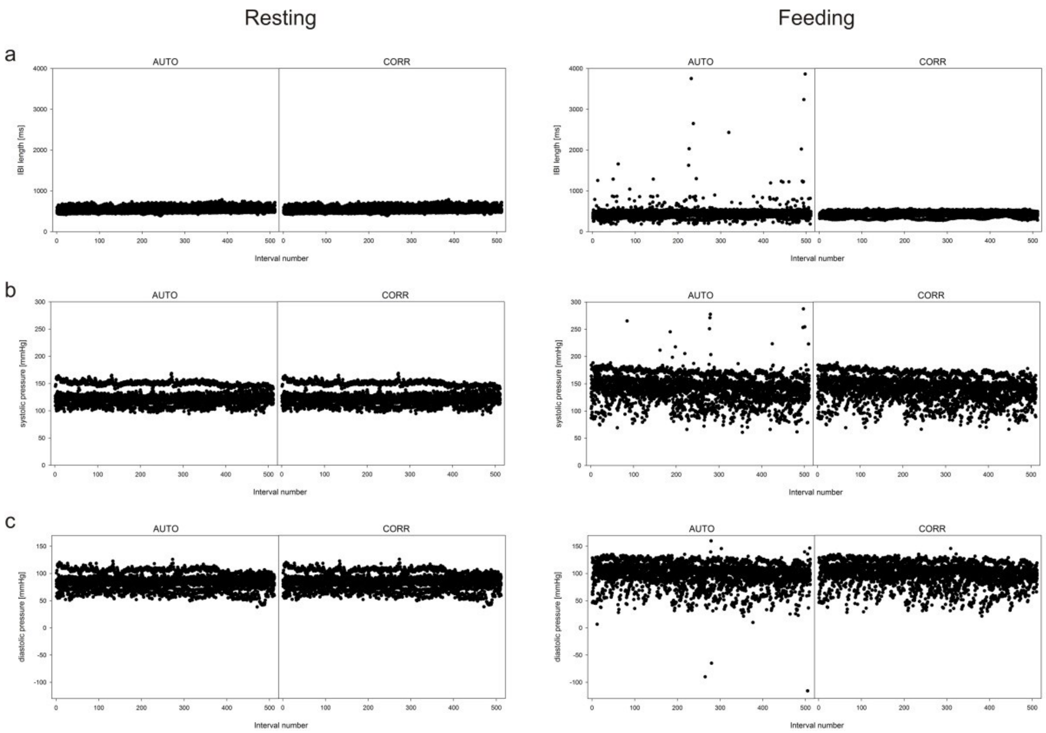

Figure 3. Plots of 512-beat intervals of interbeat interval length (a), systolic (b), and diastolic (c) blood pressure tracings in seven pigs during resting (left) and feeding (right), with automatic triggering (AUTO) and manually corrected values (CORR). Correction of values was performed by either manually adjusting falsely detected beats (adjustment), or by calculating the mean of three adjacent and three following values (interpolation). The horizontal axis denotes consecutive interval numbers.

There were no apparent differences when treatment groups were compared during resting. Automatic triggering was more successful for BP measurement (SBP and DBP) compared to IBIs derived from ECG during feeding. ANOVA indicated that IBI, SBP and DBP were significantly affected by behaviour (IBI, $F_{1,14298} = 8457.9$, $P<0.001$; SBP, $F_{1,14298} = 13367.7$, $P<0.001$; DBP, $F_{1,14298} = 9971.3$, $P<0.001$) and IBIs were additionally affected by the interaction of treatment group × behaviour ($F_{1,14298} = 32.1$, $P<0.001$). SBP and DBP values did not differ significantly between the treatment groups (SBP, $F_{1,14298} = 1.7$, $P = 0.19$; DBP, $F_{1,14298} = 0.3$, $P = 0.61$).

HR, HRV and BP, BPV during resting and feeding

The mean values (± standard error, SE) of all calculated parameters within the 512-beat interval during resting and feeding are presented in Figure 4a and b.

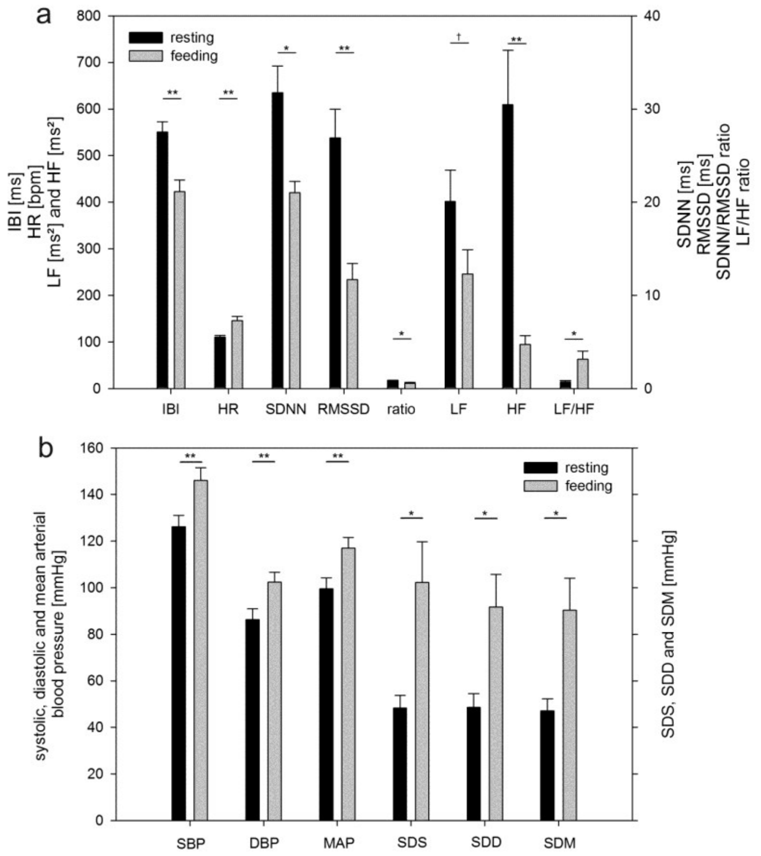

Figure 4. Mean values (± standard error) for (a) heart rate and its variability and (b) blood pressure and its variability recorded by ECG and blood pressure tracings within one 512-beat interval during resting and feeding. Only manually corrected values were included. Calculation was averaged over individuals (n = 7). Differences were determined to be statistically relevant at $P < 0.05$ (*); a trend was set at $P < 0.1$ (†). IBI, inter-beat interval in ms; HR, heart rate in beats per min (bpm); SDNN, standard deviation of successive IBIs (ms); RMSSD, the root mean of the squared distances of subsequent IBIs (ms); LF, low frequency power in absolute units (ms^2); HF, high frequency power in absolute units (ms^2); LF:HF, power content ratio; SBP, systolic BP (mmHg); DBP, diastolic BP (mmHg); MAP, mean arterial BP (mmHg); SDS, standard deviation of systolic BP (mmHg); SDD, standard deviation of diastolic BP (mmHg); SDM, standard deviation of mean arterial BP (mmHg).

HR values increased during feeding compared to resting ($F_{1,6} = 18.4$, $P<0.01$), while the SDNN ($F_{1,6} = 11.2$, $P<0.05$), the RMSSD ($F_{1,6} = 19.9$, $P<0.01$) and the RMSSD/SDNN-ratio ($F_{1,6} = 10.0$, $P<0.05$) decreased significantly during feeding. These results were also reflected by the data obtainedfrom spectral power analysis. Both the HF and LF power were higher during resting, although this effect was more pronounced for HF power ($F_{1,6} = 16.8$, $P<0.01$). Consequently, the LF/HF ratio increased significantly during feeding ($F_{1,6}=7.0$, $P<0.05$). Additionally, main effects of behaviour were found for SBP ($F_{1,6}=24.7$, $P<0.01$), DBP ($F_{1,6}=15.2$, $P<0.01$) and MAP ($F_{1,6}=18.3$, $P<0.01$). These changes were accompanied by an increase in BPV during feeding (SDS: $F_{1,6}=7.2$, $P<0.05$, SDD: $F_{1,6}=7.1$, $P<0.05$, SDM: $F_{1,6}=6.3$, $P<0.05$).

Discussion

Our study presents the systematic establishment of a new invasive telemetric method for the simultaneous assessment of ECG and BP in freely moving pigs to ensure reliable recordings in experimental contexts. Inter-beat intervals, SBP, DBP and MAP were measured via telemetry and underwent subsequent HRV and BPV analyses. Generally, when calculating such indices, data segments that are free from ectopic beats are mandatory for analysis, as the occurrence of artefacts interrupts normal inter-beat variability (Marchant-Forde et al., 2004). Failure to accurately locate the position of individual components of the telemetric device can result in the generation of anomalous waveforms, which exacerbates correct identification by the software. Therefore, successful implantation surgery procedure is essential if reliable data are to be obtained.

Fifteen days after implantation, data transmission in four animals was impaired, as two ECG signals and two BP signals ceased to function. The tip of the negative electrode in two animals was disconnected from the muscle tissue, which may have been caused by suture failure and/or tension on the electrode leads.We assume these problems were due to the longer distance that was covered by the negative electrode inside the animal. The negative electrode was tunnelled twice and proceeded from the transmitter position on the left side of the neck along the ventral aspect of the neck towards the sternum, whereas the positive electrode was tunneled once in a straight line to approximately 25 cm from the transmitter body. Furthermore, the negative electrode was more exposed to body movements because of its position at the left side of the neck. As a consequence, the tension on the negative lead during movement and growth was increased compared to the positive lead. Accordingly, the number of sutures to secure the negative ECG lines in muscle tissue was increased to ensure stability, especially in larger animals of >35 kg at the time of surgery. We recommend placing the ECG leads in loops to allow them to unwind inside the animal without resistance and to avoid tension on the leads during this process. In case of pig 6, the absence of a BP signal arose from the formation of clots in the lumen of the artery, as revealed during the post mortem examination. This condition had no effect on overall health, as the animal was exhibiting normal behaviour, ECG and temperature values throughout the entire experiment. Thrombus formation may disable data transmission by encapsulating the tip of the catheter. Therefore, a drop of heparin was pipetted onto the catheter tip to prevent thrombosis (Brockway et al., 1991). To ensure that sufficient heparin was used, we modified this procedure in the remaining pigs by rinsing the catheter in heparin instead of pipetting droplets of heparin onto the catheter tip. Other reasons for the absence of a BP signal could include catheter damage during surgery, as catheter tips and leads are fragile and sensitive to contact. In our case, technical issues induced data loss in pig 9.

The application of different detection methods (AUTO, CORR) had no impact on the detected parameters during resting. During feeding, treatment group had a significant effect on IBIs, but not on BP values; hence, manual correction of IBI data resulted in different values between treatment groups. Therefore, we can conclude that automated triggering was sufficiently reliable to eliminate errors in BP recordings during active behaviour, but this was not the case for ECG recordings. If skeletal muscle is activated, muscle fibres generate their own electrical potentials, which 'superimpose' and distort ECG waves (movement artefacts). BP showed less susceptibility to artefacts than ECG. In any case, visual inspection and manual correction of ECG data were essential and are strongly recommended to obtain reliable data, at least during active behaviour, since if there is a high proportion of artefacts HRV values are overestimated (Storck et al., 2001).

HRV and BPV values showed significant differences in nearly all calculated parameters between the two behaviour categories. During feeding, tachycardia was accompanied by a decrease in RMSSD and HF, indicating parasympathetic withdrawal (Langbein et al., 2004). Considering the accompanying decline of the RMSSD:SDNN ratio, analogous to an increase in LF:HF ratio, the shift of the autonomic balance towards predominantly sympathetic control became apparent. The strong increases in BP and BPV during feeding indicated sympathetic activation and support the results of previous studies which reported cardiovascular changes in response to feeding in different species, including humans (Bloom et al., 1975; Scalzo, 1992).

Conclusions

The established surgical implantation of a telemetric device in combination with the functional assessment of detected parameters enabled the reliable registration of cardiovascular parameters, including HRV and BPV, during resting and feeding behaviour in freely moving pigs. These results indicate the usability of a telemetric system for the measurement of autonomic responses in freely moving pigs and could serve as a basis for future studies of autonomic control during various behavioural situations in the context of emotional states, stress and animal welfare.

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2.2 Study 2: Interchangeability of electrocardiography and blood pressure measurement for determining heart rate and heart rate variability in free-moving domestic pigs in various behavioral contexts

This section includes the publication: Krause, A., Tuchscherer, A., Puppe*, B. and Langbein*, J. (2015) Interchangeability of electrocardiography and blood pressure measurement for determining heart rate and heart rate variability in free-moving domestic pigs in various behavioral contexts. *Frontiers in Veterinary Science* 2 : 52.
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*Corresponding authors

Annika Krause designed and performed the experiment, analysed the data and wrote the manuscript with the support of and in agreement with the co-authors of this manuscript.

Abstract

This study assessed the interchangeability between heart rate (HR) and heart rate variability (HRV) measures derived from a series of interbeat intervals (IBIs) recorded via electrocardiogram (ECG) and intra-arterial blood pressure (BP) in various behavioral contexts. Five minutes of simultaneously recorded IBIs from ECG and BP signals in 11 female domestic pigs during resting, feeding, and active behavior were analyzed. Comparisons were made for measures of HR, the standard deviation of IBIs, and the root mean of the squared distances of subsequent IBIs derived from ECG and BP signals for each behavior category using statistical procedures with different explanatory power [linear regression, intraclass correlation coefficient (ICC), Bland and Altman plots, and analysis of variance (ANOVA)]. Linear regression showed a strong relationship for HR during all behaviors and for HRV during resting. Excellent ICCs [lower 95% confidence intervals (CI) >0.75] and narrow limits of agreement in all behavior categories were found for HR. ICCs for HRV reached the critical lower 95% CI value of 0.75 only during resting. Using Bland and Altman plots, HRV agreement was unacceptable for all of the behavior categories. ANOVA showed significant differences between the methods in terms of HRV. BP systematically overestimated HRV compared with ECG. Our findings reveal that HR data recorded via BP agree well those recorded using ECG independently of the activity of the subject, whereas ECG and BP cannot be used interchangeably in the context of HRV in free-moving domestic pigs.

Keywords: domestic pig, electrocardiogram, blood pressure, heart rate variability, interchangeability, Bland and Altman plot.

Introduction

Fluctuations between heartbeats in mammals are predominantly regulated by the constant interplay between the parasympathetic and sympathetic branches of the autonomic nervous system (ANS). The assessment of heart rate variability (HRV) is widely used as an indicator of autonomic function in the analysis of physiological signals in humans and animals (1–3). The mean heart rate (HR) can be interpreted as a reflection of the net effects of the interaction between both branches of the ANS. Parameters of HRV derived from cardiac interbeat interval (IBI) data provide information regarding the complex interaction between both branches (SDNN: the standard deviation of all IBIs of the data set) and regarding parasympathetic activation alone (RMSSD: the square root of the mean of the sum of the squares of differences between successive IBIs) (3).

Most research on HRV primarily occurs in humans and focuses on the relationship between autonomic functioning and diseases, such as cardiac dysfunction (4) and sudden cardiac death (5). Within the field of farm animal research, analyses of HRV have been used to investigate changes in sympathovagal balance due to pathology (6), stress (7, 8), housing and management conditions (9, 10), learning (11), pain (12, 13), and emotional states (14, 15).

Advances in telemetric technology enable the use of mobile, invasive telemetric systems that can be used to (not only) verify IBIs via the simultaneous recording of electrocardiogram (ECG) and blood pressure (BP) data in free-moving animals. This enables both the evaluation of physiological variables with a minimum of disturbance to the animal and complex experimental designs in which animals are able to range freely in groups, for example, during social interactions. In the last two decades, a number of investigations have used invasive telemetric systems in the context of pharmacological studies and cardiovascular diseases (16, 17), circadian rhythms (18), and stress (19) and were used in animals, such as rabbits (20), goats (21), monkeys (22), rats (23), dogs (24), and pigs (25). Studies using telemetry to evaluate HRV in the context of autonomic regulation usually make use of IBIs derived from ECG to calculate parameters of HRV. However, invasive telemetric technology also provides the assessment of IBIs from BP signal. One IBI is the time in milliseconds between two consecutive R-peaks in an ECG or between two consecutive systolic pressure peaks in arterial BP. As HRV may easily be biased by measurement errors in IBIs, preferably only segments of data that are free from artifacts and ectopic or anomalous beats should be included in the analysis. Additionally, it is not possible to simply omit segments of data that contain artifacts as this would interrupt the fundamental time series of the data on which the analysis is based (26, 27). Postrecording editing of the data is an indispensable procedure for the assessment of reliable data (28). As found in a recent study, the telemetric measurement of ECG in free-moving domestic pigs is likely to result in the generation of anomalous waveforms that compound the correct identification of IBIs by the software [Krause et al., under revision (29)]. Some of the errors may be explained as artifacts originating from physical activity. These movement artifacts in ECG are not present in BP signals. Therefore, BP signals are potentially useful for the collection of IBI data for further analysis of HRV, especially in experimental settings in which behaviors with an elevated level of activity, such as social interaction, are being studied. If BP signals are valid and reliable in measuring IBIs in pigs, then there are obvious potential benefits in using BP instead of ECG. To our knowledge, however, there is a lack of research on the comparability of IBI data derived from ECG and BP for measuring HR and HRV. The accuracy of BP signals in terms of HR and HRV measurement remains to be determined prior to considering any further application in the context of HRV analysis.

The aim of this study was to assess the relationship, agreement, and interchangeability between HR and HRV data derived from a time series of IBIs recorded using ECG and BP in pigs during various behavioral situations with various levels of physical activity by applying statistical methods with different explanatory powers. This would help to clarify the possibility of using data derived from BP signals in the event of data loss or a high proportion of erroneous beats in the ECG signal (or vice versa).

Animals, materials and methods

Animals

Data from 11 female domestic pigs (*Sus scrofa*, German Landrace) from the experimental facilities for swine of the "Leibniz Institute for Farm Animal Biology in Dummerstorf" were included in the study. The pigs were housed in individual pens (2.67–3.31 m^2) with solid and partially slatted floors and visual, olfactory, and partially tactile contact with conspecifics (snout contact) in the adjacent pen. Pigs had free access to water (nipple drinker) and were fed twice per day. The temperature was held constant at 20°C. After 1 week of acclimatization, the pigs were weighed, and their health was checked. At 11 weeks of age, the pigs underwent surgery for implantation of a telemetric device capable of recording ECG, BP, and body temperature (29). The crucial factor for the time of surgery was for the pig's weight to exceed 30 kg, which is when the animals' artery is sufficiently large to insert and advance the catheter inside the vessel without resistance. Prior to surgery, the pigs were handled three times daily for 1 h to socialize and habituate them to human contact and equipment monitoring.

Instrumentation

The telemetry unit, individual receivers, data acquisition device (Power Lab), and analysis software (LabChart) were provided by Telemetry Research (Auckland, New Zealand) and ADInstruments (Oxford, UK). The implantable transmitter unit (Telemeter model TRM84PB) weighed 64 g and measured 90 mm × 45 mm × 10.5 mm. Two flexible biopotential leads (30 cm in length) and a fluid-filled catheter [1 mm OD (3Fr) Catheter, Millar Instruments, Houston, TX, USA] extended from the body of the transmitter. A temperature sensor embedded in the implant case was used to measure the animal's body temperature. Using an individual frequency, each implanted transmitter relayed digital signals to a receiver unit attached at a 2.42-m height in front of each single housing pen. A switch at the receiver enables the transmitter to be turned on and off in vivo without affecting the animal. The receivers were wire connected to two digital data acquisition systems (PowerLab 16/35 and 4/35, ADInstruments) that enabled the simultaneous real-time recording of up to six pigs at a sampling rate of 2 kHz. The sampled data were stored and displayed on a PC and analyzed using LabChart Pro (Version 7.0, ADInstruments).

The pigs were fasted 12 h prior to surgery but allowed access to water. The animals were subsequently implanted with the telemetric device by the veterinarian of the institute under sterile conditions. For details regarding surgery and implantation, see Krause et al. (29). Here we give a brief summary. A subcutaneous pouch was formed at the left side of the neck for placement of the telemeter body. The pressure sensor was inserted into the left carotid artery. The negative electrode was placed lateral to the sternum, and the positive electrode was located ~2 cm lateral to the left scapula. The tips of both electrodes were enclosed by muscle tissue. Figure 1 presents the locations of the four components of the telemetric system exemplary for one pig. After completing surgery, pigs were returned to their respective home pens and allowed to recover under a heat lamp to maintain body temperature after surgery. They were observed continuously until they recovered consciousness and could stand and move safely on their own. Body temperature, ECG, and BP were monitored for 5 min every hour until the pig exhibited complete recovery from anesthesia. The pigs were administered a postsurgical medication of 50 mg/kg metamizole (Metapyrin, Serumwerk Bernburg AG, Germany) and a combination of 20 mg/kg sulfadimidine and 4 mg/kg trimethoprim (Trimethosel, Selectavet, Otto-Fischer GmbH, Weyarn-Holzolling, Germany). This regimen was continued every 24 h for 5 days postsurgery. During recovery period of 14 days, health status, behavior, impact of the transmitter and surgery on the animals, and functionality of the telemetry system were checked four times daily. Skin sutures were removed on days 10 and 11 postoperatively.

Figure 1. Locations of the four components of the telemetric system exemplary for one pig 5 days after surgical procedure: positions of the transmitter body (1), positive electrode (2), catheter (3) and negative electrode (4). (Upper left) Transmitter body with according wires [positive electrode (black), catheter (white), and negative electrode (red)].

Data acquisition

Behavior, ECG, and BP data of 11 animals were monitored for 30 days after surgery. For the present study, data acquisition was performed on day 15 to provide sufficient time for recovery from surgery, wound healing, and examination of the compatibility of the device within the animal before data collection was started. A familiar person entered the experimental room at 8.15 a.m. and consecutively fed the animals. Both behavior and physiological responses (ECG and BP) were continuously recorded for 2 h prefeeding, during food intake and 1 h after feeding. Pig behavior was assessed using video cameras (Panasonic WV-CP500, EverFocus Endeavor SD + HD DVR) attached to each single housing pen and analyzed using the Observer XT (Version 11, Noldus, Wageningen, Netherlands). Three behavior categories, each 5 min in length, were defined: lying inactive, feeding (food intake), and active behavior (locomotion, drinking, and scratching, whereas locomotion was mostly dominated by exploration with snout contact to the ground or walls of the home pen). If one 5-min interval was continuously categorized as "resting," "feeding," or "active behavior," it was chosen for further analysis or more precisely, behavioral analysis required every individual to exhibit each behavior category (resting, feeding, active) for not <5 min continuously. These different activities were chosen to assess whether agreement between the different signals was stable across a wide range of data. For each individual and behavior, one 5-min segment of IBI data from both ECG and BP signals was selected. A total of 33 5-min segments of IBI from ECG signals and 33 segments of IBI from BP signals provide the basis for all further comparisons.

Interbeat intervals derived from the ECG were calculated as the time between two consecutive R-peaks in the QRS complex, and IBIs derived from the BP signals were defined as the time between two consecutive systolic pressure peaks. QRS complexes and systolic pressure waves were automatically detected using the software. Subsequently, every 5-min segment was visually checked for correct marking. If the markings of a valid

QRS complex or systolic pressure peak were missing or set falsely, they were manually adjusted using the software. If a QRS complex or systolic pressure peak was completely missing, it was corrected via interpolation calculated from the mean of three previous and three subsequent values. If more than three consecutive IBIs were missing or >5% needed interpolation, the 5-min segment was discarded from further analysis, following the recommended criteria for HRV calculations (3). HR, SDNN (an indicator of sympathetic and parasympathetic activation), and RMSSD (an indicator of parasympathetic activation) were computed by LabChart.

Statistical analysis

All statistical analyses were conducted utilizing SAS (Version 9.3, 2009, SAS Institute Inc., Cary, NC, USA). The normality of distribution of each parameter was assessed using a Kolmogorov–Smirnov test. Where normality assumptions were not met, data were logarithmically transformed if necessary.
A linear regression analysis was used to quantify the strength of the relationship between HR, SDNN, and RMSSD derived from the ECG and BP signals. To evaluate the agreement between the two different signals, we calculated the intraclass correlation coefficients (ICCs) and their 95% confidence intervals (CI) for each behavior category according to Shrout and Fleiss (30). ICCs >0.8 are considered to indicate good to excellent relative agreement, whereas coefficients between 0.6 and 0.8 are considered to be substantial (31, 32). Interchangeable use has been suggested to exist when the lower 95% CI value exceeds 0.75 (33). Additionally, levels of agreement between ECG and BP data were assessed using Bland and Altman plots with 95% limits of agreement (LoA) with the criteria of Altman and Bland (34). For BP being used interchangeably with ECG, 95% of the differences should fall within the LoA, and the width of LoA was also expected to be clinically acceptable. Bland and Altman plots illustrate the difference between paired observations on the y-axis (BP – ECG), plotted against their mean value on the x-axis [(BP + ECG)/2]. This provides a template with LoA and makes it possible not only to evaluate the agreement between the methods over different values of x but also to assess whether the range between the limits is acceptable. Furthermore, all parameters were analyzed using repeated measurements analysis of variance (ANOVA) according to the MIXED procedure included in SAS. The model included the fixed effects behavior (resting, feeding, and active), signal (ECG and BP) and behavior × signal. The analysis included the subject as a repeated factor. Least-squares means (LSM) and their standard errors (SE) were computed for each fixed effect in the models. If the fixed effect was significant, pair-wise differences of LSM were tested using the Tukey–Kramer correction. The SLICE statement of the MIXED procedure was used to conduct only specified comparisons of LSM. Effects and differences were considered significant if $P < 0.05$. Results are presented as LSM ± SE.

Ethical statement

All procedures involving animal handling and treatment were approved by the Committee for Animal Use and Care of the Ministry of Agriculture, Environment and Consumer Protection of the federal state of Mecklenburg-Vorpommern, Germany (Ref. Nr. 7221.3-1.1-037/12). After completing the entire experiment, the pigs were sedated with a combination of 2 mg/kg xylazine (Xylariem) and 20 mg/kg ketamine (Ursotamin) prior to being euthanized with an intravenous injection of 0.5 mg/kg tetracaine hydrochloride, 5 mg/kg mebezonium iodide and 20 mg/kg embutramide (T61, Intervet International GmbH, Unterschleißheim, Germany).

Results

The 11 pigs showed a good and uncomplicated healing process. Even after the postsurgical medication was concluded, body temperature remained constant and did not change over the 15-day observation period. Despite the stringent correction threshold of 5% and/or the requirement of missing no more than three consecutive beats, none of the 5-min segments had to be excluded from further analysis. The simultaneous measurement of BP and ECG in 11 subjects during the three different behavior categories (5 min each) provided an overall 40,124 IBIs [mean IBI count ± SD during resting = 527.5 (±69.1), during feeding = 637.2 (±48.4), and during active behavior = 659.1 (±59.3)]. In ECG, 2.5% of the IBIs needed manual correction; only 0.6% was interpolated. Regarding the BP signal, 0.3% of IBIs had to be manually corrected, and none of the beats needed interpolation.

Linear regression

A significant relationship between IBIs derived from ECG and BP data was found in terms of HR for all behavior categories (resting: $r^2 = 0.9999$, $P < 0.001$; feeding: $r^2 = 0.9994$, $P < 0.001$; active: $r^2 = 0.9992$, $P < 0.001$; Figure 2). The coefficient of determination for SDNN was significant only in terms of resting and feeding behavior (resting: $r^2 = 0.978$, $P < 0.001$ and feeding: $r^2 = 0.656$, $P < 0.01$), whereas only a weak association was found during active behavior ($r^2 = 0.045$, $P = 0.7$). In terms of RMSSD, a strong relationship between ECG and BP data was found during resting behavior ($r^2 = 0.943$, $P < 0.001$) but not during feeding ($r^2 = 0.146$, $P = 0.3$) or active behavior ($r^2 = 0.001$, $P = 0.9$).

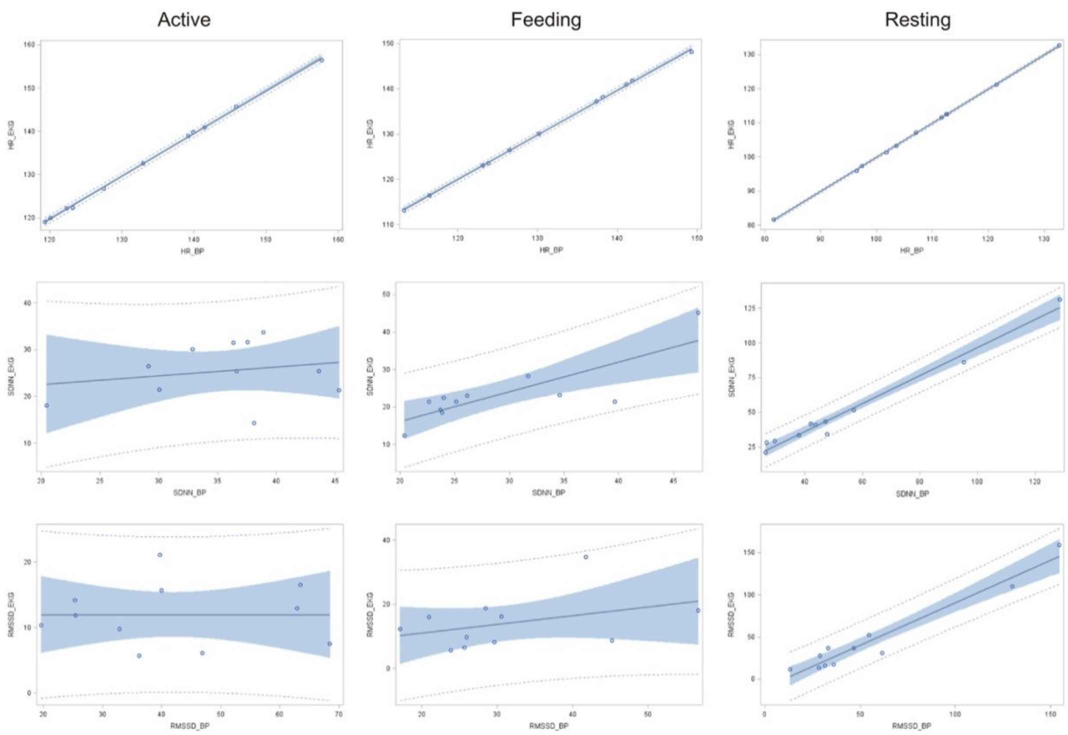

Figure 2. Linear regression of HR, SDNN, and RMSSD obtained via ECG in function of those obtained using BP ($n = 11$) with according 95% confidence limits (gray area) and 95% prediction limits (dotted line) during active behavior, feeding, and resting.

ICC

Intraclass correlation coefficient revealed a strong agreement between the HR data derived from ECG and BP for all behaviors (Table 1). ICC values >0.998 were obtained independent of activity level. Ninety-five percent CI for ICC varied scarcely between behavior categories, indicating that the true difference between these measurements was marginal. Notably, in terms of SDNN and RMSSD, interchangeable agreement was achieved only during resting behavior; none of the parameters reached the critical lower 95% CI value of 0.75 during feeding or active behavior.

Table 1. Intraclass correlation coefficients (ICCs) of heart rate (HR), SDNN and RMSSD derived from ECG and BP (n=11) with 95% confidence intervals during active behavior, feeding and resting.

	HR	SDNN	RMSSD
active	0.9989	0.0008 [a]	-0.0004 [a]
	(0.9976-1.0001)	(-0.5332-0.5332)	(-0.0932-0.0932)
feeding	0.9993	0.6027 [a]	-0.0004 [a]
	(0.9986-1.0001)	(0.2547-0.9507)	(-0.0932-0.0932)
resting	0.9998	0.9821	0.9466
	(0.9997-1)	(0.9627-1.0015)	(0.8898-1.0034)

[a] denotes ICCs which do not reach a value of 0.75 at a lower 95% confidence interval

Bland-and Altman plots

Bland and Altman plots (35) of HR, SDNN, and RMSSD in the three behavior categories are presented in Figure 3. A narrow LoA and a small bias (mean difference) were found for the HR calculated from IBIs derived from ECG and BP with the lowest values when animals were resting. In contrast, a wider LoA and a larger bias were found for the SDNN and to an even greater extent for the RMSSD with the highest values when animals were active (Table 2). Furthermore, as can be visually observed in Figure 3, an overestimation of RMSSD and SDNN was evident when calculated from IBIs derived from BP signals.

Table 2. Mean values of the differences (mean Δ) between HR, SDNN und RMSSD derived from ECG and BP (BP-ECG) and the respective standard deviations (SD) of the differences, as well as 95% limits of agreement (LoA) are presented for each parameter during active behavior, feeding, and resting.

			Active	**Feeding**	**Resting**
HR	Mean Δ		0.42	0.22	0.13
	SD		0.38	0.33	0.17
	95% LOA	Upper	1.17	0.87	0.46
		Lower	-0.32	0.25	-0.19
SDNN	Mean Δ		9.97	5.69	3.61
	SD		8.27	5.12	4.70
	95% LOA	Upper	26.18	15.73	12.82
		Lower	-6.24	-4.34	-5.61
RMSSD	Mean Δ		29.92	17.31	9.67
	SD		17.38	11.50	11.05
	95% LOA	Upper	63.99	39.85	31.33
		Lower	-4.14	-5.23	-11.98

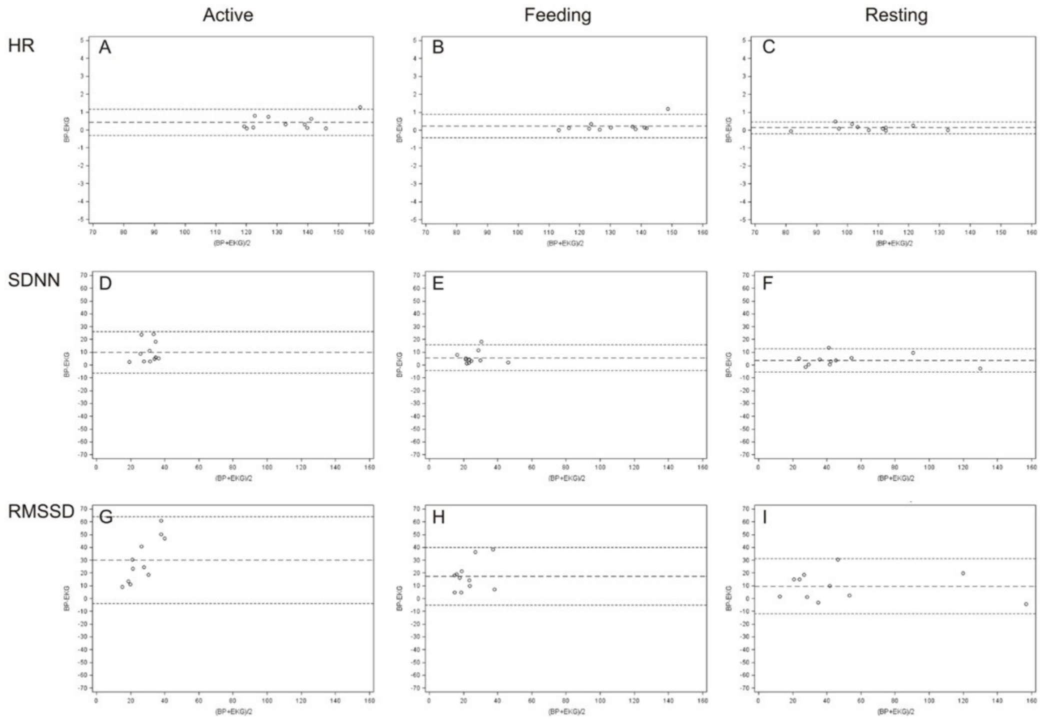

Figure 3. Bland and Altman plots of differences between ECG and BP measurements in terms of HR (A–C), SDNN (D–F), and RMSSD (G–I). The plots illustrate, in beats per minute [HR (bpm)] and in milliseconds [SDNN and RMSSD (ms)], the differences between values derived from ECG and BP on the *y*-axis (BP–ECG) against their average values on the *x*-axis for every behavior category [active (A,D,G), feeding (B,E,H), and resting (C,F,I)].

Analysis of variance (ANOVA)

Analysis of variance showed a significant effect of behavior on the mean HR ($F_{2,50}$ = 88.6, P < 0.001), but no effect of signal ($F_{1,50}$ = 0.02, P = 0.88; Figure 4). In terms of SDNN, both signal ($F_{1,50}$ = 10.7, P < 0.01) and behavior ($F_{2,50}$ = 26.9, P < 0.001), but not their interaction (P = 0.3), had a significant impact. *Post hoc* tests revealed differences between resting and feeding behavior (P < 0.001) as well as between resting and active behavior (P < 0.001) for ECG but only between resting and feeding for the BP signal (P < 0.001). The most pronounced effects were found in terms of RMSSD, which was affected by signal ($F_{1,50}$ = 59.8, P < 0.001), behavior ($F_{2,50}$ = 17.1, P < 0.001), and their interaction ($F_{2,50}$ = 7.0, P < 0.01). RMSSD differed significantly between the signals during feeding (P < 0.001) and active behavior (P < 0.001); however, no differences were found during resting behavior (P = 0.57). RMSSD derived from ECG showed significant differences between resting behavior and both feeding (P < 0.001) and active behavior (P < 0.001), whereas these effects dispersed in regards to RMSSD derived from the BP signal.

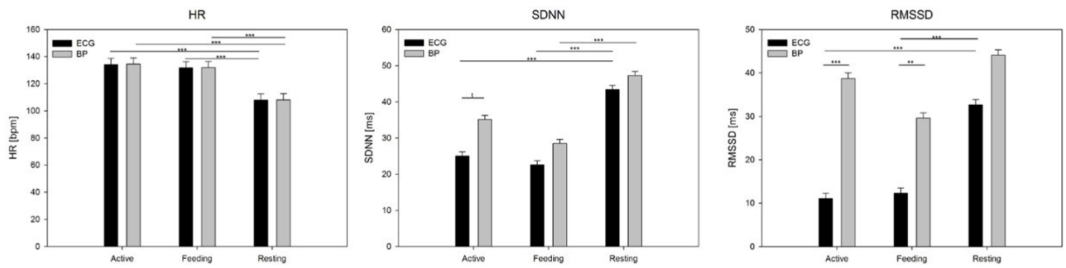

Figure 4. Mean values (±SE) of HR, SDNN, and RMSSD derived from ECG and BP during active behavior, feeding, and resting. Calculation was based on 5 min segments per behavior and averaged over individuals. Significance is given as follows: ***P < 0.001; **P < 0.01; *P < 0.05; and $^{\dagger}P$ < 0.1.

Discussion

The current study presents the first attempt to systematically investigate the usability of intra-arterial BP for the measurement of HR and HRV in comparison to ECG in free-moving domestic pigs in order to determine whether they agree sufficiently for BP to replace ECG in experimental settings. We used various statistical procedures to assess the relationship, interchangeability, and agreement of data derived from ECG and BP signals with different explanatory power. Our findings reveal strong association and agreement between ECG and BP data in terms of HR throughout all statistical procedures with high values for coefficients in linear regression, high ICCs (>0.9) and low biases with narrow LoA in all behavior categories. Regarding HRV, linear regression at least demonstrates a connection between the signals during resting, supported by results from ICCs. An ANOVA elucidated the discrepancies between ECG and BP measurements especially in terms of RMSSD. Bland and Altman plots demonstrated differences between ECG and BP in terms of HRV; large biases and wide LoA were found independently of the activity level.

There are several theoretical advantages to the use of BP in the assessment of HR and HRV in free-moving animals. BP was found to be less susceptible to movement artifacts than ECG signals (29) whereas ECG signals are more likely to develop anomalous or ectopic beats based on the frequent biological and system constraints that may generate and perpetuate the occurrence of these artifacts in cardiac signals (26). Therefore, automatic triggering of BP (systolic pressure waves) by the software requires less manual editing of falsely triggered beats and decreases the requirement of interpolation. If BP provides results comparable to those of ECG signal in HR and HRV measures, then the goal could be to replace ECG recordings with BP recordings in the event of imprecise ECG or complete malfunction. To answer the question of whether the two methods can be used interchangeably, Lee et al. (33) proposed three criteria for agreement: first, the lower limit of the 95% CI of the ICC should be at least 0.75, second, there should be no marked systematic bias and thirdly, and there should be no statistically significant difference between mean readings obtained by the two methods. However, investigations comparing of two or more variables usually utilize linear regression analysis (35). Indeed, a strong relationship was found between ECG and BP signals across all behavioral contexts in regards to HR. The coefficient of determination is the ratio of the variation to the total variation, which indicates the percent of the data that is closest to the line of best fit. Therefore, in the case of HR, it can be deduced that the line that the points fit accounts for 99% of the variation of the points from their mean. In terms of SDNN and RMSSD, >90% of the variability was explained by the linear model only during resting behavior. Feeding and active behavior severely reduced the coefficients of determination. We can safely conclude that measurements derived from ECG and BP signals are related, at least during resting behavior. However, this does not mean that the two methods agree because *r* measures the strength of a relationship between two variables, not the agreement between them (31). A study by Lee et al. (33) advocated ICC as the optimal statistic for measuring agreement between methods. ICC reveals interchangeable use in respect to HR during all behavioral contexts. Like the regression analysis, low values were found in terms of SDNN and RMSSD for feeding and active behavior. ECG and BP may be used interchangeably for measuring HRV during resting behavior as the lower 95% CI exceeds 0.75. Bland and Altman discussed the appropriateness of ICC, in which the average correlation across all possible orderings of pairs into *x* (BP) and *y* (ECG) is a ratio of the variability between subjects to the total variability (36). The more variable the subjects are, the greater the values of ICC become. Furthermore, Müller and Büttner also illustrated the limitations in the interpretation of ICC (37). The estimates are dependent on the range of the measuring scale; the wider the range, the better the result. This may cause high ICCs during resting behavior, as the range of SDNN and RMSSD values is physiologically higher during resting than during feeding or active behavior.

Bland and Altman plots were constructed to evaluate the differences in data obtained from the same subjects using two devices (ECG and BP) that measure the same criteria (35). This alternative method meets the requirement for depending not on the range of the sample but instead considering the differences between the measurements for each subject. The mean difference (bias) and the standard deviation of the differences enable the calculation of the size of the difference that is likely to arise between the two methods. Regarding HR, we found a mean difference of 0.26 bpm, and 95% of the differences lay between −0.37 and 0.89 bpm (LoA). Thus, it is unlikely (a probability of <0.05) that measurements using the two methods would differ by >1.26 bpm. The two methods could be used interchangeably if differences in measurements of this order did not matter. How far apart measurements can be without inducing difficulties is a question of judgment (34). If this is not sufficient to cause problems in interpretation, the old method can be replaced by the new or both can be used interchangeably. Regarding HR, differences between the measurements in all behavior categories were found to be acceptable, and the largest absolute bias of 0.42 bpm was found during active behavior. This value may be negligible for non-medical purposes in future research. In contrast, SDNN and RMSSD showed the greatest biases during active behavior, with correspondingly wide LoA. These values were not considered appropriate. Biases of 3.61 ms (SDNN) and 9.67 ms (RMSSD) during resting behavior were also not considered reasonable because these values may lead to the misinterpretation of HRV measurements in the context of autonomic control and balance. Noticeably, Bland and Altman plots illustrated a systematic overestimation of HRV parameters by BP signals throughout all behavior categories with the most pronounced effect on RMSSD. This overestimation was underpinned by the ANOVA results, which showed that the calculated HRV parameters from the two different signals consequently led to differing results within the various behavior categories. SDNN, which reflects long-term fluctuations in IBIs, was found to differ between active and resting as well as between feeding and resting, if the data were derived from ECG. Regarding SDNN derived from BP, only differences between feeding and resting were found. This effect becomes more apparent in RMSSD, which reflects short-term fluctuations in IBIs. The effect of behavior was completely absent from the BP signals, as RMSSD was not found to differ between active, feeding, or resting, whereas differences between these behaviors were found in RMSSD derived from the ECG signals.

Summarizing the results of the different statistical procedures, we conclude that ECG and BP may be used interchangeably to calculate HR; however, this is not the case for HRV measurements. This effect may be caused by the morphology of the BP signal. ECG provides very sharp and thus clearly recognizable peaks (R-waves in QRS complex), which facilitates exact peak definition. In contrast, the BP signal yields systolic pressure waves, which are substantially flatter and platykurtic compared with ECG waves. This may provide a further scope of triggering and may result in greater variation of the distances between successive IBIs. In the case of HR, this may not have consequences, as HR is measured using the mean number of beats per minute and, thus, the variance of single values is leveled. In contrast, in the context of HRV measurements, this inaccuracy in triggering leads to greater values throughout the measurement. This effect is more pronounced in RMSSD, because this short-term fluctuation is calculated from beat-to-beat variability, whereas SDNN reflects the deviation of all registered IBI within the 5-min segment. As a consequence, SDNN is less altered by this effect than RMSSD. Furthermore, a generally lower HRV is apparent during feeding and active behavior. Therefore, aberrations of trigger points in the BP signal may be more weighted in this case due to the smaller range in which variability is measured.

Overall, BP and BP variability are indispensable tools for the investigation of sympathetic activation which is not possible by measuring HRV alone. If ECG was found to be replaceable by BP in terms of HRV measurement, BP alone could be used as source for the investigation of autonomic regulation. This beneficial value could have found application in future research using non-invasive BP methods (e.g., ear worn sensors or tail cuffs). However, this study shows that ECG might be replaceable by BP recordings in terms of HR and potentially for HRV during resting conditions, but BP could not reliably be used in place of ECG in terms of HRV during feeding or active behavior in domestic pigs.

Conclusion

Considering the high values for regression and ICC as well as narrow LoA and the lack of relevant bias in the Bland and Altman plots, our results suggest, on the one hand, very good to excellent agreement between the ECG and BP recordings in terms of HR. Both signals can be considered interchangeable across all behavior categories. On the other hand, in the context of HRV, a lack of relationship and agreement between both signals during feeding and active behavior was demonstrated using all statistical approaches. High values for regression and ICC were found only during resting. This supported interchangeable use at least during resting behavior. However, large biases with accordingly wide LoA in terms of SDNN and RMSSD during resting were considered unacceptable for our purposes. We conclude that HR values derived from BP agreed well with those derived from ECG independently of the activity of the subject. Additionally, ECG and BP should not be used interchangeably in the context of HRV in free-moving domestic pigs. These findings that HRV may not readily be determinable by using BP signal contribute to the validity and application of HRV measurement in future studies, such as in the field of Animal Welfare Research.

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2.3 Study 3: Coping style modifies general and affective autonomic reactions of domestic pigs in different behavioral contexts

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*Corresponding authors

Annika Krause designed and performed the experiment, analysed the data and wrote the manuscript with the support of and in agreement with the co-authors of this manuscript.

Abstract

Based on individual adaptive strategies (coping), animals may react differently to environmental challenges in terms of behavior and physiology according to their emotional perception. Emotional valence as well as arousal may be derived by measuring vagal and sympathetic tone of the autonomic nervous system (ANS). We investigated the situation-dependent autonomic response of 16 domestic pigs with either a reactive or a proactive coping style, previously selected according to the backtest which is accepted in piglets to assess escape behavior. At 11 weeks of age, the pigs were equipped with an implantable telemetric device, and heart rate (HR), blood pressure (BP) and their respective variabilities (HRV, BPV) were recorded for 1 h daily over a time period of 10 days and analyzed in four behavioral contexts (resting, feeding, idling, handling). Additionally, the first minute of feeding and handling was used for a short-term analysis of these parameters in 10-s intervals. Data from day 1–3 (period 1) and day 8–10 (period 2) were grouped into two separate periods. Our results revealed general differences between the coping styles during feeding, resting and handling, with proactive pigs showing higher HR compared to reactive pigs. This elevated HR was based on either lower vagal (resting) or elevated sympathetic activation (feeding, handling). The short-term analysis of the autonomic activation during feeding revealed a physiological anticipation reaction in proactive pigs in period 1, whereas reactive pigs showed this reaction only in period 2. Food intake was characterized by sympathetic arousal with concurrent vagal withdrawal, which was more pronounced in proactive pigs. In contrast, neither coping style resulted in an anticipation reaction to handling. Vagal activation increased in reactive pigs during handling, while proactive pigs showed an increase in sympathetically driven arousal in period 2. Our findings confirm significant context-related differences in the general autonomic reaction of pigs with different coping styles. Additionally, the two coping styles differ in their affective appraisal over the time course of the experiment, underlining the importance of taking individual differences into account when studying affect and emotion in humans and animals.

Introduction

Individual reaction patterns have been intensively investigated in different species in behavioral and evolutionary ecology in recent years. Consistent individual differences in the average level of behavior across time and contexts are commonly referred to as "animal personality" and exist in a range of animal taxa (Dall et al., 2004; Réale et al., 2007; Biro and Stamps, 2008; Dingemanse et al., 2010). It has been widely recognized that such individual variations play an important role in health and disease in humans and in animals (Koolhaas et al., 1999). One key element in this context is "coping", which comprises a set of behavioral and physiological characteristics of an individual trying to master the challenge of an aversive situation (Lazarus, 1966). From the viewpoint of stress research, generally two fundamental coping patterns may be distinguished in animals: a "proactive or active pattern" on the one hand and a "reactive or passive pattern" on the other hand (Henry and Stephens, 1977). Despite domestication, targeted selection, genetic modification and inbreeding, the same coping strategies can also be observed in laboratory and farm animals. Studies in rodents basically distinguish between proactive and reactive coping (Koolhaas et al., 1986 [rats]; van Oortmerssen et al., 1985 [mice]) and studies in fish and birds often use the terms shyness and boldness (Wilson et al., 1994). In pigs, the concept of coping is also supported, although the extremes in the population do not represent distinct categories of pigs (Ruis et al., 2002; Zebunke et al., 2015). Studies focusing on coping styles in animals suggest that the proactive response is characterized by a typical active fight-flight response in aversive situations which is associated with territorial control, aggression and risk-taking (Cannon, 1929; Benus et al., 1990; Mount and Seabrook, 1993; Koolhaas et al., 1999). Conversely, the reactive coping style shows a conservation withdrawal response in aversive situations, which is characterized behaviorally by immobility and low levels of aggression (Engel and Schmale, 1972). Generally, the two coping styles differ fundamentally in their general adaptive response patterns in reaction to challenges (Koolhaas et al., 1999).

These challenges, just as other types of internal and external stimuli, also exert physiological effects, as they affect the activity of the autonomic nervous system (ANS). The perception of a challenging situation or stimulus leads to specific hierarchical, neurophysiological changes that originate from the brain, which is the key organ in reacting to and coping with stress. The first affective reaction was described as a fast neurophysiological reaction to prepare the body for action in a given circumstance (Posner et al., 2005). This process occurs subcortically and is therefore unconscious. Further processing of affect recruits additional brain systems and results in the experience of a subjective feeling or emotion, based on neocortical processing (Russell, 2003). This distributed neural circuitry determines what is threatening and stressful to the individual. Brain regions such as the amygdala, the hypothalamus and the brain stem are essential for autonomic responses to stressors, as they control the activity of sympathetic and vagal tone at the heart and the vessels, which affects sinoatrial node activity and blood pressure (BP; Carretié et al., 2009). The complex interaction between the two parts of the ANS produces complex variations in heart rate (HR) and BP. HR variability (HRV) as a result of this rhythmic oscillation is widely used as an indicator of autonomic function in the analysis of physiological signals in humans and animals (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; von Borell et al., 2007). HRV parameters provide information about the complex interaction between the two branches of the ANS or about vagal regulatory activity. In contrast, BP fluctuations deliver information about sympathetic control, as Hedman et al. (1992) found that blocking sympathetic nerve transmission decreased all components of BP variability

(BPV), whereas vagotomy decreased HRV but did not influence BPV. Recent autonomic blockade studies in pigs support this assumption (Poletto et al., 2011). Emotional perceptions of different natures can induce different shifts of autonomic balance, towards either a sympathetic or vagal prevalence. Therefore, analyzing cardiovascular dynamics is regarded as a suitable approach to draw conclusions about changes in sympathovagal balance related to ongoing appraisal processes and emotional states (Porges, 2003; Boissy et al., 2007; von Borell et al., 2007; Reefmann et al., 2009). From human research, it is known that emotional affective states can be defined in terms of two fundamental underlying dimensions. Emotional experiences are valenced, ranging from positive to negative and they also vary in the degree of arousal ranging from low to high (Russell and Barrett, 1999; Burgdorf and Panksepp, 2006; Mendl et al., 2010). Regarding autonomic regulation, researchers have investigated the relationship between the two dimensions and the activity of the two branches of the ANS. Several studies demonstrate a link between cardiac vagal tone and psychological components in negative contexts such as anxiety (Sleigh and Henderson, 1995) or panic attacks in humans (Friedman and Thayer, 1998) as well as in positive contexts, for example, in humans watching an emotionally positive film (Matsunaga et al., 2009) or in pigs showing a state of positive arousal when they were individually called to feeding in an operant conditioning paradigm (Zebunke et al., 2011). These studies emphasize that positive emotions significantly increase vagal tone, whereas the opposite occurs with negative emotions. Therefore, vagal tone is assumed to reflect the valence dimension of affect. The arousal dimension was investigated in numerous studies in different species describing sympathetic activation (de Boer et al., 1990) and a rise in HR (Farah et al., 2004) in reaction to negative situations, whereas other authors describe similar results in positive contexts such as appetitive conditioning in primates (Braesicke et al., 2005). The fact that both positive and negative affective states may increase sympathetic tone supports the assumption that it may represent the arousal dimension of affect (Yeates and Main, 2008; Mendl et al., 2010).

In recent years, advances in technology allow the use of mobile, fully implantable telemetric techniques that are able to verify a number of cardiovascular parameters. Usually, such systems find application in the context of pharmacological methods and cardiovascular diseases (Gelzer and Ball, 1997; Stubhan et al., 2008). Using the domestic pig as a suitable animal model, previous studies have shown that an implantable telemetric system, capable of measuring electrocardiogram (ECG) and intra-arterial BP simultaneously in free-ranging animals, resulted in the reliable assessment of both branches of the ANS while physiological variables were precisely assessed with minimal disturbance to the animal (Krause et al., 2015, 2016). Differences between the coping styles were found in numerous studies in the context of behavior (Koolhaas et al., 1999), physiology (Hessing et al., 1994a; Ruis et al., 2000), immune responses (Oster et al., 2015) and genetic variants (Ponsuksili et al., 2015). Coping behavior associated with detailed autonomic regulation remains largely understudied (for review, see Koolhaas et al., 1999, 2010; de Boer et al., 2017). In the present article, we used behavioral differences, resulting in proactive or reactive coping patterns, to identify phenotypes related to autonomic responses during different behavioral contexts. Autonomic reaction was investigated using an implantable telemetric device in free-ranging animals to assess both branches of the ANS. The first aim of the present study was the assessment of the general autonomic reaction of pigs with different coping styles in different behavioral contexts to identify the link between coping patterns and their respective autonomic reaction in relation to behavior. The second aim was the determination of short-term affective autonomic reactions of pigs during feeding and handling to reveal differences in their affective appraisal by characterizing the valence and arousal dimensions of affect in relation to coping styles. We expected context-related differences between the coping styles in their general autonomic reaction and in their affective appraisal depending on the respective behavioral situation.

Animals, materials and methods

Ethical statement

Animal care and all experimental procedures were in accordance with the German welfare requirements for farm animals and the ASAB/ABS Guidelines for the Use of Animals in Research (Anonymous, 2016). All procedures involving animal handling and treatment were approved by the Committee for Animal Use and Care of the Ministry of Agriculture of Mecklenburg-Vorpommern, Germany (Ref. Nr. 7221.3-1.1-037/12).

Animals and housing

In three identical replicates, a total of 16 female domestic pigs (*Sus scrofa*, German Landrace) from the experimental facilities for swine of the Leibniz Institute for Farm Animal Biology (FBN) in Dummerstorf were studied from 11 weeks of age. For each replicate, eight piglets were selected on the basis of their previously tested behavioral response in the backtest in order to describe their coping style as either proactive or reactive. Selected piglets were weaned at 28 days of age and kept in groups of eight (4 proactive, 4 reactive piglets) until 10th week of life, when pigs were moved to the experimental housing facilities of the institute. They were housed in individual pens (2.67–3.31 m^2) with a solid and partially slatted floor and visual, olfactory, acoustic and partial tactile contact with conspecifics (snout contact) in the adjacent pens. The pens were separated by means of transparent walls (acrylic glass) that enabled full sight of the adjacent pens. A metal food trough with a lockable cover (23 × 42 cm) was fitted next to the entrance of the pen. The animals were fed twice a day and had free access to water (nipple drinker). The individual pens were located in an experimental room, which provided an area for technical equipment, an electronic balance and pig food including the buckets. The temperature was held constant at 20°C. During 1 week of acclimatization, the pigs were weighed and handled three times daily for 1 h to socialize and habituate them to human contact and equipment monitoring. At 11 weeks of age (approximately 30 kg weight), 4–6 pigs in each replicate underwent surgery for the implantation of a telemetric device capable of recording ECG, BP and body temperature (Krause et al., 2016). After surgery, pigs were returned to their familiar individual pen for the following 3 weeks. Based on technical issues concerning the telemetric system, two transmitters did not transfer any cardiovascular data. After these animals were excluded from further analysis, the data of 14 pigs were included in the study.

Backtest and classification

The backtest was carried out at the ages of 5, 12, 19 and 26 days, according to the methodology described by Zebunke et al. (2015). The piglets were housed in a commercial farrowing pen measuring 2 × 3 m with a crate and creep area. The pens included a fully slatted floor (except for the creep area, which contained a heating panel and a metal plate for the sow's front legs). A V-shaped cradle fixed on a table was positioned in front of each farrowing pen and used for the backtest. Each piglet was put in the cradle in a supine position on its back for 1 min. Three parameters were recorded: (i) latency (time span until the pig showed the first struggling attempt); (ii) total duration; and (iii) frequency of all struggling attempts within 60 s. The classification of piglets in either proactive or reactive coping styles was based on previously defined upper and lower quartiles of the recorded parameters to determine the respective classification ranges (latency: 5 and 35 s, duration: 5 and 25 s, frequency: 1 and 4; Oster et al., 2015; Ponsuksili et al., 2015). A total of 12 measures per piglet (three recorded parameters in four repetitions) were considered for classification into proactive ("high resisting"), reactive ("low resisting") or doubtful. If the classification was statistically significant (binomial test), the piglet fell in one of the categories. For each replicate, 80 female piglets were tested in the backtest, while eight piglets (4 reactive and 4 proactive) were selected for the study and finally 4–6 pigs underwent surgery and were used for the experiment.

Telemetric equipment and signal monitoring

The telemetric system consisted of four components: the implantable device (transmitter), individual receivers, data acquisition device (Power Lab) and analysis software (LabChart) and was provided by Telemetry Research (Auckland, New Zealand) and ADInstruments (Oxford, UK). The implantable transmitter unit (Telemeter model TRM84PB, 90 mm × 45 mm × 10.5 mm, 64 g) measures and transmits ECG using two flexible biopotential leads (30 cm in length) and intra-arterial BP using a fluid-filled catheter (1 mm OD (3Fr) Catheter, Millar Instruments, Houston, TX, USA) via telemetry from within the animal. A temperature sensor embedded in the implant case was used to measure the animal's body temperature. Each implanted transmitter relayed digital signals to a receiver unit using an individual frequency. A switch at the receiver enabled the transmitter to be turned on and off *in vivo* without affecting the animal. The receivers were connected by wire to two digital data acquisition systems (PowerLab) that allowed the simultaneous real-time recording of up to six pigs at a sampling rate of 2 kHz. The sampled data were stored and displayed on a PC and analyzed using LabChart Pro (Version 7.0, ADInstruments).

Transmitter implantation

For detailed information regarding surgery, please see Krause et al. (2016). Here, we give a brief overview of the implantation process. The pigs were fasted 12 h prior to surgery but allowed access to water. The animals were subsequently implanted with the telemetric device by the veterinarian of the institute under sterile conditions. Prior to surgery, the pigs were anesthetized in their home pen with a combination of xylazine 2 mg/kg (Xylariem, Riemser Arzneimittel) and ketamine 20 mg/kg (Ursotamin, Serumwerk) intramuscularly. For local anesthesia, procaine 4 mg/kg (Isocain, Selectavet, Dr. Otto-Fischer) was injected subcutaneously according to the planned incision lines. During surgery, general anesthesia was maintained using ketamine 4 mg/kg/h (Ursotamin, Serumwerk) and diazepam 0.4 mg/kg/h (Faustan, AWD-pharma) intravenously in a 5% glucose solution. The telemetric device was placed in a subcutaneous pouch at the left side of the neck. The positive electrode was positioned dorsal ~2 cm lateral to the left scapula, while the negative electrode was located lateral to the sternum. Both electrode tips were enclosed by muscle tissue. The pressure sensor was inserted into the left carotid artery. After completing surgery, pigs were returned to their respective home pens and allowed to recover under a heat lamp to maintain body temperature after surgery. They were observed continuously and body temperature, HR and BP were controlled every hour until they recovered consciousness and exhibited complete recovery from anesthesia. The pigs were administered a postsurgical dose of 50 mg/kg metamizole (Metapyrin, Serumwerk) and a combination of 20 mg/kg sulfadimidine and 4 mg/kg trimethoprim (Trimethosel, Selectavet, Otto-Fischer GmbH) every 24 h for 5 days. Skin sutures were removed on days 10 and 11 postoperatively.

Data acquisition

Behavior, ECG, BP and body temperature were recorded for 1 h daily from 08.00 a.m. to 09.00 a.m. over a time period of 10 days after surgical implantation of the transmitter. A familiar person entered the experimental room at 08.15 a.m. daily to feed and at 08.45 a.m. to handle the pigs in each individual pen (2 min each). The handling procedure started with entering the single pen. Once the animal had approached the familiar person, including tactile snout contact, it was gently stroked and scratched. Pig behavior was assessed using video cameras (Panasonic WV-CP500, EverFocus Endeavor SD + HD DVR) attached to each single housing pen and analyzed using the Observer XT (Version 11, Noldus, Wageningen, Netherlands). Cardiovascular data were calculated using LabChart. Mean HR and the short-term variation of HR (RMSSD = root mean of the squared distances of subsequent inter-beat-intervals, an indicator of vagal activation) were obtained from ECG recordings. Using the BP signal, systolic BP (SBP) and its standard deviation (SD_{SBP}, indicative of sympathetic activation) were assessed.

Analysis of the general autonomic reaction in different behavioral contexts
Cardiovascular data during four behavior categories, each 5 min in duration (recorded daily over a time period of 10 days), were chosen for analysis: resting (lying inactive during the prefeeding period), feeding (08.15 a.m.), idling (locomotion, exploration of floor or wall after the feeding period) and handling (08.45 a.m., 2 min).

Analysis of the affective autonomic reaction during feeding and handling
Additionally, to evaluate affective appraisal in the pigs, a time span of 50 s each from the feeding and handling situations was chosen for more detailed analysis of the autonomic activity in 10-s intervals. For the feeding situation, we chose the time period from 20 s before feeding to 30 s after feeding. Time interval (TI) 1 included 10 s before a familiar person entered the room (TI 1: –20 to –10 s). Only pigs with active (idling) behavior during this TI were included in the analysis (pigs that were lying down were excluded) due to the large effect of physical activity on cardiovascular parameters. TI 2 (–10 to 0 s) included the 10 s immediately before feeding. The person entered the room, and prepared the buckets for feeding. During this TI, the pigs had the opportunity to develop an anticipation reaction over the time course of the experiment. With the beginning of TI 3, the pigs were fed. TI 3–5 involved food intake itself.

In the handling situation, the time span from 20 s before entering the single pen to 30 s after entering the single pen was chosen. TI 1 included the time before the familiar person was present (–20 to –10 s). Here, again, only pigs with active behavior were included in the analysis. During TI 2 (–10 to 0 s), the familiar person was standing in front of the single housing pen. With the beginning of TI 3, the person entered the single pen for handling. During TI 3, 4 and 5 (0–30 s), the person was present in the single pen. To assess the development of the autonomic reaction over the time course of the experiment in both repeated situations (feeding and handling), data from day 1–3 and from day 8–10 were grouped into two separate periods (period 1: day 1–3, period 2: day 8–10).

Approach latencies in the handling context
Approach latencies which comprised the time from entering the single pen until first tactile snout contact towards the familiar person were recorded.

Statistical analysis

All statistical analyses were conducted using SAS (version 9.3, 2009, SAS Institute Inc., Cary, NC, USA). The normality of the distribution of each parameter was assessed using Kolmogorov-Smirnov tests. Where normality assumptions were not met, data were logarithmically transformed. The data were evaluated by analysis of variance (ANOVA) using the GLIMMIX procedure in SAS/STAT software. All analyses included the animal as a repeated factor and mean differences with a $p < 0.05$ were considered significantly different.

Analysis of the general autonomic reaction in different behavioral contexts

The model for the measured parameters HR, RMSSD, SBP, SD_{SBP} and body temperature contained the main effects replicate (1–3), experimental day (1–10), behavior (resting, feeding, idling and handling), coping style (reactive, proactive) and their respective two-way interactions. Least squared means (LSMs) and their standard errors (SEs) were computed for each fixed effect in the model. All pairwise differences of these LSM were tested using the Tukey-Kramer method, the procedure for pairwise multiple comparisons with the best power. In addition, the slicediff option of the LSM statement of the GLIMMIX procedure was used for partitioned analyses of the LSM for the two-way interaction of coping style × behavior (i.e., comparisons between the coping styles within the respective behavioral context and vice versa).

Analysis of the affective autonomic reaction during feeding and handling

The statistical model consisted of fixed effects of the replicate (1–3), period of the experiment (period 1, period 2), TI (1–5), coping style (reactive, proactive) and their respective interactions, including three-way interaction. LSM and SE were computed for each fixed effect in the model and all pairwise differences of these LSM were tested using the Tukey-Kramer method. For comparisons of the coping styles within each TI and each period (i.e., performing partitioned analyses of the LS-means for the interaction of period × TI × coping style), the slicediff option of the LSM statement of the GLIMMIX procedure was applied.

Approach latencies in the handling context

Data on approach latency were analyzed for the repeated handling situation, using a model with the fixed factors replicate (1–3), period of the experiment (period 1, period 2), coping style (reactive, proactive) and the interaction of coping style × period. LSM and SE were computed for each fixed effect in the model. Pairwise differences between LSMs were evaluated using the Tukey-Kramer method.

Results

Impact of surgical procedure on cardiovascular parameters and body temperature in a 10-day convalescence period

Within the different behavioral contexts, the cardiovascular parameters did not change over the 10-day period after the surgical procedure (interaction of the fixed effects day × behavior: HR: $F_{(27,397)} = 0.94$, $p = 0.5$; RMSSD: $F_{(27,338)} = 0.23$, $p = 0.99$; SBP: $F_{(27,321)} = 0.74$, $p = 0.83$, SD_{SBP}: $F_{(27,301)} = 1.1$, $p = 0.34$). Body temperature was found to be affected by the day ($F_{(9,108)} = 4.4$, $p < 0.001$), whereas the coping style did not have any effects ($F_{(1,10)} = 0.66$, $p = 0.43$). The lowest body temperature was measured at day 1 (39.3 ± 0.06°C), but an increase was found at day 3 (39.6 ± 0.06°C), followed by a stable course until the end of the experiment. After the postsurgical medication was concluded at day 5, body temperature remained constant. All pigs showed an uncomplicated healing process, as they appeared in good health with fast wound healing, normal grooming behavior and the absence of any inflammation reactions or complications.

General autonomic reaction in different behavioral contexts

We found a significant effect of the interaction of coping style × behavior on HR ($F_{(3,209)} = 3.7$, $p < 0.05$), RMSSD ($F_{(3,343)} = 9.09$, $p < 0.001$), SBP ($F_{(3,322)} = 6.9$, $p < 0.001$) and a tendency on SD_{SBP} ($F_{(3,303)} = 2.3$, $p < 0.1$). SD_{SBP} was significantly affected by the factor behavior ($F_{(3,303)} = 108.9$, $p < 0.001$). As shown in Figure 1, the results revealed a significantly higher HR in proactive pigs during resting ($p < 0.01$), feeding ($p < 0.05$) and handling ($p < 0.05$) compared to reactive pigs. The difference in HR during resting was accompanied by lower RMSSD values in proactive than reactive pigs ($p < 0.001$). During feeding, the differences in HR between the coping styles were associated with higher SD_{SBP} ($p < 0.01$) and a trend to lower RMSSD ($p < 0.1$) in proactive pigs. The elevated HR in proactive pigs ($p < 0.05$) during handling was exclusively accompanied by elevated SD_{SBP} values ($p < 0.01$). During idling, no differences in autonomic regulation were found between the coping styles (all parameters: $p > 0.1$).

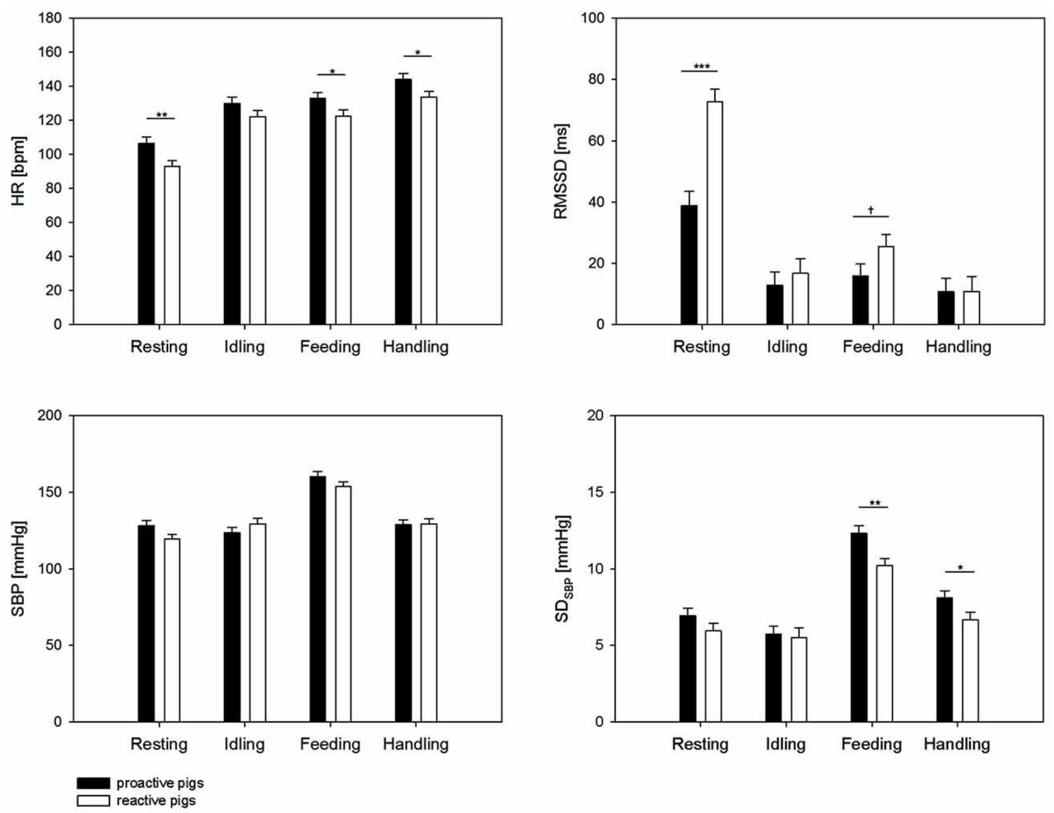

Figure 1. Heart rate (HR [bpm]), root mean square of successive differences (RMSSD [ms]) systolic blood pressure (SBP [mmHg]) and standard deviation of SBP (SD_{SBP} [mmHg]) during 5 min of resting, idling, feeding and 2 min of handling. Black bars: proactive pigs, white bars: reactive pigs. Data are presented as least squared means and standard errors (LSM ± SE). Significant differences between the coping styles during the respective behavior are indicated by asterisks ($^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) and trends are indicated by † ($^{\dagger}p < 0.1$).

Affective autonomic reaction during feeding

The detailed analysis of the feeding situation showed that all cardiovascular parameters changed significantly over the TIs (HR: $F_{(4,245)} = 42.9$, $p < 0.001$; RMSSD: $F_{(4,193)} = 15.6$, $p < 0.001$; SBP: $F_{(4,196)} = 74.8$, $p < 0.001$, SD_{SBP}: $F_{(4,129)} = 14.7$, $p < 0.001$; Figure 2). In period 1, proactive pigs showed an increase in HR in anticipation of food (TI 2) when the person entered the room to prepare the buckets. This effect was consistent over the periods and was accompanied by a parallel increase in RMSSD ($p < 0.01$) and SD_{SBP} ($p < 0.001$) in proactive pigs. Similar autonomic activation was found in reactive pigs, but not until period 2. In period 2, reactive pigs developed higher RMSSD ($p < 0.001$) and SD_{SBP} values ($p < 0.01$) during TI 2 compared to period 1. With the beginning of feeding in TI 3 (0–10 s), HR rises to its maximum in both coping styles and both periods. The period was found to have an effect on RMSSD ($F_{(1,208)} = 7.7$, $p < 0.01$). RMSSD showed a general increase in period 2 compared to period 1 ($p < 0.001$). This effect was primarily accompanied by higher RMSSD values in reactive pigs compared to proactive pigs ($p < 0.001$) during food intake (TI 5) in period 2. Nevertheless, this effect was not reflected in HR.

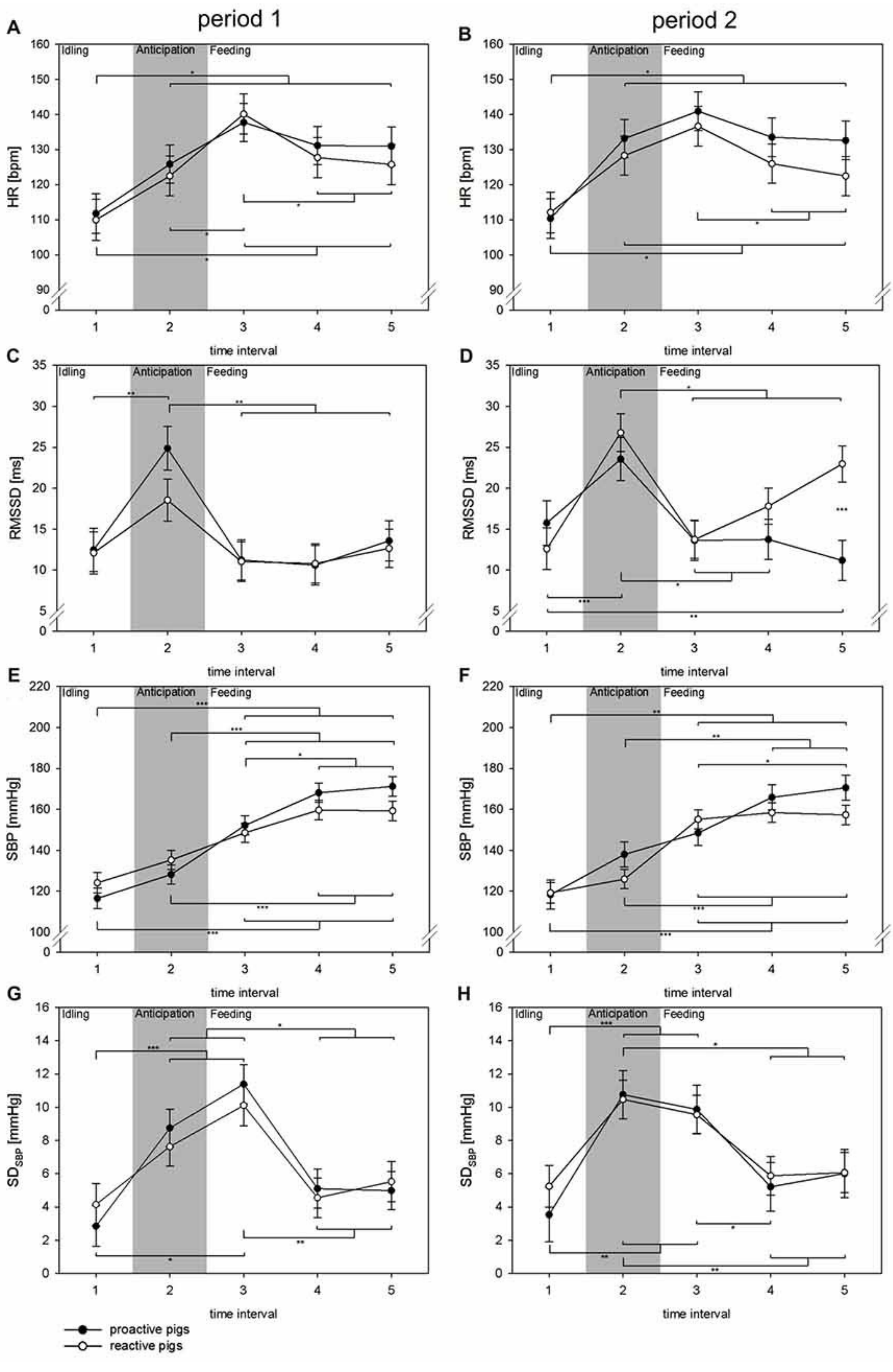

Figure 2. Changes in HR (A,B [bpm]), RMSSD (C,D [ms]), SBP (E,F [mmHg]) and SD_{SBP} (G,H [mmHg]) in time intervals (TIs) of 10 s (TIs 1–5) in experimental period 1 (left: A,C,E,G) and experimental period 2 (right: B,D,F,H) in the context of feeding. Black dots: proactive pigs, white dots: reactive pigs. Data are presented as LSM ± SE. Significant differences (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) between TIs are shown above (proactive pigs) and below (reactive pigs) the dots. Differences between the coping styles in each TI are presented between the dots. Gray area: the person is entering the room and preparing food buckets.

Affective autonomic and behavioral reactions during handling

Significant effects of the interaction of period × coping style ($F_{(1,68)} = 6.1$, $p < 0.05$) were found on the approach latency towards the familiar person in the pen. Reactive pigs showed longer latencies to approach the person in period 1 compared to proactive pigs ($p < 0.05$) and compared to period 2 ($p < 0.001$), as shown in Table 1. The difference between the coping styles disappeared in period 2.

Table 1. Approach latencies: time (s) from a familiar person entering the individual pen until the pig has approached and touched the person with the snout in period 1 (first 3 days of experiment) and period 2 (last 3 days of experiment).

	period 1	period 2
proactive pigs	3.4 ± 1.5^{a}	2.4 ± 1.5^{a}
reactive pigs	9.5 ± 1.5^{b}	2.2 ± 1.5^{a}

Data are presented as least squared means and standard errors (LSM ± SE). Different letters (a, b) indicate statistically significant differences ($p < 0.05$) across lines (coping styles) and columns (periods).

Concerning the autonomic reaction during handling, the fixed factor TI showed a significant effect on all parameters (all: $p < 0.001$). The interaction of period × coping style was found to have a significant impact on HR ($F_{(1,379)} = 7.7$, $p < 0.01$) and SD_{SBP} ($F_{(1,142)} = 4.9$, $p < 0.05$), whereas the interaction of TI × period × coping style was significant in terms of RMSSD ($F_{(4,315)} = 2.6$, $p < 0.05$). Additionally, the interaction of TI × coping style showed a significant effect on HR ($F_{(4,376)} = 3.3$, $p < 0.05$). As shown in Figure 3, a strong increase in RMSSD ($p < 0.05$) was found at the moment the person was standing in front of the single pen (TI 2) in period 1. This was more pronounced in reactive pigs than in proactive pigs ($p < 0.05$). In both coping styles, no related changes in SD_{SBP} or HR were found in this TI. In period 2, RMSSD also increased during TI 2, but the differences between the coping styles disappeared in this TI. As the person entered the single pen for handling (TI 3), HR increased markedly in period 1 in both coping styles (proactive: $p < 0.05$, reactive: $p < 0.001$) accompanied by a strong withdrawal in the RMSSD, which was more pronounced in reactive pigs (TI 2 vs. TI 3: proactive: $p < 0.01$, reactive: $p < 0.001$) and a rise in SD_{SBP} only in reactive pigs ($p < 0.05$). In period 2, reactive pigs showed an elevated RMSSD during handling (TI 3) compared to period 1 and compared to TI 1, before the person was present. This effect was also reflected in HR, which remained constantly low after the person had entered the single pen in period 2. Proactive pigs showed a strong increase in SD_{SBP} and HR in period 2 during handling compared to period 1 ($p < 0.01$) and compared to reactive pigs ($p < 0.05$).

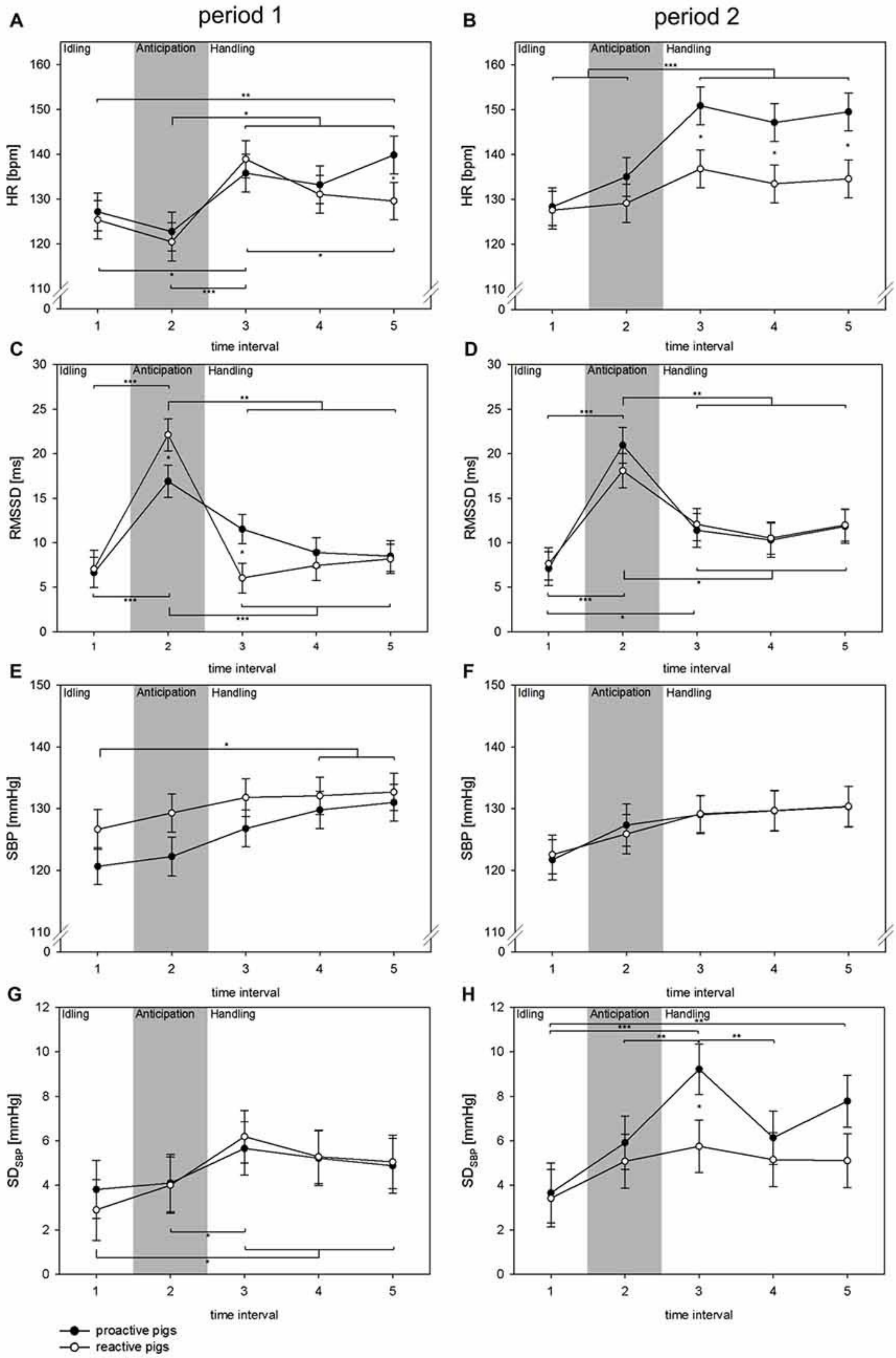

Figure 3. Changes in HR (A,B [bpm]), RMSSD (C,D [ms]), SBP (E,F [mmHg]) and SD_{SBP} (G,H [mmHg]) in TIs of 10 s (TIs 1–5) in experimental period 1 (left: A,C,E,G) and experimental period 2 (right: B,D,F,H) in a handling context with a familiar person. Black dots: proactive pigs, white dots: reactive pigs. Data are presented as LSM ± SE. Significant differences (*p < 0.05, **p < 0.01, ***p < 0.001) between intervals are shown above (proactive pigs) and below (reactive pigs) the dots. Differences between the coping styles in each TI are presented between the dots. Gray area: the person is standing in front of the single pen for 10 s.

Discussion

To our knowledge, this is the first study analyzing both branches of the ANS during different behavioral situations in regard to different coping styles in domestic pigs. Our study investigated the general autonomic activity as well as the short-term affective autonomic reaction of different coping styles to characterize the emotional valence and arousal dimensions of affect. Our study demonstrates that the determination of individual coping styles, which were classified on the basis of a proactive or reactive behavioral response in a repeated backtest, revealed different autonomic responses in selected behavioral contexts. Additionally, the coping styles also differed in their affective appraisal in two repeated situations over the time course of the experiment, as seen from different autonomic activation.

General autonomic reaction in different behavioral contexts

In the present study, the pigs' coping style was related to differences in the ANS activity in three of four behavioral contexts. The results indicated lower HR accompanied by higher vagal activity during resting in reactive pigs than in proactive ones. High vagal tone has been linked to efficient autonomic activity, which enables the individual to increase its response to physiological and environmental challenges (Friedman and Thayer, 1998). In humans, resting autonomic activity was found to be associated with several behavior traits such as cooperative behavior (Beffara et al., 2016), chronic aggression (Mawson, 2009) and impulsiveness (Mathias and Stanford, 2003). In line with our results, higher resting HR and lower vagal activity was found in impulsive cows compared to reserved individuals (Kovács et al., 2015). Similar effects were also found in the passive coping style of hens (low feather pecking line), characterized by a strong cardiac parasympathetic response during manual restraint (Korte et al., 1999). In contrast, during 5 min of feeding and handling, the elevated HR in proactive pigs was accompanied by stronger sympathetic activation compared to reactive pigs. This finding supports the assumption that proactive coping is associated with higher activity of the sympatho-adreno-medullary system (Cannon, 1929). Several studies describe higher sympathetic reactivity in proactive coping in different species and different stressful situations (Korte et al., 1997 [chicken]; Fokkema et al., 1995 [rats]). Higher HR in proactive pigs was also demonstrated during the backtest in a study by Hessing et al. (1994a). However, the authors could only indirectly conclude that the tachycardia demonstrated arousal of the sympathetic system, as they did not record specific measures for either parasympathetic or sympathetic activation. A study from Désiré et al. (2004) found increased HR in lambs in reaction to the sudden presentation of an object. During this increase in HR, there was no modification of its variability, as seen in the stability of RMSSD, which reflects vagal activity. The authors concluded that there had been a compensatory reaction by an increase in the activity of the sympathetic branch, as there is no reliable HRV indicator of the activity of the sympathetic branch. By means of the novel telemetric technique used in our study, the simultaneous measurement of ECG and BP and the subsequent calculation of HRV and BPV enabled the quantification of both, parasympathetic and sympathetic activity in free-ranging pigs.

Affective autonomic reaction during feeding

As access to food is highly intrinsically motivating in pigs (Day et al., 1995), any stimulus in the context of food is considered biologically relevant for these animals. In the current study, autonomic arousal accompanies the anticipation as well as the consumption of food. Owing to restricted feeding in this study, it is likely that the pigs were highly motivated to feed. The pigs learned that the sight of food combined with the acoustic signal of preparing the food-pellets in buckets led to access to that food soon after. This state of high motivation paired with the resulting attention of the pigs towards the food buckets induced sympathetic activation, which indicated a high state of arousal. Concomitantly, we found a parallel increase in vagal activation. Similar autonomic changes were also found in monkeys (Braesicke et al., 2005) during the sight of highly preferred food. Proactive pigs developed this anticipatory reaction in period 1, whereas the reaction did not develop in reactive pigs until period 2. This finding supports results in maze experiments in rodents showing that animals with proactive and reactive coping strategies differ in the degree to which their behavior is guided by environmental cues (Koolhaas et al., 1999, 2010). The proactive coping style is characterized by actions that are principally based on predictions. This is in contrast to the reactive coping style, which is described to respond more flexibly to changing environmental stimuli. Studies in pigs confirmed this fundamental difference in cue dependency between the two coping styles and found that proactive coping resulted in the faster development of routines than reactive coping (Bolhuis et al., 2004).

During the consumption of food, the rise in HR and sympathetic activity was present from the beginning of the experiment (period 1) in both coping styles. In period 2, reactive pigs showed higher vagal activation, which resulted in a decrease in HR during the consumption of food. The activation of vagal tone as a physiological reaction to feeding has already been described in humans (Porges, 1995a) and may be partly based on vago-vagal reflexes to coordinate digestion, including changes in gastric motility and secretion that precede feeding (Rogers et al., 1995). The positive anticipation of the event, combined with the satisfaction of the motivation to feed (Zebunke et al., 2011), may contribute to this autonomic reaction in reactive pigs in period 2 during feeding. Conversely, this vagal dominance was not found in proactive pigs during the consumption of food. The RMSSD values of proactive pigs during food intake even fell below the values during idling (TI 1) before the person was present in both periods. The sympathetic activation with accompanying vagal decrease indicated that proactive pigs were in an aroused state with a rather negative valence during feeding. One possible explanation for this effect may be jealousy over food of the adjacent pen-mates during feeding. Several studies with restrictive feeding during single housing show similar autonomic reactions, namely, a rise in HR as reaction to feeding and an elevated HR during food consumption (Marchant et al., 1997; Geverink et al., 2003). In these studies, HR was elevated during the consumption of food and did not decrease, as found in reactive pigs in the present study. In the study of Marchant et al. (1997), pigs were housed individually, so that competitive interactions were not possible, but still HR remained elevated during feeding. The authors see a major causal factor in the psychologically perceived threat, especially for subordinate pigs housed next to more dominant animals. Additionally, proactive coping strategies were found to be related to higher aggressiveness in several species (Hessing et al., 1994b; Sgoifo et al., 1996). The higher sympathetic activation in proactive pigs during feeding as found in the 5 min analysis was not reflected in the detailed 10 s analysis. This indicated that the sympathetic activation did not occur in the first 30 s of feeding but over the consumption period of 5 min. We consider that the proximity to the trough of the adjacent pen-mate paired with full sight of the feeding conspecific (transparent walls) may explain the higher sympathetic arousal during feeding in proactive pigs.

Affective autonomic and behavioral reactions during handling

A different picture regarding autonomic reaction was found during repeated handling by a familiar person. When the person was standing in front of the single pen, both coping styles demonstrated a strong vagal activation with the absence of sympathetic arousal. This reaction was already described in other studies indicating an orienting reflex, which was accompanied by bradycardia of vagal origin serving as information processing of environmental stimuli (Porges, 1995b). A parallel sympathetic activation, which would indicate an anticipation reaction, failed to appear. We assumed that neither coping style clearly anticipated the handling situation. One explanation of this effect may involve the vagueness of the stimulus. As it mainly consists of visual components, some of the pigs might have not detected or identified the person in front of the respective pen in the time window of 10 s. In contrast, the anticipation stimulus during feeding was more pronounced, as it consisted of auditory cues regarding the preparation of food pellets in the buckets.

As the person entered the pen for handling in period 1 (TI 3), HR increased markedly in reactive pigs due to the combined effect of sympathetic activation and vagal withdrawal. This may indicate an emotional state which is characterized by a negative valence and a high degree of arousal. This finding was also supported by a longer latency time to approach the familiar person in reactive pigs. This higher susceptibility to changing environmental conditions in reactive animals is supposed to result in higher behavioral flexibility (Coppens et al., 2010; Koolhaas et al., 2010).

In period 2, the rise in HR and sympathetic activation disappeared in reactive pigs. Instead, reactive pigs showed an elevated vagal tone in TI 3 compared to TI 1 before the person was present. This state is thought to occur in situations with positive emotional valence, such as pleasure or excitement (Boissy et al., 2007). Similar effects were already found in rhesus monkeys demonstrating a rise in vagal tone during grooming carried out by a familiar human (Grandi and Ishida, 2015). The autonomic reaction was also accompanied by a behavioral change in period 2, as the repeated handling procedure decreased the latency time to approach the familiar human in reactive pigs. Several (farm) animal studies demonstrate the importance of human-animal relationships (Waiblinger et al., 2006). It has been demonstrated in pigs, that early handling increased play and exploration behavior, both of which are assumed to be associated with positive emotional states (Zupan et al., 2016). In proactive pigs in period 1, the rise in HR during handling (TI 3–5) was triggered solely by vagal withdrawal as seen in the lack of sympathetic activation. Paired with short approach latencies, we assume that proactive pigs reveal less arousal in response to handling at least in this first experimental period, compared to reactive pigs.

Interestingly, proactive pigs developed strong sympathetic arousal during handling in period 2, which was reflected in a rise of HR compared to period 1 and compared to reactive pigs. Other than during feeding, this sympathetic arousal developed only in period 2 and was not present in period 1. One possible explanation for this effect may include the fact that handling resulted in a style of environmental enrichment, as it can elicit positive emotions by providing stimulations and opportunities for new behaviors. Animals quickly lose interest in simple objects in their environment if they are not relevant (Newberry, 1995). This is not the case for handling, as pigs were highly motivated to explore the familiar person, as indicated by the short approach latencies. Although tactile stimulation by a handler may not necessarily be experienced as positive by the individual, recent physiological evidence using HR and cortisol measurements in pigs (Tallet et al., 2014) suggests that it can be experienced as such. To summarize, reactive pigs seem to develop an increasing state of relaxation with positive emotional valence during a repeated handling situation, whereas proactive pigs were characterized by tachycardia and a strong arousal of the sympathetic nervous system.

Conclusion

Our study revealed significant context-related differences in the general autonomic reaction of pigs characterized as belonging to different coping styles. More specifically, the two coping styles differ in their affective appraisal in relation to a repeated feeding and handling situation with a familiar person. The present approach contributes insight into the underlying neurophysiological processes of affective appraisal related to distinct coping strategies. The comprehension of these complex relationships is of crucial importance in understanding affect and emotion in the context of human and animal health and welfare.

Author contributions

BP, JL and AK contributed to the conception and design of the study. AK performed the experiments and collected and analyzed the data. BP, JL and AK interpreted the data. AK wrote the manuscript.

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Chapter THREE.

General Discussion

Concerns for animal welfare are generally based on the assumption that animals can subjectively experience affective states and hence can suffer or experience pleasure (Dawkins, 1990, 2000; Mendl and Paul, 2004; Boissy et al., 2007b; Mendl et al., 2009). Affective states arise in situations that are 'important' to the individual and influence its behaviour in response to external stimuli occurring in its environment in order to achieve survival goals and reproductive success (Cabanac, 1992; Cardinal et al., 2002; Kringelbach and Rolls, 2004; Rolls, 2005; Mendl et al., 2009). In order to study affective states the development of accurate proxy measures is an important goal in animal welfare science (Panksepp, 1998; Lawrence, 2008; Mendl et al., 2009). One approach to objectively assess an organism's affective states is the measurement of ANS activity, which is viewed as a major component of the affective response in many theories of emotion (Porges, 1995a, 1995b, 2001, 2007; Lane et al., 1997; Thayer and Lane, 2000; Beauchaine, 2001; Ellsworth and Scherer, 2003; Kreibig, 2010). Based on this, one major aspect of the thesis was concerned with the establishment of a new methodology capable of assessing both branches of the ANS. This provides a first approach to differentiate activity arising from the two branches which contributes to identifying autonomic specificity for different affective states according to the arousal and valence dimensions of affect analogous to the differential representation that exists in the brain. However, affective states and appraisal processes may vary between individuals and several external (e.g. the nature of the stimulus) and internal factors, such as personality or, more specifically, individual coping characteristics can modulate the underlying mechanisms of the response differently (Malik et al., 1996; Schweiger et al., 1998; Boissy et al., 2007b; von Borell et al., 2007; Koolhaas et al., 2010; Kreibig, 2010). In other words: affective appraisal of a specific situation or event lies in the eye of the beholder and depends on his/her/its perspective. Therefore, the second major aspect of this thesis addressed the link between affective-autonomic reactions and individual coping characteristics as mediators of the ongoing relationship between the animal and its environment in the context of animal welfare.

According to these major topics, the following chapter was subdivided: The first section comprises a discussion of the establishment of the telemetric system addressing the methodological aspects of this thesis (study 1+2) followed by a second section in which the relationship between affective-autonomic states and individual coping characteristics are discussed (study 3). Additional sections include perspectives for application in further contexts and provide the final conclusions of the thesis.

3.1 Establishment of the methodology

In order to establish the invasive telemetric system as a valid tool for the measurement of both branches of the ANS in free-moving pigs, I a) developed a stringent surgical implantation procedure for a telemetric device used to continuously record ECG and BP in free-moving domestic pigs and b) functionally assessed the recorded parameters with special attention to data processing, detection performance, parameter reliability, and interchangeability of ECG and BP in determining HR and HRV.

The commercially available telemetry systems differ in their transmission frequency, battery life, pressure measurement, financial effort, and their application in large sized animals. Telemetry Research (and the according software partner AD Instruments) have developed a telemetric device for measuring large animals, such as domestic pigs and dogs, and offered a solution for assessing several animals at the same time by providing individual receivers operating on different frequencies. This was an indispensable feature for our studies.

3.1.1. Implantation surgery

The key element for achieving reliable data is an adequate implantation surgery procedure including the placement of the ECG electrodes, BP catheter, and transmitter body itself. Different electrode configurations are described in the literature for rats (Sgoifo et al., 1996b; Tontodonati et al., 2011) or guinea pigs (Shiotani et al., 2007). In domestic pigs, a few similar procedures were found, but these either use telemetric systems from different manufactures (Poletto et al., 2011), pigs kept in a sling during recordings (Mesangeau et al., 2000) or conduct either BP (Mesangeau et al., 2000) or ECG (de Jong et al., 1998, 2000) measurement, not both.

Overall, 21 surgical implantations were performed within the scope of this thesis. A detailed surgical implantation procedure was developed based on the knowledge gained from pre-studies (unpublished data) and the first eleven surgical implantations (study 1). Based on the experiences I made with the implantation procedure, I can give a number of recommendations to improve implantation success. In order to obtain a reliable ECG, which enables the calculation of heart dynamics, the attachment of the ECG leads to muscle tissue, one at the dorsal aspect of the animal lateral to the left scapula, and the other at the ventral aspect of the animal, close to *processus xiphoideus* of the sternum, was found to be a suitable approach to obtain electrical signals along the cardiac axis (base/apex). I recommend using at least three sutures to fix the electrode in muscle tissue in order to minimize the risk for disconnection. Additionally, it is recommended to form a pouch of muscle tissue around the tip of the electrode in order to increase conductivity. The implantation of intra-arterial BP catheter into the external carotid artery resulted in a valid arterial BP curve with characteristic slope. The catheter tip should not be inserted as far as the aortic arch, since the larger diameter of this vessel may allow excessive movement or vibration of the tip leading to a degradation or loss of the BP signal. Advancing the catheter tip approximately 8 cm into the vessel, at least in animals with a weight of approximately 30 kg, was shown to generate a valid and resilient BP curve. As a reference to verify this distance, I recommend attaching a suture or a sleeve at the catheter, 8 cm from the tip. This would also facilitate the attachment of the catheter at the arteriotomy site. Rinsing the catheter in heparin instead of only pipetting droplets onto the catheter tip is a recommended procedure to overcome loss of catheter patency (Van Vliet et al., 2000).

With regard to the fast growth of domestic pigs and depending on the duration of the experiment, it is recommended to place the leads of the electrodes and BP catheter in loops close to the telemeter body as this may allow uncomplicated unwinding if necessary. For the subcutaneous placement of the transmitter body the left side of the neck was suitable for this setup. It is recommended to surround the transmitter body with surgical mesh to improve ingrowth in connective tissue und to apply a running suture for attaching the transmitter body subcutaneously.

One further factor that was emphasised here included an adequate recovery period after surgery. In several studies in mice, depending on the study contexts and extent of the intervention, different recovery times are reported that range from 24 h (Budgett et al., 2007: rat), 5 days (Janssen et al., 2000: mouse), 7 days (Farah et al., 2004: mouse), 10 days (Gross et al., 2002: mouse), 2 weeks (de Jong et al., 1998: domestic pig; Kano et al., 2005: minipig), 3 weeks (Sgoifo et al., 1996b: rat; Stubhan et al., 2008: minipig; Poletto et al., 2011: domestic pig), up to 4-10 weeks (Gelzer and Ball, 1997: dog; Ward et al., 2012: dog). Cardiovascular data of four domestic pigs investigated in pre-studies of our group (unpublished data) revealed unstable cardiovascular parameters in the first five days after surgical procedure. Due to these tendencies, data collection in studies 1 and 2 was not started until 2 weeks after surgical procedure. On the other hand, the findings in study 3 showed that the cardiovascular parameters did not change over a 10 day period of convalescence indicating that the animals follow a good recovery from anaesthesia and healing process.

3.1.2. Functional assessment of recorded parameters

A second requirement for the collection of reliable data is the functional assessment of the acquired data. The main aspects were (1) processing the data (beat detection and data correction), (2) verifying detection performance (artefact susceptibility), (3) controlling the reliability of obtained parameters for application in two different behavioural situations with different physiological demands and (4) evaluating the interchangeability of ECG and BP signal in the determination of HR and HRV.

Data processing

As a first step in data processing, correct software settings are the key component in the identification of the beats to be detected. The sophisticated detailed software settings make it possible to identify almost every beat without the need to rely on manual correction, even in artefact-rich sections, at least if the beat is recognizable. Standardized, a 45 Hz low–pass filter was applied to remove high-frequency noise in the signal before beat detection. For the calculation of HR and HRV from ECG recordings, which is based on continuous R-peaks in ECG, two modules were available in LabChart: 'ECG analysis' and 'HRV analysis'. Both modules provide partly different beat (R-peak) detection settings specified for the use in pigs. To control if both modules generally agree in the calculated RR intervals (which is the basis for the subsequent HRV analysis), as promoted by the manufacturer, ten randomly selected sections of RR intervals from each module were compared. This resulted in 100% agreement and promoted the interchangeable use of both modules for R-peak detection and subsequent HRV analysis, depending on which module was able to detect more R-peak correctly (personal analysis, unpublished data). For correct results it is important that each R-peak is identified once, and once only. Most problems are due to incorrect triggering of the R-peak detector, and can be overcome by adjusting the settings and configuring them individually depending on the morphology of ECG signal. Typical QRS-width, which is used to tune the detection of R-peaks, that is to separate them from other ECG waves and from noise, was set to 40 ms, as proposed by the software. If the module did not accurately detect the R-peak, the QRS-width was calculated individually using the marker tool and adjusted in the module settings. The QRS-width was defined as the interval from the onset of the Q-wave (junction between the P–R isoelectric line and the beginning of the Q deflection) to the end of the S-wave (junction with the ST-segment). As I found the QRS-width to individually vary from 39 to 63 ms in our animals (personal observation), this manual calculation of individual QRS-widths is a highly recommended procedure in ECG recordings to enhance R-peak detection. Further beat detection settings included the minimal distance between two adjacent RR intervals (retrigger delay). This is the minimum RR interval that will be detected. It prevents an R-peak of being detected unless it occurs some minimum time after the previous one. In a very clean recording it is recommended to set this value to zero. Non-zero values help prevent the module falsely identifying T-waves and noise as QRS-complexes. A good starting point is to set this to a value close to the expected absolute refractory period of the examined species, for example 200 ms in pigs (Dean and Lab, 1990). Additional settings, such as pre-P baseline (50 ms), maximum PR (140 ms), and maximum RT (400 ms) were set according to the manufacturer's recommendations for pigs and detection threshold (maximum after zero) was usually set to 0.1 - 0.2 mV, depending on the amplitude of the R-peak. After completing beat detection, the ECG trace was visually controlled to ensure the module accurately detected the R-peaks. If many R-peaks were unmarked, or beat markers were set falsely, ECG settings had to be individually adjusted in order to increase the number of correctly triggered beats. If R-peaks were not able to be detected using the software settings due to software limitations, the RR interval was manually corrected by measuring the distance between the respective R-peaks. If the R-peak was missing or not clearly identifiable, the according RR intervals were interpolated by calculating the mean of three adjacent and three following values. This procedure was recommended in several studies concerning HRV calculation (von Borell et al., 2007), but the correction threshold of 5 % should not be exceeded as this would induce errors in the calculation of HRV indices resulting in invalid physiological interpretations.

Mean arterial BP, systolic and diastolic BP were calculated using the 'blood pressure analysis' module in LabChart. For the correct detection of the pressure waves, the minimum peak height was set to 20 mmHg and the minimum period was set to 200 ms which prevented two peaks from both being recognized within the same time period. Manual correction of incorrectly triggered pressure waves and interpolation followed the same guidelines as in R-peak detection in ECG.

Detection performance

The major findings of the detection performance (study 1) revealed that almost all beats (99%) were detected correctly using the described software settings during resting conditions in both ECG and BP signal. Data correction had no effect on cardiovascular parameters when compared between automated beat detection and manual correction during resting conditions. In contrast, this was not the case during feeding behaviour, as beat detection was more successful in BP recordings, whereas ECG needed significantly more manual correction and interpolation of falsely triggered beats. Automated triggering was sufficiently reliable to eliminate errors in BP recordings during resting and feeding behaviour, while detection performance in ECG was not adequate enough to rely on automatic triggering by the software, at least during active behaviour, such as feeding. One possible explanation is that muscle fibres which generate their own electrical potentials may distort ECG waves, if measured inside the muscle tissue, but do not affect BP measurement in the vessel. This outlines one major limiting factor in the analysis of HR and HRV parameters which are based on subsequent cardiac RR intervals. Generally, when calculating HRV indices, data segments that are free from movement artefacts or erroneous beats are essential for analysis, as the occurrence of artefacts interrupts normal interbeat variability. Marchant-Forde et al. (2004) reveals that even a small percentage of error in pig RR data can bias the outcome of HRV analysis. Several approaches in humans were suggested to limit the impact of error on indices of HRV by introducing fixed criteria for the identification of artefacts and the use of according algorithms for the recovering of anomalous intervals (Cheung, 1981; Schechtman et al., 1988). Using the gold standard ECG, as in our studies, identification of artefacts and anomalous beats followed a visual inspection to manually correct falsely triggered beats. This procedure was able to eliminate almost all errors in ECG recordings (only 1% of the beats had to be replaced by interpolated values), but was associated with great effort. As movement artefacts cannot be eliminated when measuring ECG in muscle tissue, this fact highlights one of the limitations of the telemetric system used. In any case, I recommend the visual inspection and review of the recorded parameters in order to achieve reliable data, at least during behaviours with elevated level of activity.

However, there are possibilities to circumvent the issues mentioned above, but these require the application of different surgical interventions or telemetric equipment. For example, one approach to minimize disturbances is the placement of one electrode intraperitoneally on the left lateral side of the diaphragm close to the apex of the heart, as described by Ruppert et al. (2016). In this position, artefacts deriving from muscle action potentials are highly unlikely. However, this approach brings along other disadvantages, for example, a larger surgical intervention resulting in greater health impairment and longer times of convalescence, which was not feasible for my purposes and possibilities. Another approach is the use of "solid tip electrodes", which may be introduced in the *vena cava* via the right jugular vein. It has been shown that electrode positioning in the vessel markedly improved ECG recordings, at least in guinea pigs (Ruppert et al., 2016). Furthermore, it is assumed that this would produce signals with high wave voltage (high R-peak amplitude), due to the closer position to the heart. This would facilitate data processing by the software. However, this is a very innovative and new feature of telemetric systems (for large animals) which are only recently available for purchase (e.g. PhysioTel®, DSI™ Data Science International, Minnesota, USA). The telemetric system used in my studies did not provide such a solid tip electrode, but this would be a conceivable approach for further studies concerning ECG measurement in free-moving animals.

Parameter reliability

Mean HR, systolic, diastolic and mean arterial BP as well as numerous parameters using time domain and frequency domain analysis in terms of HRV and BPV were calculated in order to draw conclusions about the underlying autonomic control in domestic pigs during basic (resting) and feeding conditions concerning the reliability of the calculated parameters in reflecting vagal and/or sympathetic activity. Our major findings reveal:

(1) Baseline values during resting were characterised by decreased HR, elevated HRV, and low BP and BPV levels reflecting high vagal and low sympathetic activity. This indicated a relaxed state with low arousal, whereas (2) the consumption of food was accompanied by pronounced increases in HR, BP and BPV with concurrent decreases in all components of HRV indicating sympathetic activity and vagal withdrawal.

The heart is dually innervated by both the sympathetic (which increases HR) and the parasympathetic (which reduces HR) systems that affect HR either in coupled (reciprocal, coactivated, or coinhibited) or uncoupled modes (Berntson et al., 1991; Hainsworth, 1995; von Borell et al., 2007). Mean HR, based on the detection of R-peaks in ECG (intervals between adjacent R-peaks resulting from sinoatrial node depolarisation) is therefore not informative of the respective branch's influence upon cardiac functioning (Berntson et al., 1991, 1993). Time and frequency domain analyses are two approaches that can be used to determine autonomic activity from cardiac RR interval data (Malik et al., 1996). Vagal influence on the heart may be well illustrated using both analyses, as fast changes in HR are mostly mediated vagally (Hainsworth, 1995). The power of the HF component in the power spectrum of spectral analysis is a useful tool in the quantification of vagal activity (Akselrod, 1995). The according RMSSD parameter of the time domain analysis is also generally accepted to reflect vagal activity and was found to significantly correlate with the HF component (Kleiger et al., 1991; Zebunke et al., 2011). Low resting HR values in our study were therefore, at least partly, ascribed to high vagal activation as seen in RMSSD and HF. Screening the literature on comparable studies in domestic pigs describing baseline HR values by using invasive or non-invasive measurements revealed concordant results. Using implantable devices in single-housed domestic pigs, Fedor et al. (1978) found HR values of 108 ± 4 bpm during resting and Poletto et al. (2011) reported HR values of 112 ± 3.5 bpm three weeks after surgical implantation of a biotelemetric transmitter, which are both comparable to our results. Zebunke et al. (2013) and Mahnhardt et al. (2014) found higher HR in resting domestic pigs at 9 weeks of age using non-invasive measurements. This probably indicates age-related changes, as have been described in piglets (Kuwahara et al., 1986; Webster and Jones, 1998). I assume that the low HR values accompanied by elevated vagal tone in my study may indicate a good recovery from surgical intervention as high vagal tone during baseline measurements has been described to reflect efficient autonomic control (Porges et al., 1996). Sympathetic influence on HR is much more difficult to quantify. Some authors emphasise the use of the LF component or SDNN parameter to characterise sympathetic activity (Malliani et al., 1991; Kamath and Fallen, 1995), whereas others assume also vagal contribution and question the identification of sympathetic activity based on the LF component (Akselrod et al., 1981; Cerutti, 1995; Malik et al., 1996; Janssen et al., 2000; Gross, 2005). According to the autonomic blockade study conducted by Kuwahara et al. (1999) in minipigs, vagal activation contributed to both the HF and LF components, whereas sympathetic tone only affected the LF component. Due to these ambiguous findings concerning the reliability of HRV parameters in reflecting sympathetic activation, I analysed specific components of the BP signal for the quantification of sympathetic activity. According to the physiological model of the different control branches (Pagani et al., 1986), BP is directly influenced by the sympathetic branch of the ANS (e.g. sympathetic vasoconstrictor activity) and only indirectly affected by the parasympathetic activity (e.g. via baroreflex). Mean BP level is regulated by several mechanisms, for example, the renin-angiotensin-system, aldosterone release and the baroreceptor reflex and is influenced by blood volume, cardiac output, total peripheral resistance and arterial stiffness. These mechanisms, triggered by parasympathetic and sympathetic activity, respond to and regulate all these different factors and they rarely act in isolation resulting in a wide variation of the actual BP response in a given individual.

Due to these complex mechanisms, mean pressure levels are not directly indicative for short-term sympathetic control but can be seen as a more long-term sympathetic activation. However, the short-term sympathetic control of circulation has been suggested to play an important role in the origin of BPV by controlling vasoconstriction (Laitinen et al., 1999). The knowledge about determinants and physiological correlates of BPV is limited and refers mainly to time-domain and/or frequency domain analysis of BPV in humans. Some studies focus on the association between short-term BPV and HRV (Scheffer et al., 1994; Taylor and Eckberg, 1996), while other studies investigate the relationship between short-term BPV and BP (Duprez et al., 1995; Siche et al., 1995; Su and Miao, 2001). In several approaches, BPV has been expressed as the standard deviation or/and coefficient of variation, which is the standard deviation divided by the average BP level (Mancia et al., 1983a; Su et al., 1986; Poletto et al., 2011). Hence, the coefficient of variation assumes that, at a given BP level, the changes in standard deviation are influenced by the BP level. However, the degree of BPV is not necessarily associated with the BP level as found by Jacob et al. (1989). Therefore, the standard deviation is assumed to be a better index of BPV and one should not use the coefficient of variation (Su and Miao, 2001). Short-term BPV has been calculated using the standard deviation of systolic (Pringle et al., 2003; Björklund et al., 2004; Mena et al., 2005) or both diastolic and systolic BP (Kikuya et al., 2000; Pierdomenico et al., 2006; Verdecchia et al., 2007) and is thought to be a measure of sympathetic activation, at least in humans and rodents (Clement et al., 1979; Di Rienzo et al., 1983; Mancia et al., 1983a; Parati et al., 1995; Van Vliet and Chafe, 2000; Van Vliet et al., 2000; Kanemaru et al., 2001; Delgado et al., 2014). In animal research, BPV studies are rare and mostly conducted in contexts of drug research or stress associated paradigms. Therefore, BPV related studies can primarily be found in laboratory animals like mice (Janssen et al., 2000; Farah et al., 2004), rats (Rubini et al., 1993; Van Vliet et al., 2000), and dogs (Rimoldi et al., 1990a). One study investigating BPV in domestic pigs (Poletto et al., 2011) in the context of pharmacological blockade was using frequency-domain analysis of BPV. Again, as with HRV parameters, this topic is not without debate. Some authors question the suitability of BPV components and concluded from their studies in rats that the LF component of arterial BP does not appear to be a suitable marker for the prevailing sympathetic activity (Stauss et al., 1995), while other authors emphasise the use of the LF component in reflecting sympathetic activity (Kuwahara et al., 1999; Janssen et al., 2000; Brown et al., 2012; Delgado et al., 2014). Despite these conflicting assumptions concerning spectral analysis of BPV, such an approach has the potential to extend the spectrum of parameters in evaluating sympathetic activity. Due to the fact that LabChart was not designed for BPV analysis in the frequency domain, I focussed on the standard deviations of BP to reflect short-term sympathetic contributions. In general, sympathetic activity is thought to play a minor role during resting conditions, which was confirmed by my findings showing decreased BP and BPV parameters during resting. My results showed even lower BP levels compared to the values of Poletto et al. (2011) and Houpt et al. (1983) indicating low sympathetic contribution (mean arterial pressure: 112.8 ±2.8 mmHg (Poletto et al., 2011)) and 114±2,5 mmHg (Houpt et al., 1983)). In the latter study, the activity level of the pigs was not clearly defined and pigs had been food deprived for 17 h, which may explain the higher BP values as indicative for elevated stress levels.

The feeding response was characterised by tachycardia and decreased HRV (indicating vagal withdrawal) accompanied by an increase in BP and BPV (indicating sympathetic activation) compared to the resting condition. The decline of the RMSSD:SDNN ratio in my study, analogous to the increase in LF:HF ratio demonstrated the shift of the autonomic balance towards predominantly sympathetic activity. The strong increases in BP and BPV variables support the assumption of sympathetic enhancement during feeding, which was expected to be reflected in the LF component of HR fluctuations as well. This effect on LF is probably blunted by the concomitant vagal withdrawal, which in turn reduces the LF part of the HR power spectrum. The decline in LF (from resting to feeding condition) is not as significant as in the HF component of HR fluctuations (indicative for vagal contribution), confirming that the LF component of HR fluctuations is mediated by both the parasympathetic and sympathetic branches (Akselrod et al., 1997).

My findings support that the RMSSD or the HF component of HR fluctuations as well as the standard deviations of systolic, diastolic, and mean arterial pressure are suitable parameters to reflect changes in vagal and sympathetic activity, respectively, comparing resting to feeding conditions in free-moving domestic pigs.

Interchangeability of ECG and BP in determining HR and HRV

Based on the findings of the detection performance in study 1 in relation to BP being more stable and less susceptible to movement artefacts, the question arose if the BP signal was usable for the calculation of HR and HRV parameters in comparison to ECG signal. The motivation of this study was based on the idea of substituting HR and HRV detected from ECG signal with the one provided by intra-arterial BP signal in experimental settings. The major findings reveal that HR data obtained via BP are interchangeable with those obtained by ECG signals independently of the activity of the subject, whereas this is not the case for HRV measurements. A systematic overestimation of HRV parameters by BP signal was found throughout all behaviour categories, albeit less during resting, but more apparent during feeding and active behaviour. Therefore, ECG and BP can be used interchangeably in the context of HR, but not HRV in free-moving domestic pigs.

Generally, for comparing and validating two methods measuring the same variable, several approaches based on different mathematical procedures are proposed in the literature, for example, linear regression, intraclass correlation coefficient (ICC), Bland-and Altman plots, or analysis of variance (ANOVA). Study 2 of the present thesis incorporated these suggested procedures where the ECG was assumed to provide the correct measurement (gold standard). The performance of both signals (ECG and BP) in IBI detection and subsequent analysis of HR and HRV was evaluated during three behavioural situations that modify the influence of the autonomic nervous system of the heart, reflected by changes in HR and HRV. This emphasised the consistency of the results, as HR and HRV parameters were calculated for a wide range of values. The linear regression analysis is customarily used as an indicator of the relationship and association between a scalar response (or dependent variable) and one or more explanatory variables (or independent variables). Several investigators question the usability of linear regression in the comparison of two methods and pronounce its limitations, as linear regression quantifies the strength of the relationship between two samples, not the agreement between them (Nunan et al., 2008). The ICC was introduced as a more appropriate statistic for measuring agreement between methods (Shrout and Fleiss, 1979; Lee et al., 1989). It describes how strongly units in the same group resemble each other and mostly found application in the assessment of consistency or reproducibility of quantitative measurements made by different observers measuring the same quantity. According to linear regression and ICC, IBIs deriving from ECG and BP show strong relationship and agreement for HR in all three behavioural situations and for SDNN and RMSSD at least during resting. However, the use of ICC to demonstrate agreement has been questioned as several investigations reported that the estimates are dependent upon the range of the measuring scale, the wider the range, the better the result or, in other words, the more variable the values or subjects are, the greater the values for ICC (Bland and Altman, 1990; Müller and Büttner, 1997). This may be an explanation for the high ICC values for HRV during resting in study 2. IBIs are more variable during resting compared to behaviour with elevated activity, owing to increased vagal activation when the animal is lying inactive. This is reflected by higher RMSSD values during resting. Additionally, large inter-individual variation in HRV parameters may also have an effect, a finding not uncommon in HRV analysis (Sinnreich et al., 1998). Such wide spreads of scores can inflate the values for ICC coefficients and may disguise the true magnitude of variation. Therefore, I chose a more appropriate approach that meets the requirement for not depending on the range of the sample (Bland and Altman, 1986). The Bland-and-Altman plots showed that differences between the methods were marginal in terms of HR in all behavioural situations, thus, I concluded that HR may be calculated from both methods. In contrast, in terms of SDNN and RMSSD, large biases and wide limits of agreement were found during all behavioural contexts based on a systematic overestimation of HRV parameters when calculated from BP signal. Although a relatively high ICC between ECG and BP derived data in terms of SDNN and RMSSD during resting was found, the Bland-and-Altman analysis clearly demonstrated that these parameters did not agree closely enough. Hence, reliance solely on the ICC would have resulted in incorrect assumptions being drawn from the data. These findings were also reflected in ANOVA analysis indicating statistical differences of HRV parameters between the two methods which consequently led to differing results within the behavioural contexts.

To answer the question of whether two methods can be used interchangeably, Lee et al. (1989) proposed three criteria of agreement: (1) the lower limit of the 95% confidence interval of the ICC should be at least 0.75, (2) there should be no marked systematic bias in the data and (3) there should be no statistically significant difference between main readings obtained by the two methods. These requirements were all met using two methods (ECG and BP signal) in the determination of HR in different behavioural situations; hence the BP signal could reliably be used in place of ECG signal in terms of HR, whereas this is not recommended for HRV calculations. Even though the first requirement was fulfilled at least during resting behaviour, the second and third requirements could not be addressed in either of the behavioural conditions in terms of HRV.

Comparisons with my findings are difficult due to the lack of studies assessing agreement of short-term HRV measurements related to intra-arterial BP measurements. Some few studies have addressed this topic, but differences in data collection protocols and the use of inappropriate or insufficient statistical tests complicates an appropriate comparison to my findings. For example, Carrasco et al. (1998) found unacceptable agreement in terms of HR and HRV in humans, but used an indirect measure of pulse pressure, such as the finapres monitor. This is based on the volume-clamp method proposed by Penaz (1973). They demonstrated that HR and HRV measured from the pressure wave were not interchangeable with those obtained from the RR interval using ECG. A systematic error resulted in underestimating the HR and overestimating HRV by the finaprez in comparison to ECG recordings. To my best knowledge, no studies systematically comparing HRV from ECG with that obtained from intra-arterial BP have been reported. Nevertheless, some studies make use of the calculation of HR and/or HRV parameters based on beat-to-beat recordings of BP tracings (Mancia et al., 1986; Rimoldi et al., 1990b; Cohen et al., 1998).

From a physiological perspective the RR interval variation (measured by ECG) is based on direct influences of the ANS on the sinoatrial node. This is not the case for BP intervals, where the pressure value might be affected by numerous other factors, as discussed in the previous section. The neural information is converted by the sinoatrial node of the heart into action potentials which release the myocardial contraction that elicit the BP wave propagation through the arteries to the periphery (Lakatta, 1993). This effect is based on sympathetic activity eliciting positive ionotropic effects on the ventricular myocardium which results in increased cardiac contractility by a more rapid development of intraventricular pressure. This decreases the time required to reach aortic pressure and opening of the aortic valve. This sympathetic influence contributes to the length of systolic time intervals which offer a temporal description of the sequential phases of cardiac cycle representing the duration of total electro-mechanical systole with its two major components, the pre-ejection and the ejection phases. The determination of systolic time intervals (Weissler et al., 1961, 1969; Weissler, 1977) includes for example, the calculation of left ventricular ejection time (LVET) and pre-ejection period (PEP). According to Cacioppo et al. (1994), PEP is calculated by the time from the onset of ventricular depolarization (beginning of the Q-wave on the ECG) to the beginning of left ventricular ejection (beginning of the upstroke of the invasive arterial pressure curve) and is generally accepted to reflect sympathetic activity. This sympathetic-dependent time delay between electrical and mechanical phenomena during cardiac cycle is one possible explanation for differences between HRV parameters deriving from ECG and BP signal. However, the transition between the electrical and the mechanical processes seems to be linear in the resting condition, since HR and HRV are at least highly correlated. In conditions with higher sympathetic and lower vagal activation (feeding and active), the IBIs from BP signal are more variable, consequently overestimating IBIs from ECG signal, mostly accentuated in the RMSSD parameter. Similar results were reported in a study by Carrasco et al. (1998) demonstrating a modification of pressure intervals in their spectral distribution, as indicated by the larger high frequency component in adults during exercise. They argue this may reflect the more important modulating influence that respiration exerts on pressure intervals than on ECG intervals. Casadei et al. (1996) argue in their autonomic blockade study that the HF component of HRV (related to the respiratory sinus arrythmia) is modulated by non-neural mechanisms. This would be an explanation for the strong overestimation of cardiac vagal activity during activity. One further study (Liu and Liping, 2009) compared ECG with Phonocardiogram (PCG) in terms of HRV parameters (variability in RR intervals deriving from ECG vs. variability in systolic time intervals deriving from PCG). They found increased variability in RR intervals deriving from ECG compared to PCG and stated that systolic time intervals keep a higher stability.

The underlying complex interactions of sympathetic and parasympathetic influences on regulatory mechanisms of the cardiovascular system are not entirely understood yet, as shown by divergent results in different studies. Since there has been evidence that respiration exerts a higher modulation on the IBIs from BP signal than that observed in the IBI from ECG signal during behaviours with elevated activity, the RMSSD parameter of the IBIs from BP signal could be used as a specific index of non-neural mechanisms of the respiratory sinus arrythmia. To clarify which mechanisms underlie the variability of IBIs deriving from intra-arterial BP, future studies are needed that investigate respiratory and sympathetic influences while considering parameters of systolic time intervals (PEP and LVET). The study of the IBIs from BP interval as a specific index could generate new research opportunities in the analysis of the interactions between the autonomic nervous system and the electromechanical cardiovascular phenomena.

Summary at a glance: Establishment of the methodology

- ✓ A surgical implantation procedure was developed for a telemetric system in pigs.
- ✓ Recommendations for improvement of implantation techniques were given.
- ✓ Detection performance decreased with elevated behavioural activity.
- ✓ Blood pressure signal was less susceptible to artefacts than electrocardiogram.
- ✓ Baseline values were characterised by decreased HR, elevated HRV (RMSSD, HF) and low BP and BPV levels reflecting high vagal and low sympathetic activity during resting conditions.
- ✓ Behavioural activity during food consumption was accompanied by increases in HR, BP and BPV with concurrent decreases in all components of HRV indicating sympathetic activity and vagal withdrawal.
- ✓ HR values derived from BP signal agreed well with those derived from ECG independently of the activity of the subject.
- ✓ HRV may not be determinable by using BP signal in free-moving domestic pigs due to systematic overestimation in comparison to HRV derived from ECG.

3.2 Affective – autonomic reactions and coping styles

Based on the successful establishment of the telemetric system and the validation of recorded parameters to continuously measure ECG and BP in free-moving pigs (study 1+2), the system was applied in different housing-relevant situations in order to draw conclusions about the context-specific affective-autonomic state in pigs and the relationship to their individual coping style (study 3). In this section, I will discuss the autonomic reactions of the coping styles within the framework of the valence and arousal dimensions of affect during baseline conditions (resting), active behaviour (idling), feeding and a standardized human-animal interaction (handling).

In many recent theories of emotion, ANS activity is viewed as a major component of the emotion response, but contemporary researchers in the field of emotion hold diverging positions on the degree of specificity of ANS activation in emotion, ranging from undifferentiated arousal over some degree of autonomic emotion specificity (Cacioppo et al., 2000) to highly specific predictions of autonomic response patterns for certain emotions (Woody and Teachman, 2006). According to meta-analytical studies (Cacioppo et al., 2000; Phan et al., 2002), context-specific effects of ANS activity in emotion was shown. Especially valence-specific patterning was found to be more consistent compared to emotion-specific patterning in the way that negative emotions were associated with stronger autonomic responses than positives ones (Taylor, 1991; Kreibig, 2010). One key question in this context is how to distinguish positive from negative emotional states (Boissy et al., 2007b). Patterns regarding ANS activity in terms of negative valence can be derived from stress research in humans (Johnson et al., 1992; Porges, 1995b; Brotman et al., 2007). Several specific emotions with negative valence such as anger, anxiety, disgust, embarrassment, fear, and sadness have been studied in humans according to their relationship in autonomic response and were found to be associated with decreased vagal tone (for review see Kreibig, 2010). In animals, vagal deactivation occurred during stimuli that are likely to be stressful or perceived as emotionally negative; for example in the context of fear and anxiety (Stiedl et al., 2004; Forkman et al., 2007), including separation from group members in the case of gregarious animals (Baldock and Sibly, 1990; Sandem and Braastad, 2005; Reefmann et al., 2009c). Due to the fact that vagal tone was found to play an important key role also in positive contexts (as elucidated in section 1.2.3, and see Boissy et al., 2007b; Matsunaga et al., 2009; Reefmann et al., 2009c; Kreibig, 2010; Zebunke et al., 2013), vagal activity was used as a correlate of emotional valence in my studies, despite the knowledge that it appears to be ambiguous in some studies since vagal responses can overlap for presumably negative and positive states (for review in humans, see Kreibig, 2010). On the other hand, emotional arousal seems to be associated with increased sympathetic activity (Bradley et al., 2008). In a picture viewing context, sympathetic activity was larger when viewing pleasant and unpleasant, compared to neutral pictures. This indicates that the sympathetically mediated response covaries with emotional arousal (Lang et al., 1993; Lane et al., 1997; Bradley et al., 2008, for more examples see section 1.2.3).

Dimensional models typically assume valence and arousal to be at least in part distinct dimensions (Barrett and Russell, 1999). This finding originated from neuroimaging studies in humans demonstrating that the orbitofrontal and ventral anterior cingulate cortex respond more to valence, whereas the amygdala and anterior insular cortex respond more to arousal (Posner et al., 2008; Colibazzi et al., 2010). However, when studying emotion, it is important to consider the possible interaction of emotional valence with arousal (Robinson et al., 2004; Citron et al., 2014a). Some empirical work shows that valence and arousal may interact during the processing of emotional stimuli. Negative emotional states are generally rated higher in arousal (Sinha et al., 1992; Reefmann et al., 2009c; Citron et al., 2014b). From an adaptive perspective, high arousal coincides with increased attention and mobilization of energy to cope with an aversive situation (possible threat), mainly to elicit a withdrawal tendency and facilitate fight-flight responses (Dawkins, 1998). In contrast, positive emotional states are often characterised by lower arousal and elicit an approach tendency. This is thought to occur in situations that are perceived as safe, for example, in sheep being voluntarily groomed by a familiar human (Reefmann et al., 2009c). The specific way how an individual perceives a situation and which affective state is triggered strongly depends on an individual's interplay with the environment including not only challenges and threats, but also their ability to respond to them. The concept of coping emphasises individual behavioural and physiological (autonomic) reaction patterns which are based on reducing the effect of aversive stimuli. The specific role of affective states in modulating these individual response patterns to external stressors is not well understood yet. To date, it is unknown if the different coping styles differ in their situational appraisal, contributing to their general affective state and thus, welfare.

To my knowledge, this is the first attempt to investigate the link between coping characteristics and affective-autonomic reactions in different behavioural situations using parameters that reflect both vagal (HRV) and sympathetic (BPV) activity simultaneously in free-moving pigs. The major findings confirm that the determination of individual coping styles, which are classified on the basis of a proactive or reactive behavioural response in the backtest, was related to significant context-related differences in their general autonomic state. This indicates that coping styles in pigs may play a much more pivotal role than suggested so far, as differences between the coping styles are not only apparent in reaction to stressful situations, as known from the literature, but also manifested in their general basic physiological state. Additionally, the two coping styles differed in their affective-autonomic response over the time course of the experiment in the context of a repeated handling situation indicating individual differences in their affective appraisal in this specific situation.

Resting

My results show elevated HR and lower vagal activation (but not higher sympathetic activation) during resting in proactive pigs compared to reactive ones. In several studies, proactive animals have been characterised by lower vagal activity, for example, Hessing et al. (1994a) showed that HR in proactive pigs was substantially increased in reaction to a falling novel object, while reactive pigs were characterised by bradycardia suggesting that they exhibit higher vagal reactivity in this situation. Conclusions about sympathetic activity have not been drawn, as no direct parameter for sympathetic activity has been recorded. Other studies in laying hens from two lines with high or low propensity to feather peck show individual differences in physiological and behavioural responses to stress (manual restraint) that are similar to the ones described in coping styles (Korte et al., 1998). In cows, higher HR was found in individuals with high behavioural reactivity compared to low responders (Sutherland et al., 2012). However, the aforementioned studies investigating differences between individuals in autonomic reactions were designed to study coping styles in response to aversive, stressful situations – not during resting conditions. The investigation of resting ANS activity is useful to exclude effects of changes in physical activity, excitement or acute mental effort, but instead, reveals a more long-term and general influence of different factors on ANS activity. For example, a positive effect of cognitive challenges was found in goats, which was verified by elevated vagal activity during resting conditions (Langbein et al., 2004). Since this is the first investigation of describing differences in baseline ANS function between pigs with different coping styles, it is difficult to compare the present results to earlier findings in this field. One study was found that demonstrated lower resting vagal activity in temperamental cows than in calm ones and also in impulsive cows, compared to reserved ones (Kovács et al., 2015). The physiological rationale behind these differences in the basal autonomic state may be associated to the stress concept of Porges (1995a), who defined homeostasis as an autonomic state that promotes visceral functions. This condition is reflected by a high basal vagal activity. In this context, stress is reflected by decreased vagal activity due to disturbance of homeostasis. Hence, the basic autonomic state reflects a quantity of the individual stress sensitivity. High vagal tone is thought to indicate high efficiency of autonomic control, which enables the individual to increase its reactivity to physiological and environmental challenges (Porges, 1995b; Thayer et al., 1997; Friedman and Thayer, 1998). Following this approach, reactive pigs (elevated resting vagal tone) would be characterised by a decreased sensitivity to stress. This concept was well studied in humans and it has been demonstrated that high baseline vagal tone in neonates was associated with greater mental, motor, and social skills at the age of three (Doussard-Roosevelt et al., 1997) and infants with higher baseline vagal tone showed fewer negative behaviours in social challenges (Huffman et al., 2008). In adults, high resting vagal activity was associated with cooperative behaviour (Beffara et al., 2016), whereas low basal vagal tone in infants and adolescents was related to social behavioural problems (Mezzacappa et al., 1996). All these findings in humans indicate that vagal reactivity has a close relationship to pro-social behaviour traits, which is assigned to the reactive coping pattern (Koolhaas et al., 1999). Coping styles may play a much more pivotal role than suggested so far, as differences between the coping styles are not only apparent in reaction to stressful situations, but also manifested in their general basic physiological state.

Active behaviour / Idling

My findings reveal a significant change of the influences of the two branches of the ANS, comparing rest to active behaviour (idling) in the home pen. During idling, a higher influence of the sympathetic nervous system was found and, in the same degree, a pronounced decrease in vagal activity accompanied by tachycardia. No differences in ANS activity between the coping styles were apparent during this behaviour. From human literature, it is known that differences in physical activity alone can be responsible for changes in ANS activity. Generally, HR is closely linked to body posture and behaviour, being always lower when the animal (or human) is lying down than when it is standing or moving (Pagani et al., 1986). However, changes from supine to standing position in humans lead to vagal withdrawal within the first seconds after standing up, which is probably attributed to two mechanisms: the central stimulation of the brainstem cardiovascular centres and a feedback reflex from the contracting muscles due to the activation of their mechanoreceptors (Vissing et al., 1991). Following an immediate HR increase on standing mediated by vagal withdrawal in humans, several authors found that HR decreased moderately again and stayed on elevated level during standing (Drischel et al., 1963; Ewing et al., 1980). Increased sympathetic activity has also been reported in studies showing elevated systolic BP and increased BPV in patients during tilt (Borst et al., 1982), in dogs during exercise (Rimoldi et al., 1990a), and in rabbits during activity (van den Buuse and Malpas, 1997). My findings support the view that ANS activity is strongly influenced by physical activity (Visser et al., 2002; Voss et al., 2002), but seems to be unaffected by the coping style in context of ANS reactions. One possible explanation may include the fact that idling behaviour in the home pen reflected a conglomerate of different behaviours, such as locomotion and exploration. The lack of an emotionally relevant stimulus may contribute to the lack of differences in ANS activity between the coping styles; instead, the general impact of physical activity on ANS functioning was demonstrated. This highlights that if one wants to draw conclusions about emotional valence and arousal in an experimental setting, only behaviours with a similar level of activity should be taken into account.

Feeding

Compared to idling behaviour, an increase in sympathetic activation was found during feeding in both coping styles, although proactive pigs showed an even stronger sympathetic response (and a trend to lower vagal activity), compared to reactive pigs. Furthermore, the detailed analysis of the first 30 seconds of feeding in the two experimental periods revealed an initial tachycardia in both coping styles, but reactive pigs showed a delayed vagal activation during food consumption in experimental period 2 indicating the development of a more relaxed state with positive valence during feeding, compared to proactive pigs.

ANS-related effects during food consumption have been studied in different species (sheep, calves, domestic pigs, monkeys), including humans (Bloom et al., 1975; Houpt et al., 1983; Scalzo, 1992; Marchant et al., 1997b; Robert et al., 2002; Braesicke et al., 2005; Stubhan et al., 2008; Zebunke et al., 2013), but the influence of coping style is far less understood. Feeding *per se* is known to elicit strong physiological reactions (in the context of chewing, swallowing, digestion and secretion), as well as psychological mechanisms in relation to event memory (McGaugh, 2004), decision making (Damasio, 1998), and emotional perception (Ekman et al., 1983). These mechanisms are controlled and accompanied by the differential contributions of underlying sympathetic and parasympathetic mechanisms to ingestive processes (Young and Landsberg, 1977; Steffens et al., 1986; Porges, 1995b). For example, the gastrointestinal vago-vagal reflexes in the vagal complex of the brainstem provide coordination over digestive functions (for a review on vago-vagal reflexes, see Rogers et al., 1996) and sympathetic nerve endings provide innervation to the enteric ganglia, to gastrointestinal arterioles, and to the muscle of sphincters and non-sphincter regions (Lomax et al., 2010). Despite the inhibiting effects of the sympathetic nervous system on motility, I expected sympathetic arousal during feeding due to the fact that food, as an essential resource, has a high intrinsic motivation in pigs (Day et al., 1995; Spruijt et al., 2001) and any stimulus in the context of food is considered biologically relevant for these animals and, hence, has the potential to elicit highly aroused states.

Several internal and external stimuli in the context of feeding may contribute to the ANS response by modulating the individual's affective state, for example, the incentive value of the food-item, intrinsic states (e.g. hunger), the presence of pen mates (social status), and predictability and controllability of the essential resource. For example, sympathetic activation was shown to play a pivotal role during consummatory periods in primates (Randall et al., 1974; Braesicke et al., 2005), although this reaction depended upon the overall incentive value of the reward (Braesicke et al., 2005). Autonomic arousal occurs when reward value is high. Although high food values may even enhance autonomic arousal as in the study of Braesicke et al. (2005), my results show sympathetic arousal in the pigs even if the food reward was the animal's daily food ration. Current intrinsic states, such as hunger, may contribute to this effect by increasing the rewarding value of food-items. Hunger is part of a mechanism that engages the animal to perform foraging behaviour and to restore a food deficit before it gets more critical. This reflects the anticipatory element of animal behaviour what Berridge (2004) calls 'wanting' (Anselme and Robinson, 2016; Gygax, 2017). It has been shown in pigs that if they can predict the delivery of food by performing such situational appropriate behaviour that may be innate (for example rooting) or acquired by operant learning (Ernst et al., 2005; de Jonge et al., 2008), these behaviours (serving as an investment for collecting a necessary incentive, e.g. food) are able to elicit positive emotional valences mediated by different transmitter systems, such as dopamine and opioids, activating the mesolimbic system (Berridge, 1996; Spruijt et al., 2001; Burgdorf and Panksepp, 2006; Manteuffel et al., 2009). In food-rewarded tasks, for example, when pigs were individually called to feeding in an operant conditioning paradigm, positive welfare aspects have been demonstrated, such as less fear behaviour (Puppe et al., 2007), increased immune cell proliferation, better wound healing (Ernst et al., 2006), and a state of positive arousal indicated by vagal and sympathetic activation during feeding (Zebunke et al., 2013). One main contributing factor to this result is that the pigs in the operant conditioning approach are able to control the access to an appetitive stimulus. Due to restrictive feeding, as in study 3, the animal has to wait for food, resulting in a loss of control over essential resources. Presenting a positive stimulus (food) without giving control to its access was already found in pigs to be unsuitable to elicit positive affective states or to increase welfare (Mahnhardt et al., 2014).

Based on the stronger sympathetic activation (with accompanying lack of vagal activation) in proactive pigs during feeding, one may conclude that they are in a more aroused state, compared to reactive pigs. Physiologically, the higher domination of the sympathetic nervous system in proactive pigs agrees with observations of proactive rodents (Bohus et al., 1987; Benus et al., 1991; Fokkema et al., 1995; Koolhaas et al., 1999) and pigs (Hessing et al., 1994a), which predominantly react with a sympathetic response to challenging situations. The background to this assumption involves that proactive coping requires expenditures of energy, and the functional role of the sympathetic nervous system has been viewed as one of mobilizing resources to deal with environmental demands (e.g. Beauchaine, 2001; Heimer, 2012). The increase in performance is achieved by affecting cardiac electrical activity (increase in HR) and contractility of the vessels (increase in BP) to be prepared for fight or flight. Furthermore, proactive copers engage more in aggressive behaviours (Hessing et al., 1994b; Sgoifo et al., 1996a; Ruis et al., 2001) contributing to jealousy over food of the adjacent pen-mates. Several studies with restrictive feeding in individually housed pigs show similar autonomic reactions to feeding (Marchant et al., 1997b; Geverink et al., 2003; Mahnhardt et al., 2014), although competitive interactions were not possible due to single housing. It has been assumed that one major causal factor for this is the psychologically perceived threat, especially for subordinate pigs housed next to more dominant animals. I presume that the proximity to the trough of the adjacent pen-mate paired with full sight of the feeding conspecific (transparent walls) may contribute to the enhanced sympathetic arousal during feeding in proactive pigs. Another explanation involves the fact, that loss of control over the limited resource, as mentioned above, may have a greater negative impact on the proactive coping animal as it has been reported that proactive rats develop a higher vulnerability to the formation of stress-induced ulcers during uncontrollable stress, compared to reactive ones (Murison and Skjerve, 1992).

Interestingly, the increase in vagal activation in reactive pigs during food intake only appeared in period 2 (at the end of the experiment) at the same time as an anticipatory ANS response to feeding developed across testing in reactive pigs. It is possible that there are connections between these two processes. One important function of emotion is to produce appropriate anticipatory responses to improve chances of survival (Braesicke et al., 2005). Anticipation, in turn, may influence the affective state and welfare by allowing animals to prepare for an upcoming event (Badia et al., 1979). In line with my results, it has been suggested that sympathetic activation played a pivotal role in anticipation of incentive foods in pigs (Mahnhardt et al., 2014). This reaction has been interpreted as an indicator of positive emotional arousal in dogs and marmosets (Randall et al., 1974; Braesicke et al., 2005). Equally, vagal activation has been found to occur in anticipation of important events (Thayer and Lane, 2000; Imfeld-Mueller et al., 2011). The high state of motivation (probably enhanced by restricted feeding) combined with the resulting attention of the pigs towards the food buckets and learning that the sight of food led to eventual access to that food may contribute to this autonomic anticipation. So far, reactive pigs seem to be more relaxed during feeding at the end of the experiment. The positive anticipation of the event, combined with the satisfaction of the motivation to feed may contribute to this vagal activation in reactive pigs during feeding.

Conversely, proactive pigs were able to anticipate the feeding event already at the beginning of testing (experimental period 1). According to Koolhaas et al. (1999, 2010), coping styles differ depending on the degree to which their behaviour is guided by environmental cues. In rodent maze experiments proactively coping rats were characterised by actions that were principally based on predictions, whereas reactive copers were able to respond more flexible to changing environmental stimuli. Studies in pigs confirmed this fundamental difference in cue dependency between the two coping styles and found that proactive coping resulted in the faster development of routines and anticipatory behaviour than reactive coping (Bolhuis et al., 2004), which was supported by my findings in the context of anticipatory ANS response in proactively coping pigs.

Handling

In anticipation of the handling procedure, pigs did not show an autonomic anticipatory response, instead, an autonomic state interpreted as orienting reflex was found, which was accompanied by bradycardia of vagal origin. Porges (1995b) specified two sources of vagal efference to the heart, one originating in the dorsal motor nucleus (vegetative vagus) and the other in the nucleus ambiguous (smart vagus). The vegetative vagus mediates reflexive cardiac activity, including bradycardia associated with orienting. This serves as information processing of environmental stimuli (Porges, 1995b). After orienting, the smart vagus mediates cardiac activity when environmental demands require coping and the animal must either attend to and engage with the initial stimulus or resort fight-flight-freeze responding (Beauchaine, 2001; Bijttebier et al., 2009). It seems that both coping styles cannot properly define the stimulus which might explain the pronounced orienting response. One major factor for this finding may include the differences in vagueness between the stimuli, as pigs were able to use more sensory cues (visual, olfactory, auditory) to identify the feeding situation, compared to handling, where the visual cue was the only source for information.

After this short-term orienting response, the handling situation was characterised by elevated HR and a higher sympathetic activity in proactively coping animals compared to reactive coping animals indicating a more aroused response in proactive pigs, considering all experimental days. The detailed analysis revealed that the autonomic reaction of the coping styles differed between the periods of the experiment. Proactive pigs did not show any pronounced autonomic response to the handling procedure at the beginning of the experiment, but this changed to a high aroused state at the end of the experiment. Reactive pigs, however, showed a contrary reaction: handling initially elicited a highly aroused state with rather negative valence in reactive pigs (supported behaviourally by longer approach latencies) which shifted to enhanced relaxation (low arousal) with positive valence at the end of the experiment. This may indicate individual differences in affective appraisal in the context of human-animal interactions in pigs with different coping styles.

Several studies addressed human-animal interactions in farm animal species (for review see Waiblinger et al., 2006), observing, for example, the reaction of animals towards approaching humans (Murphey et al., 1981; Marchant et al., 1997a; Waiblinger et al., 2003), familiar or unfamiliar humans (Terlouw and Porcher, 2005), and the effect of different handling procedures on behaviour (Hemsworth et al., 1981, 1987; Waiblinger et al., 2004; Probst et al., 2012; Zupan et al., 2016), intra-specific communication (Langbein et al., 2018), growth (Pearce et al., 1989), meat quality (Probst et al., 2012), HR and/or HRV (Rushen et al., 1999; Tallet et al., 2014; Grandi and Ishida, 2015), and affective states by using a judgement bias paradigm (Baciadonna et al., 2016). Despite domestication and selective breeding the potentially most frightening events that many farm animals are likely to experience are exposure to human beings and to sudden changes in their social or physical environments (Hemsworth et al., 1994; Boissy, 1995; Jones, 1996). The predominant reaction of most farm animals to humans is still strongly characterised by fear (Duncan, 1990; Hemsworth, 2003; Zulkifli, 2013), unless the animals have become accustomed to human contact (Wechsler and Lea, 2007). Indeed, many of the occasions on which animals and humans interact in current farm practice are negatively reinforcing, e.g., veterinary treatment, restraint, depopulation. An animal may perceive an interaction with a human as negative, neutral, or positive, depending on its existing relationship with humans, which is based on previous experiences with humans (Waiblinger et al., 2004). Within the framework of animal personality, tests like the novel human test are conducted with farm animal species to measure the reactivity of an individual toward an unknown person (reviewed by Finkemeier et al., 2018). In most cases, the measured trait is suggested to mirror fearfulness when describing an animal having a higher latency in approaching an unknown person (Janczak et al., 2003). In this context, proactive coping was shown to be associated with shorter latencies to approach a novel human (pigs: Zebunke et al., 2017) and shorter avoidance distance (cows: Kovács et al., 2015) compared to the reactive coping style. Although the person was not unknown to the pigs in my study, this human-animal relationship was influenced by positive (feeding), but also negative (separating from group members, veterinary interventions) experiences; procedures that are common and usual in animal husbandry. Therefore, I estimated the valence of this stimulus to be rather negative (or at least ambiguous) to the animals, especially in the first handling sessions. The specific way individual pigs responded when exposed to this situation was shown to be associated with their coping style: longer approach latencies, which indicated fearfulness in reactive pigs, was accompanied by an aroused state of negative valence, a reaction which has already been described in the context of fear in rats (Inagaki et al., 2004). Proactive pigs, on the other hand, approached the person faster, indicating less fearfulness and they did not show any enhanced autonomic response to the same situation. Although they lacked a vagal increase (only numerical increase, not statistically significant), their vagal tone was elevated compared to reactive pigs indicating a more positive affective state in proactive pigs. Therefore, I concluded that they do not perceive the same situation as stressful or alarming, compared to their reactive conspecifics. This finding supports the assumption that the observed behavioural differences between the coping styles are not merely reflexive in nature but are associated with a subjective interpretation of the stimuli, based on a differential affective-autonomic response. According to Gosling (2001), fear is an important component of animal personality and there have been several approaches to quantify the relationship between coping characteristics and emotionality (fearfulness), for example the three-dimensional model of Koolhaas and Van Reenen (2016, see above, p.10). The evolutionary rationale behind this concept is that fearfulness allows individuals to avoid potential threat or danger. Individual variation in the threshold for when a stimulus becomes inhibitory or stimulatory is therefore likely to be linked to the subjective experience of that stimulus in a particular situation. My results suggest that the relationship between coping characteristics and affective states is a widespread phenomenon and not only apparent in humans.

Repeated handling was thought to influence the affective state in the pigs in a positive way, as it can reduce an individual animal's fear of humans (Tanida et al., 1995; Day et al., 2002; Terlouw and Porcher, 2005). In sheep, grooming by a familiar person induced a positively valenced affective state (Reefmann et al., 2009c) and gentle touching in early life reduced avoidance distance and slaughter stress in cattle (Probst et al., 2012). Interestingly, the repeated experience with human contact over the time course of the experiment resulted in differential affective-autonomic states in the coping styles. Other than expected, proactive pigs showed an increase in sympathetically driven arousal with a lack of vagal activation during handling at the end of the experimental period. One may conclude that proactive pigs develop a high aroused state which was not characterised by positive valence. The according behavioural results regarding low approach latencies towards the familiar person seem contradictory to these findings. According to Koolhaas et al. (1999), proactive individuals respond generally more sympathetically driven to external stimuli. This assumption can be supported by my results, as proactive individuals showed sympathetic activity during feeding and handling, rather than vagal reactivity. One possible explanation may include the fact that proactive pigs were highly active during handling, changing from one side of the person to the other while exploring the person (personal observation). This may result in higher activity in general and may have affected the ANS response, but further investigations are needed to answer this question.

On the other hand, reactive pigs seem to develop a state of positive emotional valence with low arousal over repetitions of human handling. Similar increases in vagal tone were demonstrated in rhesus monkeys during grooming carried out by a familiar human (Grandi and Ishida, 2015). The autonomic reaction was also accompanied by a behavioural change, as the latency time to approach the familiar human in reactive pigs decreased indicating a shift from avoidance to attraction. An increased willingness of pigs to move voluntarily to a human experimenter after re-exposure was also found by Zebunke et al. (2017). It is known that 'pleasant' handling in the early life environment can influence an animal's subsequent behavioural development (Gonyou et al., 1986), and it has been demonstrated in pigs that early handling increased play and exploration behaviour, both of which are assumed to be associated with positive emotional states (Zupan et al., 2016). This is in line with recent physiological evidence using HR and cortisol measurements in pigs suggesting that tactile stimulation by a handler can be experienced as positive by the individual (Tallet et al., 2014). Handling can result in a type of environmental enrichment, as it can elicit positive emotions by providing stimulations and opportunities for new behaviours. Animals quickly lose interest in simple objects in their environment if they are not relevant (Newberry, 1995). This was not the case for handling, as pigs were highly motivated to explore the familiar person, as indicated by the short approach latencies.

The major findings point to the fact that the individual variation in how different situations were interpreted (appraisal) is linked to the individual's coping style. This link between coping style and the subjective experience in relation to the valence and arousal dimensions of affect may have consequences for different elements of coping ability related to stress reactions and situational appraisal in the context of animal welfare.

Summary at a glance: Affective-autonomic states and coping styles

- ✓ The respective coping style was related to significant context-related differences in their general autonomic response.
    - ❖ Reactive pigs showed higher vagal activity during resting compared to proactive pigs.
    - ❖ During idling, the coping styles did not differ in their ANS response.
    - ❖ Both coping styles showed a pronounced sympathetically driven arousal during feeding, which was stronger in proactive pigs, whereas reactive pigs developed a vagal activation during food consumption.
- ✓ The two coping styles differed in their behavioural and affective-autonomic response over the time course of the experiment in the context of a repeated handling situation indicating individual differences in their affective appraisal.
    - ❖ The ANS response of proactive pigs developed from low to high sympathetically aroused with no apparent changes in vagal activity.
    - ❖ The ANS response of reactive pigs developed from a high sympathetic arousal with rather negative valence to a more relaxed, low-aroused state with vagal activation indicating a positive affective state.
- ✓ Proactive pigs developed an anticipatory ANS response in the context of feeding faster than reactive pigs, whereas both coping styles did not clearly anticipate the handling situation, instead, a vagally mediated orienting response was shown.

3.3 Perspectives

The study of affective states in farm animals - especially the relationship to individual coping characteristics is still in development, but has received increased attention in recent years. The studies presented in this thesis reflect a first approach to measure both branches of the ANS to investigate affective-autonomic reactions in domestic pigs and help to gain deeper insight in the complexity of autonomic functioning in emotion.

3.3.1. Methodological perspectives

Using direct (invasive) telemetric methods to measure ANS-related correlates of affective states has a great potential unravelling the underlying mechanisms. However, as pointed out in section 3.1.2, ECG recordings are likely to suffer from movement artefacts. One possible approach to improve ECG signal quality is the use of solid tip electrodes, such as provided by DSI. This would contribute to lower correction effort and enhance parameter reliability. Moreover, the controversies regarding the interpretation of HRV in reflecting cardiac sympathetic activity show that HRV measures are not universally accepted to provide sympathetic insight (Billman, 2013). Besides using parameters that reflect vascular sympathetic activity (standard deviations of BP, as in this thesis), an alternative approach supports the use of systolic time intervals, in particular the pre-ejection period (PEP). PEP was demonstrated to directly reflect cardiac sympathetic influences on myocardial contractility (Cacioppo et al., 1994; van Dijk et al., 2013; Michael et al., 2017), as discussed in section 3.1.2. Thus, assessing PEP during positive and negative affective states may provide insight into cardiac sympathetic activity (inotropic influences) in order to augment HRV measurements of cardiac parasympathetic modulation (chronotropic influences). Generally, an interesting step towards more detailed information about autonomic function in emotion can integrate further well-established indices in the analysis. For example, T-wave amplitude, an index of sympathetic influence on the heart (Furedy et al., 1992) has been observed in several studies in humans in emotional paradigms, such as anxiety (for review see Kreibig, 2010). In the context of vagal activity, baroreflex sensitivity (de Boer et al., 1987; Parati et al., 2004) and respiratory sinus arrythmia (Huffman et al., 2008; van Dijk et al., 2013) have been used to quantify vagal activity in human research. All these autonomic indices have been established in human medical and/or emotional paradigms and offer a great potential for transfer to farm animals.

On the other side of the coin, according to the ethical principle of “3R”, I assume that future research will and should direct back to the use of less invasive procedures to measure affective states in animals. The fundamental issue with this progress is that less invasive as well as non-invasive systems are not yet able to keep up with the invasive telemetric method concerning parameter variety and reliability. For example, a minimally invasive sensor is commercially available for the use in large animals (DST milli-HRT, 13 mm × 39.5 mm; 11.8 g, Star Oddi, Iceland). It has been subcutaneously implanted in elephant seals (Chaise et al., 2017) but this device only provides information about HR (and body temperature). This is not sufficient to differentiate activity arising from the two branches of the ANS. The application of non-invasive systems implies disadvantages in experimental settings (as discussed above), but at least some systems are able to record continuous ECG tracings, for example Bioharness (BioHarness™ Telemetry System, Zephyr Technology Corporation, Annapolis, MD, U.S.A.), enabling the calculation of a variety of HRV parameters and also T-wave amplitude. In the context of BP recordings, the application of external methods is a possible approach to avoid surgical intervention, for example the use of tail-cuffs (based on oscillometry or Doppler flow detection) or specific ear clips (based on photo-plethysmography). Such systems have been utilised primarily in humans (Teng and Zhang, 2003; Zheng and Zhang, 2003; Espina et al., 2006; Guo et al., 2008), but their application in cats (Binns et al., 1995), rabbits (Kurashina et al., 1994) and rodents (Whitesall et al., 2004) has also been validated. However, these methods cannot replace the invasive telemetric system regarding its informational content, but careful validation, a step that can be accompanied and improved by the telemetric system, can enhance the reliability and applicability of non-invasive methods in experimental designs.

3.3.2. Animal welfare perspectives

The established surgical implantation of a telemetric device in combination with the functional assessment of detected parameters enabled the reliable registration of cardiovascular parameters during rest and behaviour patterns with elevated activity levels in free-moving pigs. The clear description of the use of the system in pigs, including surgical intervention, data processing, beat detection, data analysis, and application in experimental contexts provide a solid basis to successfully adopt the system in any desired situation. By comparing and validating several HRV and BPV parameters in known (or better: assumed) positive or negative situations, future research can synchronize these responses to unknown situations in experimentally-controlled conditions to determine the animal's affective state and emotional appraisal in the context of animal welfare.

The application of the telemetric system to clearly define positive and negative situations from the animals' point of view may facilitate the development of experiments investigating more complex situations in the context of affective-autonomic responses in pigs, such as social interactions (e.g. agonistic fights, dominance structure, socio-positive interactions). This topic is hardly feasible using external hardware in pigs, which are known for their destructive potential resulting from their high explorativeness. Furthermore, the animal's behaviour may be influenced by external equipment during social interactions (e.g. restricted mobility) or otherwise be affected (e.g. disruptive belts or cables during fights with high physical contact between encounters). The relationship to individual coping characteristics would be of particular interest in social contexts, as the concept of coping in animals was initially based on studies in rodents demonstrating aggressiveness as one general component of coping patterns (Van Oortmerssen, 1989; Benus et al., 1990, 1991; De Boer et al., 2003). Individual pigs, regardless of their weight or size, were found to differ considerably in how much aggression they show when confronted with conspecifics (Hessing et al., 1993). Aggressive individuals are more likely to adapt a proactive coping style, whereas pro-social behaviours were assigned to the reactive coping animal (Koolhaas et al., 1999). In this context, the literature consistently supports the model relating autonomic state in humans to social engagement behaviours and emotion regulation proposed in the polyvagal theory (Porges, 2007). It is assumed that similar relationships exist in domestic pigs. To date, it is unknown if specific autonomic processes may underlie these individual differences in the context of social behaviour and whether animals with different coping styles also differ in their affective appraisal during social interactions. Preliminary results on this topic let carefully assume that the coping style and dominance state play an important role in modulating behaviour and affective-autonomic states during social confrontation and, more specifically, success rather than coping style seems to influence the affective-autonomic reaction of pigs during agonistic interactions.

The application of the system in social contexts could provide valuable insight in understanding the potential of social aversive (e.g. fighting) as well as positive factors (e.g. grooming, playing, lying together, social support) and would fundamentally contribute to the assessment and understanding of animal welfare.

3.4 Conclusions

Gaining better insight in affective states of farm animals is of importance for understanding their welfare state. One important step in this context is to establish valid proxy measures to objectively assess and interpret an individual's subjective perception of its environment including its ability to respond to external and internal stimuli and cope with challenges. This thesis presents a reliable tool for the objective evaluation of affective-autonomic states in free-moving pigs and gains insight into the neurophysiological mechanisms underlying the processing of affective states. The detailed methodological information on the establishment of a telemetric system provided surgical instructions for the implantation of a telemetric device including the appropriate configuration of electrodes and catheters. Combined with the functional assessment of recorded parameters including the finding that HRV may not readily be determinable by using BP signal and the identification of reliable indices of vagal and sympathetic activity, this approach provides a solid basis to successfully adopt the system in any desired situation.

The ability to clearly differentiate activity arising from the two branches of the ANS enabled the interpretation of affective states according to the framework of the valence and arousal dimensions of affect. The context-related differences in the ANS response of pigs with different coping styles indicate that they may play a much more pivotal role than suggested so far, as differences between the coping styles are not only apparent in reaction to stressful situations, but also manifested in their general basic physiological state. This highlights the importance of taking individual differences into account when selecting animals for studies or interpreting the data. The specific way how individual pigs responded behaviourally and physiologically when exposed to the same situation suggests differences in their situational appraisal based on a differential affective-autonomic state. This individual variation in how specific situations were interpreted seems to be linked to the individuals' coping style reflecting the importance of two processes, affective appraisal and coping, as mediators of the relationship between the animal and its environment.

Understanding affective states and appraisal processes is of elemental interest in the context of animal welfare. If we were able to clearly identify the affective character of a situation, we would have the possibility to avoid negative and promote positive situations and therefore improve the life of animals in human care. Gaining better knowledge on the complexity of affective states and their underlying mechanisms is important to reveal new perspectives to both current and future animal welfare studies and will (hopefully) direct our perception of pigs from being a production species more to being complex individuals with each having their own individual characteristics, emotions and needs. This would be a significant step in the direction of improving farm animal husbandry and welfare.

Summary

This thesis focusses on the investigation of affective-autonomic states in domestic pigs (*Sus scrofa domestica*), by using an implantable telemetric technology capable of assessing both branches (parasympathetic and sympathetic) of the autonomic nervous system in the context of individual coping and animal welfare.

Gaining better insight into affective states of (farm) animals plays an important role in terms of understanding and improving animal welfare. One approach to objectively assess an organism's affective state is the measurement of autonomic activity, which is viewed as a major component of the affective state in many theories of emotion. Common approaches in this field measure cardiac activity by the means of external systems providing reliable parameters indicative for parasympathetic activity, whereas there does not appear to be any valid index that adequately reflects sympathetic modulation of the heart. Advances in technology have provided implantable telemetric systems which enable the additional analysis of vascular fluctuations. This allows examining sympathetic function exclusively, which complements information about subtle changes in autonomic activity in the context of affective states and enables conclusions about the valence and arousal dimensions of affect.

Based on this, the first aim of this thesis comprised the establishment of an implantable telemetric method for measuring both branches of the autonomic nervous system in order to provide a valid tool for the objective evaluation of affective-autonomic states in free-moving pigs.

However, affective states may vary between individuals based on the perception of individually relevant interactions with the environment, including the ability to respond to and cope with challenges. Thus, individual reaction pattern, such as coping styles, may influence the underlying mechanisms of the affective state in a specific situation. Therefore, the second major aim of this thesis was the investigation of individual coping styles and their specific role in the context of affective-autonomic reactions of pigs in different housing-relevant situations within the two-dimensional model of affect. The thesis is divided into three chapters.

Chapter I provides a general introduction into the topic of affective states, their relevance for the evaluation of animal welfare as well as their interaction with autonomic functioning and individual coping styles. This is followed by a description of the proximate neurophysiological pathways underlying affective states. After illustrating the current methods to measure autonomic activity in the context of affective states including their implication, the key structure of this thesis is highlighted in the overarching context of the integrated studies, as well as the according hypotheses.

Chapter II provides three studies that were published as part of this thesis.

Study 1 addresses the establishment of a new telemetric technology as a valid tool for the objective investigation of both branches of the autonomic nervous system in domestic pigs and has been published in *The Veterinary Journal* 207, 140-146 (2016). The first aim in this study was the development of a stringent surgical procedure for the implantation of a telemetric device for the continuous measurement of electrocardiogram (ECG) and blood pressure (BP) in free-moving pigs. The second aim comprised the functional assessment of the recorded parameters which included data processing, detection performance, and controlling the reliability of obtained parameters for application in two different behavioural situations with different physiological demands (resting vs. feeding). The major findings revealed technical and surgical issues in four pigs concerning catheterization and detachment of ECG leads. These issues were solved and a list of recommendations for future studies was given. Detection performance decreased with elevated activity level, but manual correction was able to reliably eliminate errors. Generally, blood pressure was less susceptible to movement artefacts compared to ECG. This study demonstrated the usability of the telemetric system for the reliable registration of cardiovascular parameters in pigs permitting to draw conclusions about both branches of the autonomic nervous system and hence, the current affective state of the animal. This served as a basis for study 2 and 3 of this thesis.

Study 2 provides a mathematical-statistical approach concerning the calculation of cardiac parameters and has been published in *Frontiers in Veterinary Science* 2, 52 (2015). Based on the findings from study 1 regarding BP-signal being less susceptible to movement artefacts than ECG, the major aim of study 2 was to clarify whether the BP-signal can be used instead of the ECG to determine heart rate (HR) and its variability (HRV) in pigs in different behavioural situations. Several statistical and mathematical methods were applied with different explanatory power. The major findings showed that HR data recorded via BP agree well with those recorded using ECG independently of the activity of the subject, whereas ECG and BP cannot be used interchangeably in the context of HRV in domestic pigs. This finding contributes to the validity and application of cardiac parameters in future studies.

Study 3 transfers the methodological findings from study 1 and 2 in to the application in an experimental context addressing the link between affective-autonomic states and individual coping characteristics in domestic pigs and was published in *Frontiers in Behavioral Neuroscience* 11, 103 (2017). The main aim was the investigation of affective-autonomic states using parameters that reflect both branches of the autonomic nervous system simultaneously in pigs in different housing-relevant situations and the relationship to their individual coping styles. The major findings confirm that the respective coping styles were related to context-specific differences not only in the general autonomic response during resting and feeding conditions, but also in the behavioural and affective-autonomic response in the context of a repeated human-animal interaction. This indicates individual differences in the subject's affective appraisal of the situation.

Chapter III provides the general discussion of the findings from the three studies and comprises a discussion of the establishment of the telemetric system addressing the methodological approach of this thesis followed by a section on the relationship between affective-autonomic states and individual coping styles. Additional sections include perspectives for future studies and provide the final conclusions of the thesis.

Understanding affective states and appraisal processes is of elemental interest in the context of animal welfare. Gaining better knowledge of the complexity of affective states regarding their underlying mechanisms and their individual perception and processing is important to reveal new perspectives to both current and future animal welfare studies. This will (hopefully) direct our perception of domestic pigs from being a production species more to being complex individuals with each having their own individual characteristics, emotions and needs. This would be a significant step in the direction of improving farm animal husbandry and welfare.

Zusammenfassung

Der Fokus dieser Arbeit liegt auf der Untersuchung affektiver Zustände domestizierter Schweine mittels einer implantierbaren telemetrischen Methodik zur Erfassung autonomer Reaktionen im Kontext individueller Reaktionsmuster (*Coping*) und Wohlbefinden.

Die objektive Erfassung affektiver Zustände bei Nutztieren spielt eine entscheidende Rolle in Hinblick auf das Verständnis und die Verbesserung von Wohlbefinden. Ein Ansatz dazu ist die Messung der Aktivität und Balance der beiden Zweige des autonomen Nervensystems (ANS), dem Parasympathikus und Sympathikus, die in vielen Emotions-Theorien als zentrale Komponenten betrachtet werden. Gängige Untersuchungen in diesem Bereich messen die Herzaktivität via Elektrokardiogramm (EKG) durch externe Systeme. Dabei können zuverlässige Rückschlüsse über die parasympathische Aktivität gezogen werden. Durch den technologischen Fortschritt der letzten Jahre stehen heutzutage implantierbare telemetrische Komplettsysteme zur Verfügung, die durch die zusätzliche Analyse des Blutdrucks (BP) Aussagen über die sympathische Aktivität des ANS zulassen. Damit wird die Information über subtile Änderungen im ANS erweitert und ermöglicht Rückschlüsse über die Wertigkeit (Valenz) und Erregung (*arousal)* des affektiven Zustandes.

Das erste Ziel dieser Arbeit war daher die Etablierung einer telemetrischen Methodik zur kontinuierlichen Erfassung kardiovaskulärer Parameter (EKG, BP) beim Schwein, durch die beide Zweige des ANS abgebildet werden können. Damit wird ein valides Werkzeug zur objektiven Evaluierung affektiver Zustände bei freibeweglichen Schweinen bereitgestellt.

Affektive Zustände sind komplex und variieren zwischen Individuen – basierend auf der unterschiedlichen individuellen Wahrnehmung relevanter Interaktionen mit der Umwelt einschließlich der Fähigkeit auf Reize zu reagieren und Herausforderungen zu bewältigen. So können zum Beispiel individuelle Reaktionsmuster (*Coping*-Strategien) einen Einfluß darauf haben, welche zugrundeliegenden Mechanismen affektiver Zustände situationsbedingt aktiviert werden. Das zweite Ziel dieser Arbeit greift diesen Aspekt auf und konzentriert sich auf die Untersuchung des Zusammenspiels von individuellen *Coping*-Strategien und ihrer Rolle bei der Ausprägung kontext-spezifischer, affektiv-autonomer Reaktionen von Schweinen. Die Arbeit ist in drei Kapitel unterteilt.

Kapitel I leitet in die Thematik der affektiven Zustände ein, setzt ihre Relevanz in Zusammenhang mit der Evaluation von Wohlbefinden und stellt die direkte Verknüpfung zu autonomer Funktion und individuellen *Coping*-Strategien vor. Darauf folgt eine Beschreibung der proximaten neurophysiologischen Mechanismen, die den affektiven Zuständen zugrunde liegen. Nach der Vorstellung gängiger Methoden zur Messung autonomer Aktivität im Kontext affektiver Zustände wird die übergreifende Struktur der Arbeit im Kontext der integrierten Studien beleuchtet sowie die entsprechenden Ziele und Hypothesen vorgestellt.

Kapitel II stellt drei Studien vor, die als Teil dieser Arbeit publiziert wurden.

Studie 1 befasste sich mit der Etablierung einer telemetrischen Methode beim Schwein und wurde in *The Veterinary Journal* 207, 140-146 (2016) publiziert. Das erste Ziel dieser Studie umfasste die Entwicklung eines präzisen chirurgischen Eingriffs zur Implantation eines Transmitters zur kontinuierlichen Erfassung von EKG und BP. Das zweite Ziel dieser Studie war die funktionale Berurteilung der Parameter, einschließlich Datenbearbeitung, Detektionsleistung und Überprüfung der Zuverlässigkeit der Parameter in Bezug auf die

Anwendung in unterschiedlichen Verhaltenskontexten (Liegen vs. Fressen). Die Ergebnisse zeigten technische und chirurgische Probleme bei vier (von 11) Tieren. Eine Reihe an Empfehlungen für zukünftige Implantationen konnte erarbeitet werden. Insgesamt sank die Detektionsleistung mit steigendem Aktivitätslevel der Tiere; die manuelle Korrektur konnte Fehler allerdings zuverlässig eliminieren. Insgesamt war der BP im Vergleich zum EKG weniger anfällig für Bewegungsartefakte. Diese Studie zeigte die Verwendbarkeit des telemetrischen Systems zur zuverlässigen Erfassung kardiovaskulärer Parameter beim Schwein, die Rückschlüsse über die Aktivität beider Zweige des ANS zulassen – und damit über den aktuellen affektiven Zustand. Dies diente als Basis für die Studien 2 und 3.

Studie 2 stellte einen mathematisch-statistischen Ansatz hinsichtlich der Berechnung der Herzaktivität dar und wurde in *Frontiers in Veterinary Science* 2, 52 (2015) veröffentlicht. Basierend auf dem Ergebnissen aus Studie 1, bezüglich der geringeren Anfälligkeit für Bewegungsartefakte, wurde in Studie 2 überprüft, ob das BP-Signal anstelle des EKGs verwendet werden kann um Herzfrequenz (HR) und dessen Variabilität (HRV) bei Schweinen in unterschiedlichen Verhaltenssituationen zu bestimmen. Dazu wurden verschiedene Parameter aus EKG und BP berechnet und mittels statistischer und mathematischer Methoden mit unterschiedlicher Aussagekraft verglichen. Die Ergebnisse wiesen darauf hin, dass die HR, die mittels BP-Signal berechnet wurde, sehr gut mit der aus dem EKG übereinstimmt – unabhängig von der Aktivität des Tieres. Das BP-Signal konnte das EKG allerdings nicht in Hinblick auf die Berechnung von HRV ersetzen. Dies trägt zur Validität und Anwendung von Herzaktivitätsparametern in zukünftigen Studien bei.

Studie 3 diente der Anwendung der Methodik aus Studien 1 und 2 in einem experimentellen Kontext und wurde in *Frontiers in Behavioral Neuroscience* 11, 103 (2017) publiziert. Das Ziel war die Untersuchung affektiv-autonomer Zustände bei Schweinen in unterschiedlichen Haltungssituationen sowie speziell der Zusammenhang mit individuellen *Coping*-Strategien. Die Ergebnisse zeigten deutliche kontextbezogene Unterschiede zwischen Tieren mit individuell unterschiedlichen *Coping*-Strategien in ihrer autonomen Aktivität. Zudem unterschieden sich Schweine mit unterschiedlichen *Coping*-Strategien in ihrer ethologischen und affektiv-autonomen Reaktion während einer wiederholten Mensch-Tier-Interaktion. Dies weist auf individuelle Unterschiede in der affektiven Bewertung der Situation hin.

Kapitel III beinhaltet die übergreifende Diskussion der Ergebnisse aus den vorgestellten Studien. Dies umfasst eine Diskussion über die Etablierung des telemetrischen Systems hinsichtlich des methodischen Ansatzes dieser Arbeit, gefolgt von einem Abschnitt, in dem der Zusammenhang zwischen individuellen *Coping*-Strategien und affektiv-autonomen Zuständen in unterschiedlichen Verhaltenssituationen diskutiert wird. Zusätzliche Abschnitte weisen auf den Einsatz in zukünftigen Studien hin und beenden das Kapitel mit dem finalen Fazit der Arbeit.

Das Verständnis affektiver Zustände und Bewertungprozesse ist von großem Interesse im Kontext von Wohlbefinden. Durch genauere Kenntnisse über die Komplexität affektiver Zustände hinsichtlich ihrer zugrundeliegenden Mechanismen und ihrer individuellen Wahrnehmung und Verarbeitung können neue Perspektiven für zukünftige Studien im Rahmen von Wohlbefinden aufgezeigt werden. Dies wird (hoffentlich) unsere Wahrnehmung von Schweinen als Produktionstier zu einem komplexen Individuum lenken, von dem jedes seine eigenen individuellen Merkmale, Emotionen und Bedürfnisse hat. Dies wäre ein bedeutender Schritt in Richtung Verbesserung der landwirtschaftlichen Nutztierhaltung und Wohlbefinden.

List of Abbreviations

ANOVA	*analysis of variance*
ANS	*autonomic nervous system/ autonomes Nervensystem*
BP	*blood pressure / Blutdruck*
BPV	*blood pressure variability*
CAN	*central autonomic network*
CI	*confidence interval*
cm	*centimetre(s)*
DBP	*diastolic blood pressure*
ECG	*electrocardiogram*
EKG	*Elektrokardiogramm*
h	*hours*
HF	*high frequency*
HPA	*hypothalamic pituitary adrenal*
HR	*heart rate / Herzfrequenz*
HRV	*heart rate variability / Herzfrequenzvariabilität*
IBI	*interbeat interval*
IM	*intramuscular*
ICC	*intraclass correlation coefficient*
IV	*intravenous*
kg	*kilogram(s)*
kHz	*kilohertz*
LF	*low frequency*
LoA	*limit of agreement*
LSM	*Least squares mean*
LVET	*left ventricular ejection time*
m	*metre(s)*
mm	*millimetre(s)*
mmHg	*millimetre of mercury*
ms	*millisecond(s)*
mV	*millivolt*
MAP	*mean arterial blood pressure*
mg	*milligrams*
min	*minutes*
PCG	*phonocardiogram*
PEP	*pre-ejection period*
RMSSD	*root mean square of successive differences*
RR interval	*time between two consecutive R-peaks in an ECG*
s	*seconds*
SBP	*systolic blood pressure*
SC	*subcutaneous*
SDD	*standard deviation of diastolic blood pressure*

SDM	*standard deviation of mean arterial pressure*
SDNN	*standard deviation of all interbeat intervals of a data set*
SDS / SD_{SBP}	*standard deviation of systolic blood pressure*
SE	*standard error*
TI	*time interval*
VLF	*very low frequency*

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Theses

Objectives of research

Gaining better insight into affective states of (farm) animals is increasingly important in terms of understanding and improving animal welfare. One important step in this direction is to establish valid and accurate proxy measures to objectively assess an organism's affective state. Evaluating the activity of the autonomic nervous system (ANS) and the balance of its two branches (parasympathetic and sympathetic branch) is a promising approach, as they are viewed as a major component of the affective state in many theories of emotion. Common studies in this field evaluate cardiac activity by the use of external systems providing reliable parameters indicative for parasympathetic (vagal) activity, whereas there does not appear to be any valid index that adequately reflects sympathetic modulation. Due to advances in technology it is now possible to detect both branches of the ANS by the additional assessment of blood pressure (BP), which complements information about subtle changes in ANS activity. The objective assessment of these underlying neurophysiological mechanisms makes it possible to draw conclusions about how affective states may vary both in terms of valence (pleasantness/unpleasantness) and arousal, which are viewed as the core dimensions of affective states.

Moreover, affective states may vary between individuals and it has to be considered that individuals differ in their general adaptive response to challenges. This comprises individual behavioural reaction patterns, such as coping styles (proactive vs. reactive). However, only little is known about the possible link between affective states and coping styles. This thesis combines a methodological and an experimental approach by using an implantable telemetric system to investigate affective-autonomic states in free-moving domestic pigs in the context of coping and animal welfare. Two major aims were addressed:

(1) The establishment of a telemetric method for measuring both branches of the ANS in order to provide a valid tool for the objective evaluation of affective-autonomic states in free-moving pigs. This included (a) the development of a reliable surgical procedure for the implantation of a telemetric device for the continuous recording of ECG and BP and (b) the functional assessment of recorded parameters to ensure reliability of the acquired data.

(2) The assessment of affective-autonomic responses of pigs in different housing-relevant situations and the relationship to their individual coping characteristics within the two-dimensional model of affective states.

Main findings

In the context of the establishment of the telemetric method the following key findings were published in *The Veterinary Journal* 207, 140–146 (2016) and in *Frontiers in Veterinary Science* 2, 52 (2015).

- ✓ A detailed surgical protocol for the implantation of a telemetric device for the continuous recording of electrocardiogram (ECG) and BP in free-moving pigs was developed. For the generation of valid signals it has proven advisable to place electrodes in muscle tissue (the negative electrode lateral to the sternum and the positive electrode lateral to the scapula), to insert the BP catheter in the external carotid artery, and to place the telemetric device subcutaneously at the left side of the neck of the animal.

- ✓ Technical and surgical issues concerning catheterization and detachment of ECG leads impeded data transmission in four (out of 11) pigs and a list of recommendations for future implantation studies was generated.
- ✓ Detection performance decreased with elevated behavioural level comparing resting to feeding conditions, but manual correction was able to reliably eliminate errors.
- ✓ Blood pressure signal was more stable and less susceptable to movement artefacts compared to electrocardiogram.
- ✓ Cardiovascular parameters during baseline (resting) conditions indicated high vagal and low sympathetic activity.
- ✓ Cardiovascular parameters during behavioural activity in the context of food consumption indicated decreased vagal and increased sympathetic activity.
- ✓ BP signal was reliable in calculating HR values, but may not be used for the determination of HRV due to systematic overestimation of the values in comparison to calculations derived from ECG (gold standard).

The investigation of affective-autonomic responses of pigs in different housing-relevant situations and the relationship to their individual coping style yielded the following findings which were published in *Frontiers in Behavioral Neuroscience 11, 103 (*2017):

- ✓ The respective coping style was related to significant context-related differences in their general autonomic state.
- ✓ The two coping styles differed in their behavioural and affective-autonomic response over the time course of the experiment in the context of a repeated handling situation indicating individual differences in their affective appraisal.
- ✓ Proactive pigs developed an anticipatory ANS response in the context of feeding faster than reactive pigs, whereas pigs of both coping styles did not clearly anticipate the handling situation. Instead, a vagally mediated orienting response was shown.

Conclusions

This thesis presents a valid tool for the objective evaluation of affective-autonomic states in free-moving pigs. This provides insight into the neurophysiological processes underlying affective responses and situational appraisal. The link between context-specific affective-autonomic states and individual coping characteristics indicates that coping may play a more pivotal role than suggested so far and highlights the importance of both, as mediators of the relationship between the animal and its environment. Understanding affective states and appraisal processes is of elemental interest in the context of animal welfare. Gaining better knowledge of the complexity of affective states regarding their underlying mechanisms and their individual perception and processing is important to reveal new perspectives to both current and future animal welfare studies. This will (hopefully) direct our perception of domestic pigs from being a production species more to being complex individuals with each having their own individual characteristics, emotions and needs. This would be a significant step in the direction of improving farm animal husbandry and welfare.

List of publications

Peer-reviewed journal articles

Krause, A., Puppe, B., Langbein, J., 2017. Coping style modifies general and affective autonomic reactions of domestic pigs in different behavioral contexts. Front. Behav. Neurosci. 11, 103. doi: 10.3389/fnbeh.2017.00103

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Zebunke, M., Krause, A., Langbein, J., Puppe, B. 2018. Gibt es individuelle Strategien im Umgang mit Stress? – Untersuchungen zum Coping-Verhalten beim Schwein. Nutztierhaltung im Fokus, Animal Personality – Persönlichkeit bei Nutztieren, Internationale Gesellschaft für Nutztierhaltung e.V.

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Krause, A., Puppe, B., Langbein, J. 2016. Implantable transmitters for measuring both branches of the autonomic nervous system to describe affective reactions in domestic pigs. Oral presentation, Workshop Measuring Animal Emotion, Edinburgh, United Kingdom.

Krause, A., Puppe, B., Langbein, J. 2016. Changes in autonomic balance of pigs in different behavioral contexts with special focus on coping type. Oral presentation, Proceedings of the 50th Congress of the International Society for Applied Ethology 12-15th July, 2016, Edinburgh, United Kingdom.

Krause, A., Zebunke, M., Langbein, J., Puppe, B. 2014. Etablierung eines invasiven Telemetrie-Systems zur Erfassung kardiovaskulärer Parameter beim Schwein. Oral presentation, 7. Agrosnet Doktorandentag, Matrin-Luther- University in Halle-Wittenberg, Germany.

Krause, A., Zebunke, M., Langbein, J., Puppe, B. 2014. Verhalten und autonome Reaktion von Schweinen im Kontext der Fütterung. Poster presentation (Poster award: 2nd place), Aktuelle Arbeiten zur artgemäßen Tierhaltung, KTBL-Schrift 505, 242-243. Internationale Arbeitstagung Angewandte Ethologie bei Nutztieren der Deutschen Veterinärmedizinischen Gesellschaft e.V.

Krause, A., Zebunke, M., Langbein, J., Puppe, B. 2013. Etablierung eines invasiven Telemetrie-Systems zur Erfassung kardiovaskulärer Parameter beim Schwein. Poster presentation (Poster award: 3rd place), Aktuelle Arbeiten zur artgemäßen Tierhaltung, KTBL-Schrift 503, 240-241. Internationale Arbeitstagung Angewandte Ethologie bei Nutztieren der Deutschen Veterinärmedizinischen Gesellschaft e.V.