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On assessment methods related to pain in dogs with osteoarthritis

ANN ESSNER

ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2018

ISSN 1651-6206
ISBN 978-91-513-0199-0
urn:nbn:se:uu:diva-334679

Dissertation presented at Uppsala University to be publicly examined in Gustavianum, Akademigatan 3,753 10 Uppsala, Uppsala, Friday, 16 February 2018 at 10:00 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in Swedish. Faculty examiner: Docent Patricia Hedenqvist (Sveriges lantbruksuniversitet, Institutionen för kliniska vetenskaper).

Abstract
Essner, A. 2018. On assessment methods related to pain in dogs with osteoarthritis. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine* 1415. 70 pp. Uppsala: Acta Universitatis Upsaliensis. ISBN 978-91-513-0199-0.

There is a need of valid and reliable assessment methods that are clinically applicable in canine rehabilitation practice. The aim of this thesis was to psychometrically evaluate measurement properties in assessment methods related to pain in naturally occurring canine osteoarthritis. Assessment methods developed for heart rate variability analysis, i.e. Polar heart rate monitor, and owner-reported perceptions of pain severity and pain interference with functionality, i.e. Canine Brief Pain Inventory, were tested.

Methods: Four observational studies were conducted. Study I was a cross-sectional study consisting of two groups of consecutively recruited dogs. The Canine Brief Pain Inventory was administered to owners of dogs with naturally occurring osteoarthritis (n=61) and clinically sound dogs (n=21). Study II was a descriptive and correlative cross-sectional study based on the same sample of dogs with osteoarthritis (n=71), assessing chronic pain behavior and associations between explanatory variables and chronic pain behavior. Study III and IV were correlative studies, assessing Polar heart rate monitor measuring interbeat intervals and time- and frequency-based heart rate variability parameters, compared to simultaneously recorded electrocardiogram in dogs (n=11).

Results: High internal consistencies and ability to discriminate sound dogs from osteoarthritis dogs were found. The hypothesis of the presented two-factor structure of the Canine Brief Pain Inventory was rejected. Owners reported higher proportions of chronic pain behavior in items targeting physical activities, e.g. getting up, moving after rest and moving after major exercise. A minor proportion of dogs with osteoarthritis showed no owner-perceived behavioural signs of chronic pain. Owner observations were not associated with ongoing antiinflammatory medications. In Study III and IV, 595 errors (12.3%) were identified in Polar data. The number of errors were unequally distributed among the dogs. Interbeat intervals and heart rate variability parameters from electrocardiogram and Polar were strongly associated. Standard error of measurements were high among some heart rate variability parameters in Polar and electrocardiogram.

In conclusion, this thesis contributes to our knowledge about assessment methods related to diverse components of pain in dogs with osteoarthritis, allowing improved pain management in clinical practice.

Keywords: assessment methods, behavior, canine, chronic pain, heart rate variability, measurement properties, osteoarthritis, physiotherapy, rehabilitation

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ISSN 1651-6206
ISBN 978-91-513-0199-0
urn:nbn:se:uu:diva-334679 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-334679)

Till min fantastiska tvåbenta och fyrbenta familj

” I have never tried that before, so I think I should definitely be able to do that…”.

Pippi Longstocking
(Astrid Lindgren)

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals (Study I-IV).

I Essner A, Zetterberg L, Hellström K, Gustås P, Högberg H, Sjöström R. Psychometric evaluation of the canine brief pain inventory in a Swedish sample of dogs with pain related to osteoarthritis. Acta Vet Scand. 2017;59:44.

II Essner A, Högberg H, Zetterberg L, Hellström K, Sjöström R, Gustås P. (2017). Owner-perceived chronic pain behavior and associated factors in canine osteoarthritis – an observational study. Submitted.

III Essner A, Sjöström R, Ahlgren E, Gustås P, Edge-Hughes L, Zetterberg L, Hellström K. Comparison of Polar RS800CX heart rate monitor and electrocardiogram for measuring interbeat intervals in healthy dogs. Physiol Behav. 2015;138:247-53.

IV Essner A, Sjöström R, Gustås P, Edge-Hughes L, Zetterberg L, Hellström K. Validity and reliability properties of canine short-term heart rate variability measures – a pilot study. J Vet Behav. 2015;10:384-90.

Contents

Preface 11

1 Introduction 13
1.1 Canine osteoarthritis 13
1.2 Pain mechanisms in canine osteoarthritis 13
1.3 Definition of pain in animals 14
1.4 Components of pain in animals 14
1.5 Biophysiological responses to pain 16
1.6 Cognitive and emotional responses to pain 16
1.7 Pain-related overt canine behaviors 17
1.8 Assessing pain in canine osteoarthritis 18
1.8.1 Heart rate variability analysis 18
1.8.2 Owner-reported pain and disability questionnaires 20
1.9 Psychometric properties of assessment methods 20
1.9.1 Validity 22
1.9.2 Reliability 22
1.9.3 Responsiveness 23
1.9.4 Interpretability 23
1.10 Rationale for this thesis 24

2 Aims 25
2.1 Specific aims 25

3 Methods 26
3.1 Design 26
3.2 Ethical considerations 26
3.3 Subjects and procedures 26
3.3.1 Study I and II 26
3.3.2 Study III and IV 28
3.4 Data collection 29
3.4.1 Pain severity and pain interference with function (Study I and II) 29
3.4.2 Chronic pain behavior (Study II) 30
3.4.3 Body condition score (Study I and II) 31
3.4.4 Interbeat intervals (Study III) 31
3.4.5 Heart rate variability parameters (Study IV) 31
3.4.6 Anthropometric measure (Study I-IV) 31

3.5 Data management and analysis 31
3.5.1 Study I 32
3.5.2 Study II 32
3.5.3 Study III 33
3.5.4 Study IV 33

4 Results 35
4.1 Psychometric properties of the CBPI 35
4.1.1 Construct validity; structural validity 35
4.1.2 Construct validity; hypothesis testing 35
4.1.3 Internal consistency and interpretability 36
4.2 Owner-perceived chronic pain behavior in canine osteoarthritis 37
4.2.1 Presence of owner-perceived chronic pain behavior 37
4.2.2 Differences between dogs with and without chronic pain 37
4.2.3 Association between chronic pain and explanatory variables 37
4.3 Measuring interbeat intervals 41
4.3.1 Relationship and relative reliability between ECG and Polar interbeat intervals 41
4.4 Analyzing heart rate variability 45
4.4.1 Associations and agreement between HRV parameters from Polar and ECG 45
4.4.2 Within-group variation in Polar and ECG measurements 45

Discussion 47
5.1 Translation of owner-perceived questionnaires 48
5.2 Psychometric properties of the CBPI (Study I) 48
5.3 Owner-perceived chronic pain behavior in canine OA (Study II) 49
5.4 Impact of pain on body function and activities (Study I and II) 50
5.5 Measuring interbeat intervals (Study III) 51
5.6 Analyzing heart rate variability (Study IV) 51
5.7 Methodological considerations 52

6 Conclusions 55
6.1 Implications for clinical practice 56
6.2 Implications for future research 56

7 Svensk sammanfattning (Swedish summary) 57

8 Acknowledgments 59

9 References 61

Abbreviations

CBPI	Canine Brief Pain Inventory
CFA	Confirmatory factor analysis
CI	Confidence interval
ECG	Electrocardiogram
EFA	Exploratory factor analysis
HCPI	Helsinki Chronic Pain Index
HF	High frequency
HF n.u.	High frequency normalized units
HRV	Heart rate variability
IBI	Interbeat interval
ICC	Intraclass correlation coefficient
LF	Low frequency
LF n.u.	Low frequency normalized units
LF/HF	Ratio low frequency power/high frequency power
LoA	Limits of agreement
OR	Odds ratio
RMSSD	Square root of the mean squared differences of successive normal-to-normal interbeat intervals
SAM	Sympatho-adreno-medullary
SD	Standard deviation
SDNN	Standard deviation of normal-to-normal interbeat intervals
SEM	Standard error of measurement

Preface

This thesis is based on my clinical experience as an animal physiotherapist, practicing within veterinary medicine for the past 15 years. From my experience, many dogs with musculoskeletal disorders are affected by pain at some point during the treatment process. Recognition of adaptive and maladaptive pain and pain-related disability is key to adequately manage canine osteoarthritis. Pain in canine osteoarthritis may be complicated and therefore challenging to treat. There are sometimes diverse opinions among dog owners and animal health care professionals about how to interpret signs of pain in a potentially chronic pain condition, such as osteoarthritis. Lack of valid and reliable assessment methods makes it difficult to evaluate outcome from interventions targeting the multiple aspects involved in the canine chronic pain experience. In physiotherapy, there is a clear connection between theory and practice, and in the four studies included in this thesis, a multi-dimensional approach is applied to evaluate assessment methods related to pain in canine osteoarthritis.

1 Introduction

1.1 Canine osteoarthritis

Osteoarthritis (OA) in domestic dogs (*canis familiaris*) is a common and chronic disease of movable joints [1-3]. The prevalence of canine OA is about 20% to 30% in the adult dog population [4-6]. Osteoarthritis is characterized by diverse changes in joint tissue metabolism, cartilage degradation, modified bone remodeling, osteophyte formation, joint inflammation and loss of normal joint function [1,6-8]. The most frequently associated consequences of canine OA are pain, disability and decreased quality of life [9-11].

Disability refers to the dogs' function in three levels: the body or a body part, the whole individual and the whole individual in a social context, and life activities [12-14]. Osteoarthritis negatively impacts local and global function, causing disability, i.e. impairments of body structure or function, activity limitations and participation restrictions [12-14]. The clinical signs of naturally occurring canine OA are e.g. reduced pain-free range of motion in affected synovial joints, reduced muscle flexibility, modified weight-bearing of a limb during standing or moving, reduced level of performance in activities of daily living e.g. running, walking, rising, climbing and gradual changes of the dogs' behavior in e.g. various social contexts [10,12,15]. However, pain and disability do not always correlate with structural joint changes detected by radiography, i.e. in joint space narrowing, osteophyte formation, bone sclerosis and bone cysts, pathological bone contour alterations and joint malalignment [1,16-19].

1.2 Pain mechanisms in canine osteoarthritis

Pain in OA is mediated by diverse mechanisms [9,20,21]. Excessive mechanical stress, e.g. in weight bearing and movement, subjected to a joint affected by OA may lead to nociceptive input and pain [22,23]. Canine OA pain is categorized as nociceptive and inflammatory in origin [24]. Inflammatory mediators may sensitize the neural pathways leading to increased sensitivity to stimuli in nociceptive afferent neurons and contributing to peripheral sensitization [24,25]. Pain-induced sensitization of nociceptor transmission in the spinal cord, i.e. central sensitization, is also associated with inflammation and with development and maintenance of chronic (maladaptive) pain [26]. Adaptive OA pain

may convert to maladaptive pain by pain-induced changes in the nervous system [24]. Links between pain related to OA and central sensitization in the dorsal horn in the spinal cord, leading to altered spinal and supraspinal processing of sensory input and pain perception, have been presented in dogs and cats [27,28]. The central augmentations, modulated by descending and facilitating pathways in the central nervous system, may cause increased excitability leading to pain by a stimulus that does not normally lead to pain, i.e. allodynia, and increase the response to a stimulus that is normally painful, i.e. hyperalgesia [29]. Recent progress suggests that inflammation of tissue within the peripheral nervous system and central nervous system – neuroinflammation – has a key role in the development of chronic pain [30,31]. Osteoarthritis is considered a major cause of chronic pain in dogs and is therefore a threat to health-related quality of life and animal welfare [4,32,33].

1.3 Definition of pain in animals

The International Association for the Study of Pain has defined pain in humans as *"An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage"* and also states that *"The inability to communicate verbally does not negate the possibility that an individual is experiencing pain"* [34] , which allows for the definition to be applied to animals [26,35,36]. The definition of chronic pain in animals corresponds to the definition endorsed by the International Association for the Study of Pain, and is that *"pain that extends beyond the period of tissue healing and/or low levels of identified pathology that are insufficient to explain the presence and/or extent of pain"* [26]. Determining the end of the healing phase is difficult and chronic pain is often described over a duration of more than three months [37]. Acute and chronic pain differ in pathology, and as such chronic pain in dogs may be considered a separate disease state [26].

1.4 Components of pain in animals

Historically, it has been debated to what extent animals experience pain [38]. It has now been concluded that, beyond any doubt, dogs experience pain. There are arguments for parallel pain experiences in dogs and humans, since the neuroanatomy and physiology of pain are similar [35]. In concordance with the definition of pain in animals, pain is a multidimensional experience involving several components [25,26]. Despite the extensive research on behavior and pain in animals in experimental and clinical trials conducted over the years, there is a lack of unified agreement on a conceptual model of pain related to OA in dogs [20,25,26,39]. It is important for animal health care professionals and researchers to consider how these different components may affect the dogs' responses

to pain, to assess for any indication of pain in each of the components and tailor treatment case-by-case. Several conceptual models of pain have been adopted in companion animals in the current literature [9,39,40]. At its simplest, pain in animals has been described as a two-component structure: a sensory-discriminatory component and an emotional component [40]. A three-component model, based on a seminal model by Melzack, involving sensory-discriminatory, emotional-motivational and cognitive-evaluative components has been described in dogs and cats [41-43]. Recently, a conceptual model of pain, integrating sensory, emotional, cognitive and behavioral components of pain experience was applied to dogs with chronic pain related to OA [9,44] (Figure 1). The model was originally described in a seminal work by Loeser [44].

From an animal welfare perspective, it is essential to respect the rights of animals to live according to the five provisions of animal welfare and accordingly to recognize, assess, reassess and treat dogs for signs of chronic pain [33,45,46]. Despite potential barriers to adopt a multidimensional approach to chronic pain conditions in veterinary clinical practice, e.g. due to the time required to conduct assessments, there is a need to consider all components involved in the pain experience, to implement a thorough approach and tailor treatments in evidence-based clinical practice [9].

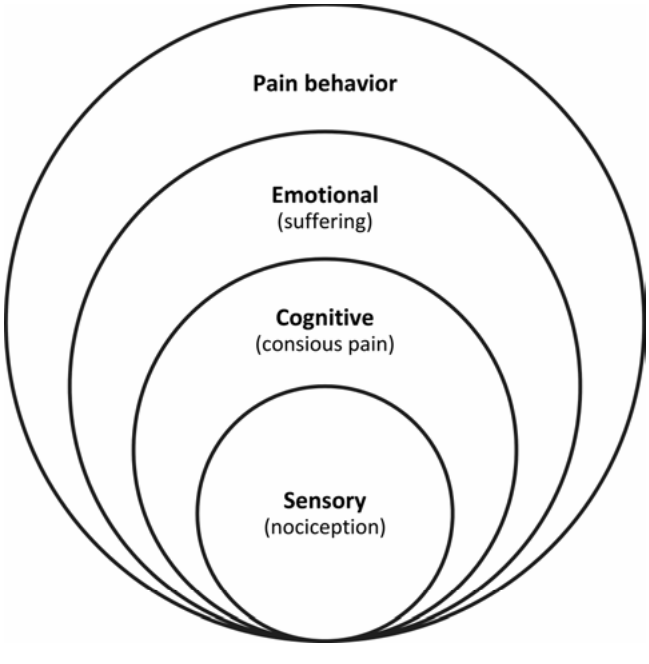

Figure 1. *Integration of the physical, cognitive, emotional and behavioral components in a conceptual model of chronic pain, based on the seminal work by Loeser and adopted to dogs by Fox* [9,44].

1.5 Biophysiological responses to pain

To maintain homeostasis, mammals adapt to physiologic and psychogenic stressors that are part of normal life. Maintenance of stability in the adaptive systems are active processes achieved through physiologic responses in diverse body systems, i.e. the autonomous nervous system (ANS), the immune system and the endocrine system [47-49]. There is a widely accepted relationship between stress response and pain, and pain itself is a stressor. When homeostasis is threatened or when the responses are restricted, and not able to adapt to the stressors, there is a state of distress in the body as functioning is challenged [36]. The physiological systems responding to stress exposure e.g. the hypothalamic-pituitary-adrenal axis and the sympatho-adreno-medullary (SAM) axis, are characterized by biologically normal fluctuations during a day, the circadian rhythm [50,51]. The ANS is a regulatory system responsible for adaptive regulations to stress in peripheral target organs, e.g. cardiovascular alterations [52]. Functionally, the ANS consists of two systems with reciprocal physiological effects: the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS). The relationship between SNS and PNS activity in the ANS, i.e. the sympathovagal balance, is essential for homeostasis [52,53]. Sympathetic activation in the ANS is crucial to prepare the body for physical and mental challenges. The peripheral expression of SNS to stress response is modulated via e.g. the SAM axis. Manifestations of SAM axis activation include e.g. increased heart rate, decreased heart rate variability (HRV), increased blood pressure, and increased plasma glucose. In addition to the regulatory effect on heart rate and the variability of heart rate, via the SAM axis, the ANS is also influenced by descending input from the limbic system and the cortex [53-55]. Therefore, changes in cardiac activity, i.e. heart rate and variability of heart rate, are influenced by emotional states [52,56-58]. Enhanced parasympathetic activity decreases heart rate and increases HRV [52]. In contrast to the SNS, the PNS dominates the ANS activity during rest and sleep, and promotes functional recovery and anabolic processes. Clinical biomarkers used to monitor interventions and to identify dogs at risk for developing chronic pain are scarce.

1.6 Cognitive and emotional responses to pain

To understand and explain the lack of correlation between radiographic findings in canine OA and pain-related behavior and disability, there is a need to integrate several components of pain into the clinical assessment. Pain perception is induced by a noxious stimulus and the stimulus draws the attention of the dog. Directing attention to the noxious stimulus is required for the dog to perceive the stimulus as painful. Once the dog with an intact nervous system attends the stimulus the dog will try to interpret the sensory experience, which

requires a cognitive-evaluative process. Pain perceived by the dog may cause negative emotions, e.g. fear and anxiety, which influence the cognitive interpretation of the stimulus [11]. Cognitive and evaluative processes are involved in the canine behavioral expression linked to OA pain, e.g. memory of earlier experiences [39]. Facilitation of emotional responses are expressed by e.g. sleeping disturbances, changes in general activity, changes in mood and impairments in social functioning [59-63]. There is no identified objective marker for pain responses related the emotional component of chronic pain in canine OA [61,64].

1.7 Pain-related overt canine behaviors

Canine behavior is defined as *"the internally coordinated response (action or inaction) of whole living organisms (individuals or groups) to internal and/or external stimuli"* [65]. This definition includes the ways dogs interact with other dogs, interaction with individuals from other species, and with the environment [66]. Some canine behaviors are innate, i.e. reflexes and fixed action patterns [67], whereas others are learned, i.e. developed through experience. Overt canine behaviors usually consist of intertwined innate and learned components [66,68]. Understanding the behavioral biology of a given species is helpful during pain assessment because pain may modify species-specific behavior [35]. The domestic dog is a social and territorial omnivore that occasionally exhibits predatory behavior [66,69-71]. Behaviors related to pain are nonspecific, i.e. there is no core sign sufficient to indicate pain and there is no specific behavioral sign that is necessary to indicate pain. Instead, there are several sufficient signs that, if present, may indicate that there is a pain condition [41]. There are motivational factors involved in the likelihood of the dog performing a particular behavior at a certain time [68,72]. For example, the withdrawal reflex is a highly predictable innate behavior induced by a sensory stimulus [68]. Subsequently, when a dog experiences pain induced e.g. when jumping into a car, the dog may learn to avoid pain by not jumping into the car, a behavioral change that can be explained by respondent and operant conditioning (associative learning) [68,73,74]. Some of the behavioral changes related to pain in canine OA are subtle and develop over time. Because pain is experienced subjectively and varies considerably among individuals, it has been suggested that behavioral pain assessment in companion dogs should include the owner [25,26]. To cover different aspects of a pain experience, behavioral changes occurring in dogs with OA should be assessed and evaluated in terms of diverse components, i.e. sensory, cognitive, emotional and behavioral [39,60-62,66,68].

1.8 Assessing pain in canine osteoarthritis

There are three major categories of rehabilitation measures: biophysiological, self-reporting and observational measures [75]. Mechanical or electrical devices used to obtain the measurements, i.e. goniometry and heart rate monitoring, are classified as biophysiological attributes. Self-reporting measures require that the participant being assessed describes the phenomenon measured, i.e. in a written survey or self-reporting items in an interview or in a pain scale. Observational measures involve a human instrument, i.e. the observer, as an examiner. The examiner observes overt behaviors in the participant, e.g. a dog, and sometimes actively allows the participant execute physical activities, as items in a structured test battery [12].

To cover the broad spectrum of pain perception and the health status of osteoarthritic dogs, several assessment measures should be implemented. Techniques for quantitative sensory testing have been used to assess neural changes in dogs with pain related to OA [21,76-78]. Assessment methods focusing on body structure and function, e.g. joint range of motion, should preferably be used together with valid measures of activity and participation, e.g. functional test batteries and health-related quality of life [79]. Pain is a subjective unpleasant sensory and emotional experience in dogs; and dogs' inability to communicate their experience in words makes it impossible to use self-reporting instruments to directly assess pain [26]. Instead, instruments designed for completion by a proxy, e.g. the dog owner, who knows the dog well are being used [66,80]. Owner-reported pain instruments are based on canine behavioral changes affected by pain and the ability of the naïve observers, i.e. the owners, to recognize the behavioral signs in their dogs [60,62,81-83] (Figure 1). Heart rate variability parameters have been used as biophysiological proxy variables of sympathovagal balance in chronic pain conditions in cows [84], humans [85,86], and in long-term stress in dogs [87]. Further, HRV analysis may be a potential assessment method of the emotional component in canine chronic pain conditions (Figure 1).

1.8.1 Heart rate variability analysis

Heart rate variability is defined as the variability of time intervals in consecutive heart beats [52]. The sinoatrial node generates an intrinsic heart rate of about 100 beats per minute in absence of neural influence [88]. Fluctuations between heart beats are caused by autonomic cardiac modulations, mainly via increased sympathetic or reduced vagal activity in efferent nerves, to the sinoatrial node of the heart. By analyzing fluctuations in series of interbeat intervals (IBI), various parameters indicate modulations and activity in the ANS [52,89]. Heart rate variability may be analyzed in statistical time-based parameters, i.e. variance, and in frequency-based parameters obtained from mathe-

matical algorithms in a power spectral density analysis [52]. The interplay between the SNS and the PNS is complex, and HRV analysis allows detailed information about modulations in the ANS [52]. The guidelines on HRV [89] specifically recommend the standard deviation of normal-to-normal IBIs (SDNN) and the square root of the mean squared differences of successive normal-to-normal IBIs (RMSSD) from the time-based parameters, and low frequency (LF) power, high frequency (HF) power, low frequency power in normalized units (LF n.u.), high frequency power in normalized units (HF n.u.), and the ratio of low frequency power/high frequency power (LF/HF) from the frequency-based parameters in a short-term, e.g. five minutes, HRV analysis. There are short-term HRV parameters specifically of interest for the evaluation of physiotherapeutic interventions targeting the PNS as some interventions may potentially reflect the activity in the ANS [84,90]. To provide information on the contribution of the neural control of heart rate, as in evaluating interventions targeting the PNS, the RMSSD, HF and HF n.u. are clinically relevant. The SDNN is an overall measure of HRV and the LF-to-HF ratio has been proposed to provide information on the sympathetic influences of the neural control of heart rate [91].

Heart rate variability analysis has been used as a quantitative marker of autonomic activity in clinical and experimental research in humans [85,86, 90,92-94] and different animal species [95-98]. As changes in cardiac activity are influenced by emotional states there are potential clinical applications for short-term HRV parameters as outcome measures for the relief of pain and/or stress in animals [56,58,99,100]. Within the field of canine behavioral science, a growing number of professionals and scientists include biophysiological assessment methods such as heart rate and HRV analysis to report autonomic responses [96,101-105]. The relationship between short-term HRV parameters and the level of stress [87], fear [106,107], anxiety [57], responses to human–dog contact [103,108,109] and physical as well as mental activities [105] have been studied in dogs of various breeds and of differing ages. In addition, HRV has been used as an outcome measure in various physical interventions and exercise regimens for the possible effect on the ANS system in humans [110-112] and in dogs [113]. Heart rate variability analysis may be a potential clinical assessment method in interventions addressing the ANS in dogs [114-117]. The cost and complexity of electrocardiogram (ECG) have made HRV analysis difficult outside laboratory environment. However, in the last two decades some studies have used different Polar heart rate monitors to record cardiac activity in several different species. Polar heart rate monitors have been tested for validities and reliabilities, against ECG, for recording short-term HRV data in humans [118-121], dogs [122-125] and horses [126,127]. Preferably only segments of IBIs completely free from error and/or nonsinus beats should be included in an HRV analysis. The time- and frequency-based parameters in HRV analysis may easily be biased by measurement errors in IBIs. It is recommended to assess the accuracy of IBI measurements with equipment designed to record IBI series by comparing to a gold

standard method, i.e. ECG [89]. Results are conflicting and researchers have raised concerns about whether Polar heart rate monitors should be used interchangeably with ECG [126,128,129].

1.8.2 Owner-reported pain and disability questionnaires

The ability of dog owners to report the level of pain severity on a visual analog scale is limited [130]. This may be because they do not recognize subtle signs derived from the emotional and behavioral factors as sufficient signs of pain. Pain related with OA may be manifested as changes in movement behavior in the dog, and gait evaluation during pain management is widely used in clinical settings. However, visual movement assessment and assigning levels and grades of lameness have shown poor intra- and interrater reliability among owners [131] and veterinarians [131,132]. Hence, there is a challenge in constructing owner-reported instruments that prove adequate measurement properties. Several owner-reported instruments intended to capture diverse dimensions of dog owners' perceptions of canine osteoarthritic pain have been developed [62,81,133]. Items targeting the dogs' general activity, enjoyment of life, mood and playfulness have been included in the questionnaires together with items covering movement behavior [62,81]. To assess chronic pain, the answers of the items in questionnaires are given by a person living in the same household as the dog of interest, i.e. the owner of the dog [20,26,134]. Despite the challenges to owners to estimate pain experienced by their dogs, using visual analog scale, psychometric testing of the Canine Brief Pain Inventory (CBPI) [62,135] and the Helsinki Chronic Pain Index (HCPI) [81], have shown adequate construct and criterion validity to assess owner-perceived pain-related behaviors in untreated dogs with OA pain. The CBPI has not been psychometrically tested for construct validity in a more diverse group of dogs with OA pain, e.g. dogs presented for animal physiotherapy.

In this thesis, a multidimensional approach of chronic pain [9] (Figure 1) is applied in the evaluation of psychometric properties in clinically applicable assessment methods related to diverse components of the pain construct in canine OA, i.e. the CBPI (*Study I*) and HRV analysis measured by Polar heart rate monitor RS800CX (*Study III* and *IV*), and to describe pain-related overt behaviors and disability in dogs with OA (*Study II*).

1.9 Psychometric properties of assessment methods

For clinical practice and research, the selection of assessment method needs to be based on a clearly defined variable. That is, first one needs to know what to measure. Further, an assessment method refers to how the variable is measured. Psychometric testing involves evaluating the measurement properties, i.e. validity, reliability and responsiveness of an assessment method (Figure

2) [136]. Sometimes the variable measured is a phenomenon that cannot be observed directly, for example health-related quality of life [60,137] or chronic pain [62,81], and it should be clarified which subdomains are relevant for the target population in the specific context of interest [138]. Psychometric properties can be evaluated in various ways. In this thesis, classical test theory is applied [139].

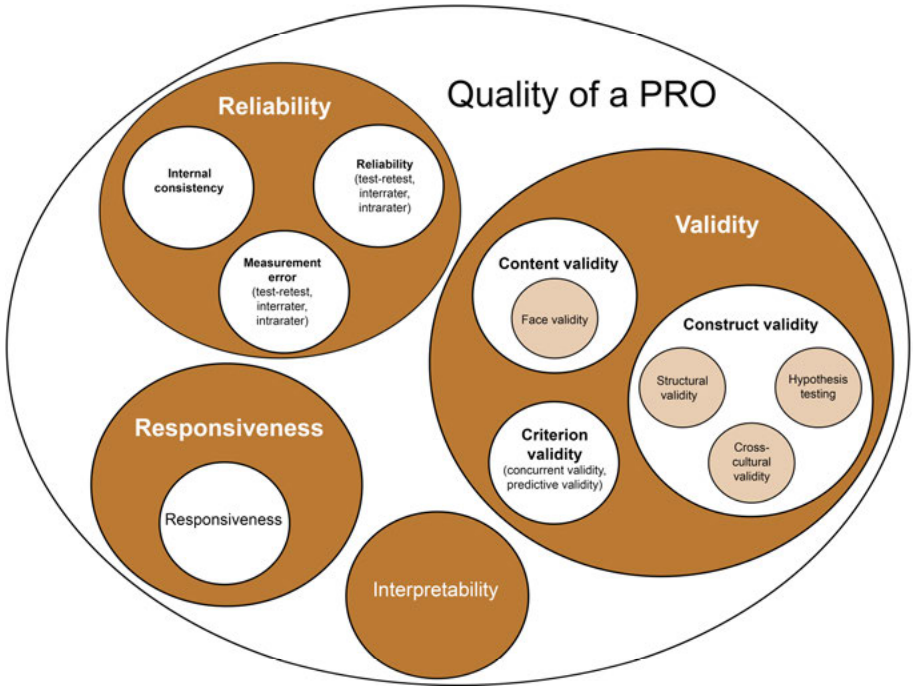

Figure 2. *Relationships of measurement properties patient-reported outcome (PRO) in the COSMIN taxonomy. Mokkink et al. J. Clin. Epidemiol. 63, 737–745 (2010)* [136]*, with permission from Elsevier.*

1.9.1 Validity

Construct, content and criterion validity of assessment methods are fundamental properties because evidence about the extent to which an assessment method measures what it is intended to measure is provided [75,79]. Construct validity of an owner-reported questionnaire refers to the extent to which the scores of the instrument are consistent with hypotheses based on the assumption that the instrument validly measures the construct to be measured, i.e. with regard to internal relationships, relationship to other instruments and differences between groups (Figure 2) [136]. The construct validity of owner-reported questionnaires, measuring pain [62,81] and health-related quality of life [60,137] in dogs, is under investigation during the development process. Psychometric testing concerns the construction and internal relationships, i.e. structural validity, and relationships to scores of other instruments or differences between known groups, i.e. hypothesis testing and cross-cultural validity. Content validity of an owner-reported instrument focuses on items in a questionnaire and their relevance to the tested attribute. Criterion validity of an instrument refers to the relationship between one assessment method against another, which intends to assess the same variable. To determine criterion validity of a new measurement method, correlational coefficients are used for comparison to the gold standard method [75,140].

1.9.2 Reliability

Methodological studies should provide information about whether an instrument can measure accurately and repeatedly, including estimates on the level of agreement and the amount of systematic and random errors in a score or measurement in a sample [141]. All measurements consist of several sources of variability within the observed score. Specifically, the observed score contains a true component and an error component [75]. In addition, there is also a source of variability, usually biological, within each subject being measured [142,143]. Reliability testing addresses the extent to which scores for subjects who have not changed are the same for repeated measurements and various contexts (Figure 2) [136]. Defined statistical methods are to be used to assess the different components of reliability: for example, using different sets of items from the same owner-reported outcome measure i.e. internal consistency, over time, i.e. test-retest, by different persons on the same occasion i.e. interrater, or by the same persons on different occasions i.e. intrarater [136]. For owner-reported questionnaires, the internal consistency can be estimated to examine the extent to which items in the questionnaire are correlated and measure the same concept [140,144]. The relative reliability is the estimate of the degree of association between repeated measurements or concurrent measures. Two or more measurements are to be examined on the relationship, by correlational estimates [75,79]. Important additional information on measurement variability is indicated

by the standard error of measurement (SEM), which indicates the absolute reliability of the measurement. The values of SEM indicate to which extent an assessment method varies on repeated measurement and provide meaningful clinical information about possible true changes in the variable of interest [145,146].

1.9.3 Responsiveness

Responsiveness is defined as the ability of an assessment method to detect change over time in the construct to be measured [136]. Knowing the amount of change needed in a measured score is essential to be able to interpret whether differences overcome measurement errors and reflect true changes. Responsiveness of an assessment method is evaluated with several different statistical methods. Some parameters proposed in the literature to assess responsiveness are considered inappropriate. Several measures of responsiveness are considered measures of the magnitude of change due to an intervention or other event, rather than measures of the quality of the assessment method [147,148]. Responsiveness is related to construct and criterion validity, in such a way that construct and criterion validity refers to the validity of a cross-sectional single score, and responsiveness refers to the validity of a changed score. Appropriate measures used to evaluate responsiveness in an assessment method are the same as for hypothesis testing and criterion validity [136]. In repeated measurements the test-retest reliability also needs be considered prior to any conclusions being drawn about changes in a measured score [75,79].

1.9.4 Interpretability

Interpretability is considered an important characteristic to an instrument, referring to the degree to which one can assign clinical meaning to the quantitative scores or change in scores. Hence, interpretability is not referred to as a psychometric property. However, the interpretation of results in a study may be inadequate, e.g. if there are marked floor or ceiling effects in a sample. Floor and ceiling effects indicate that there may be more variance in the concept being measured by the assessment method [139,140].

In this thesis, the construct validity, the internal consistency and the interpretability of the CBPI in a new target group was addressed in *Study I* and the interpretability of the HCPI was explored in *Study II*. In *Study III* and *IV*, the criterion validity, level of agreement and the relative and absolute reliabilities of Polar RS800CX measuring IBI and HRV parameters were assessed.

1.10 Rationale for this thesis

Sharing life with dogs is associated with positive human health benefits. Higher levels of physical activity, lower blood pressure, diminished responses to stress, improved lipoproteins and a reduced incidence or severity of depression are confirmed biological, psychological and social benefits [149-151]. An important aspect of the human-dog cohabitation is the human responsibility for the health and welfare of the dog [33]. Quality of life of the dog, and the owner, are threatened when major consequences of OA, i.e. pain and disability, are present. To provide evidence-based canine physical rehabilitation and tailored pain management, aimed to alleviate pain and disability in canine OA, there is a need for valid and reliable assessment methods. Increased knowledge about the psychometric properties of clinically applicable assessment methods is essential to ensure the quality of measurements and animal health care. Companion dogs live their life with humans and more knowledge about the attributes of canine OA may contribute to better understanding of pain and disability related to human OA [152].

Prior to implementing assessment methods for pain related to canine OA, animal health care professionals and researchers should consider the context in which the measure is used [138]. The measurement properties, i.e. validity, reliability and responsiveness, should be established in the population of interest and an owner-reported instrument should be properly translated to the target language [79,139,153].

An observational assessment method of owner-perceived pain severity and the interference of pain with function, i.e. the CBPI, has been developed and tested for psychometric properties in the original language and in a homogenous group of OA dogs. To use the CBPI in a more diverse group of dogs with OA pain, e.g. dogs presented for animal physiotherapy, the instrument should to be psychometrically tested for its construct validity to determine whether it is adequate. A biophysiological assessment method linked to the emotional state in chronic pain, i.e. HRV analysis has been studied previously in dogs. However, there are concerns whether a more clinically applicable instrument, i.e. the Polar heart rate monitor, can be used interchangeably with ECG to measure time- and frequency-based HRV parameters.

2 Aims

The general aim of this thesis was to psychometrically evaluate measurement properties in clinically applicable assessment methods - owner-reported pain severity and pain interference and heart rate variability analysis - related to pain in naturally occurring canine OA.

2.1 Specific aims

I To translate the original CBPI and evaluate psychometric properties, in terms of internal consistency and construct validity, of the CBPI in a clinical sample of OA dogs referred for physiotherapy.

II To assess owner-perceived chronic pain behavior; and to investigate differences between dogs with and without owner-perceived chronic pain behavior; and to assess associations between sex, body condition, use of antiinflammatory medication and owner-perceived pain interference with function score, and owner-perceived chronic pain behaviors in a group of dogs with naturally occurring OA referred for animal physiotherapy.

III To assess the criterion validity, relative reliability and level of agreement of Polar RS800CX heart rate monitor measuring IBIs, compared to simultaneously registered ECG, in dogs during stationary standing position.

IV To compare validity and reliability properties of Polar RS800CX against simultaneously recorded ECG measuring time- and frequency-based short-term HRV parameters, in dogs during stationary standing position.

3 Methods

3.1 Design

This thesis includes four observational studies. *Study I* was a cross-sectional study on owner-perceived pain severity and interference of pain on function in dogs. In *Study II*, owner-perceived chronic pain behaviors in dogs from a cross-sectional sample were described and variables explaining the outcome i.e. chronic pain were analyzed. *Study III* was a correlative and descriptive study of IBIs from a Polar heart rate monitor analyzed against recordings from ECG. In *Study IV*, a subsample of dogs who participated in *Study III* were analyzed to compare HRV parameters using two separate technical devices. An overview of the study designs, subjects, study variables and data collection are presented in Table 1.

3.2 Ethical considerations

The study protocols were approved by the Local Ethical Committee in Uppsala, Sweden (Dnr C81/12, C111/12, C17/2016). Dog owners were given written and oral information about the studies and informed owner consent was obtained. None of the dogs reacted with aggression or fear during the studies.

3.3 Subjects and procedures

3.3.1 Study I and II

To determine the size of the sample in *Study I*, a subject-to-item of ratio 5:1 was used [154,155]. The subject-to-item ratio was determined by the number of CBPI items rated by the owners; hence 10 items generated a sample size of 50 dogs. The sample size was overestimated by 10% to cover possible losses. The inclusion criteria were as follows: dog >1 year of age, dog >9 kg body weight, clinical evidence of OA of at least one synovial joint, radiographic evidence of OA of at least one synovial joint. The following were exclusion criteria: the owner completing the questionnaire lacked an understanding of written Swedish, other concurrent disease interfering with the dogs' mobility, activity or health-related quality of life. The dogs and the owners in the control group

fulfilled the same inclusion and exclusion criteria as the OA group, except the clinical and radiographic evidence of OA. When about 20 dogs were enrolled to the control group, it became clear that the respondents in the control group scored mainly zero in pain severity and pain interference with function items, yielding no more information from the owner-perceived answers. Another ten OA dogs were included in *Study II* according to a sample size recommendation of 10:1 per explanatory variable and case. In *Study II*, we aimed for 40 dogs with owner-perceived chronic pain behavior, i.e. total HCPI≥12. In total 71 dogs referred from veterinarians for physical rehabilitation interventions due to naturally occurring OA were included in the OA group in *Study I* and *II*. *Study I* and *II* are based on the same sample, and *Study II* was expanded by 10 OA dogs. A group of 21 clinically sound dogs participated as controls in *Study I*. Before the studies, high-quality translations of the CBPI to *Study I*, and HCPI to *Study II* questionnaires was done according to the standard procedure for translation and back-translation of instruments designed for self-reported outcome [139]. The owner-reported questionnaires were translated into Swedish with a forward and backward procedure as follows: translation from English and Finnish to Swedish was done from the original languages, i.e. English or Finnish, by two independent native Swedish persons who were fluent in the target language and who had good understanding of the original language. Further, the Swedish version of questionnaires were back translated into the original language by two independent native English or Finnish persons who were fluent in the original language and had good understanding of the target language, i.e. Swedish. Permissions to translate the CBPI and the HCPI were obtained in a written consent from the copyright holders Dr. Dorothy Cimino Brown and Dr. Anna Hielm Björkman. The translated questionnaires were pretested in a pilot study. The conceptual meaning in the translated versions of the questionnaires was kept because semantic equivalents were found in Swedish.

All OA dogs and control dogs were clinically examined by a veterinarian prior to enrolment in the studies. The OA dogs were diagnosed before they were recruited to the study. None of the control dogs had a history or current clinical evidence of OA. At a visit to a registered animal physiotherapist, the clinical history was collected and the owners, whose dogs fulfilled the inclusion criteria, answered the Swedish version of the CBPI and the HCPI questionnaires. The questionnaires were administered to the owners at the veterinary clinic and the owners were instructed according to the user guide available for the CBPI and the HCPI. Collection of the questionnaires was performed on the same occasion. Nineteen of the owners of the control dogs in *Study I* received the questionnaire from a veterinarian.

3.3.2 Study III and IV

Eleven clinically sound dogs were recruited on a consecutive sample to *Study III* and *IV*. The studies were based on the same sample as a previously published study by the author of this thesis [123]. Data from 11 (6 female and 5 male) dogs from various breeds, with mean ± standard deviation (SD) age of 3.8 ± 1.3 years and mean ± SD weight of 29.9 ± 7.2 kg were included in the *Study III*, and data from subgroup (3 female and 5 male), with a mean ± SD, age of 3.5 ± 1.3 years, mean ± SD weight of 32.6 ± 6.0 kg, and normal body condition were included in *Study IV*. None of the dogs had a history or current evidence of cardiovascular or systemic diseases, as assessed by a veterinarian. Two heart beat recording devices were simultaneously applied to the dogs. Polar heart rate monitor (Polar Electro Oy) consisted of electrode belt and transmitter W.I.N.D. and heart rate monitor RS800CX. The electrode belt and transmitter supported recording and processing of IBI at a frequency of 1000 Hz and 2.4 GHz transfer between the belt and heart rate monitor. The coat was clipped at all electrode sites and the skin was cleaned with alcohol and air dried. Cefar electrode transmission gel (Cefar-Compex Scandinavia AB) was applied liberally to promote conductivity. The electrode belt was strapped around the chest of the dogs with the transmitter placed ventrally and the electrodes on each side of the sternum. Cardiostore digital ECG (Vetronic Services Ltd) was attached by three adhesive ECG electrodes (Kruuse Svenska AB).

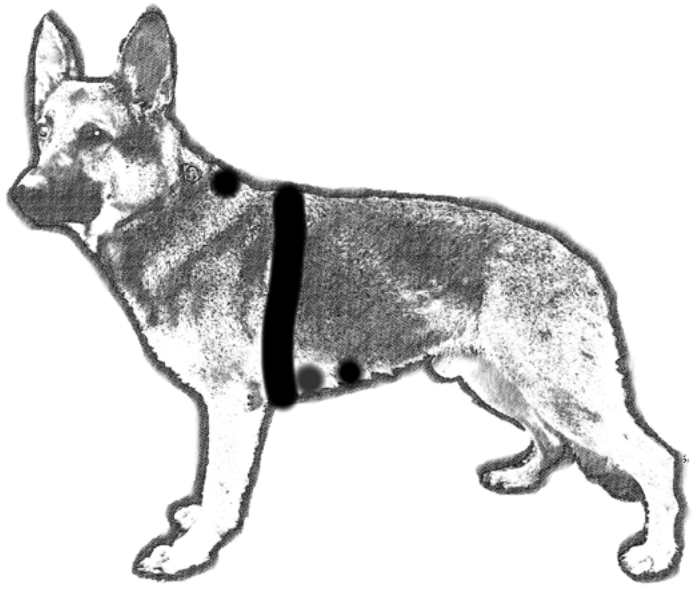

Figure 3. Picture of one of the dogs in Study III and IV, showing ECG and Polar electrode placement in the subjects.

Electrodes were placed: 1) on the right side of the dog, slightly caudal and dorsal to the point of the elbow and caudal to Polar electrode belt, 2) on the left side of the dog in level with the xiphoid process of sternum and at the lowest point on the side of the dog without being ventral and, 3) at the scruff of the neck [156] (Figure 3). The ECG recorded cardiac activity at a frequency of 600 Hz. The dogs came from their routine activities and were fed not less than two hours before the test. The experiment was conducted in calm examination room at a veterinary clinic at a room temperature of 18–22 °C.
After the Polar and ECG electrodes were placed to the skin, the dogs rested for five minutes. Recording was manually started when IBI and cardiac activity could be visually inspected in the display of each device. Each dog fully completed the recordings for seven minutes in standing on an examination table. One person was responsible for all measurements. Polar data were transmitted at the end of each recording to a laptop computer via a bidirectional infrared interface using the Polar software, Polar Protrainer 5. Computer software Cardiostore 1.33 was used to visually inspect raw ECG data and to calculate IBIs. The first 5-minute subsequent recordings from both devices were extracted and visually inspected by a veterinarian to identify technical and physiological artifacts [89]. No nonsinus beats were present in the ECG recordings. Polar and Cardiostore software were each respectively used to export IBIs as text files to Microsoft Excel and further to the Windows based software Kubios HRV for analysis of HRV time- and frequency-based parameters, *Study IV* [157,158].

3.4 Data collection

3.4.1 Pain severity and pain interference with function (Study I and II)

The *Canine Brief Pain Inventory* [15,62,133,135,159] is a 10-item questionnaire assessing pain severity and pain interference with function. The first four items consist of eleven-point (0–10) rating scales asking the owners to rate the pain intensity in their dogs during the last seven days, addressing pain "at its worst", "at its least", "on average" and "right now". Zero indicates "no pain" and 10 represents "extreme pain". The remaining six items cover the degree to which the owners rate the pain interference with function for their dog. In the interference items, 0 indicates "does not interfere" and 10 indicates "interferes completely". CBPI scores are aggregated in two dimensions: (1) *pain severity*, using the four items (item 1–4) on pain intensity, and (2) *pain interference,* using the six items (item 5–10) on pain interference with function. The minimum sum of CBPI is 0 and the maximum sum in the pain severity items is 40 and in the pain interference 60. The sums of the two dimensions

may be averaged to deliver a pain severity score and a pain interference score. In *Study II*, the pain interference score was used.

Table 1. *An overview of designs, subjects, variables and data collection in the four studies.*

	Study I	**Study II**	**Study III**	**Study IV**
Designs	Cross-sectional, case-control	Cross-sectional, correlative, descriptive	Cross-sectional, correlative, descriptive	Cross-sectional, correlative, descriptive, explorative
Subjects	OA dogs (n=61) and clinically sound dogs (n=21)	OA dogs (n=71)	Clinically sound dogs, 20-40 kg (n=11)	Clinically sound dogs, 20-40 kg, from a subgroup (n=8)
Variables	Pain severity and pain interference with function	Chronic pain behavior, pain interference with function, body condition score, sex, use of antiinflammatory medication	Pair-wise interbeat intervals (*n*= 4851) from two technical devises	Time and frequency based heart rate variability parameters
Data collection	Owner-perceived questionnaire	Owner-perceived questionnaires, body condition score	R-to-R intervals recordings	R-to-R intervals recordings and heart rate variability analysis

OA, osteoarthritis

3.4.2 Chronic pain behavior (Study II)

The presence of owner-perceived chronic pain behaviors during the last week was assessed with the *Helsinki Chronic Pain Index* [61,81,133,160,161], which is an owner-reported questionnaire consisting of 11 questions on the dog's mood and willingness to perform daily activities e.g. walking, playing and jumping. Owners were asked to describe their dogs on a 5-point descriptive scale and their answers were tied to a value of 0 to 4 and then summed. The total HCPI score ranges from 0 to 44. In each HCPI item a score of 0 and 1 is assumed to indicate normal canine behavior, whereas 2, 3 and 4 indicate increasingly severe pain-related behavior [61,81,160].

3.4.3 Body condition score (Study I and II)

Body condition score was assessed by palpation and visual inspection by two of investigators. A nine-point scale, reaching from one (severely underweight) to nine (obese) was used to assign the dogs to a body condition score [162].

3.4.4 Interbeat intervals (Study III)

Cardiac *interbeat intervals,* i.e. the time (milliseconds) between continuous R-to-R peaks in ECG, were measured with Polar heart rate monitor (Polar Electro Oy), consisting of electrode belt and transmitter W.I.N.D. and heart rate monitor RS800CX, and Cardiostore digital ECG (Vetronic Services Ltd).

3.4.5 Heart rate variability parameters (Study IV)

Interbeat interval data for analysis were derived from the first 300-second IBI segment in the ECG and the Polar IBI series respectively from each subject. The software Kubios HRV 2.0 (Department of Physics, University of Kuopio, Kuopio, Finland) generated a power spectral density analysis using fast Fourier transform [157,158], a Welsh periodogram with 256-second window and 50% overlap. Frequency-based parameters selected were LF, 0.04-0.15 cycles/beat, HF, 0.15-0.60 cycles/beat, LF n.u., HF n.u., and LF/HF. Selected time-based parameters were SDNN and RMSSD.

3.4.6 Anthropometric measure (Study I-IV)

In *Study I-IV, body weight* (kg) of the dogs were documented using a digital scale. Data on treatment with antiinflammatory medication was documented in *Study I and II*.

3.5 Data management and analysis

Statistical analyses were performed using SPSS (Version 20, IBM Statistical Package for Social Science Statistics for Windows, Armonk, NY: IBM Corp) in *Study I, II* and *IV*, AMOS (IBM, SPSS, AMOS 22.0., AMOS Development Corporation, Spring House, PA) was used in *Study I*, and Stata Statistical Software (Release 13, College Station, TX; StataCorp LP) was used in *Study III.* The statistical methods used in *Study I-IV* are presented in Table 2. Statistical significance was declared at $p<0.05$, and two-tailed assessments were used, in *Study I-IV*.

3.5.1 Study I

The internal consistency of the questionnaire was estimated to examine the extent to which items in the questionnaire correlated and measured the same concept. Cronbach's $\alpha > 0.70$ was considered acceptable [144]. Construct validity (structural validity) was assessed by confirmatory factor analysis (CFA) and exploratory factor analysis (EFA) [139,163]. A CFA by maximum likelihood method was conducted to test the hypothesis that a two-factor representation or a one-factor model in the CBPI would be confirmed [62,133]. The following goodness-of-fit indices were assessed: model-Chi^2, degrees of freedom and Chi^2/df, comparative fit index, root mean square error of approximation with 90% confidence intervals, normed fit index, and parsimony adjusted normed fit index. Because the ordered data from CBPI items are not normally distributed and not on a quantitative measurement scale, the models were also estimated by bootstrapping and by Bayesian methods [164]. An EFA by principal component model with subsequent varimax rotation was repeated to study the interitem relationship and to explore the factor structure. Factors were extracted according to Kaiser's rule; eigenvalues >1. By assessing for differences between sound dogs and dogs diagnosed with OA, using Mann-Whitney U test, the construct validity (hypothesis testing) and the ability of the CBPI to discriminate dogs with OA was tested [139,163].

3.5.2 Study II

A dichotomous variable from the HCPI score was created. Total HCPI scores 0-11 indicated no or few chronic pain behaviors, i.e. no chronic pain, and HCPI score ≥12 indicated several or many chronic pain behaviors, i.e. chronic pain [61,160]. Differences between dogs with chronic pain behavior, i.e. HCPI≥12, and dogs without chronic pain behavior, i.e. HCPI≤11, were assessed. The Pearson's Chi squared test was used to compare proportions of chronic pain behavior in categorical variables, i.e. sex, body condition and use of antiinflammatory medication. The Mann-Whitney U test was used to assess how CBPI pain interference with function scores differed. Scores of the pain interference domain in the CBPI were averaged and used in a logistic regression model [165]. Univariate and multivariate logistic regression models were used to model each explanatory variable and to assess the association between chronic pain behavior measured with HCPI and explanatory variables. Relationships among owner-perceived chronic pain behavior measured by HCPI, and the following explanatory variables were assessed: sex, body condition score, use of antiinflammatory medication and owner-reported pain interference with function score. Results from the univariate logistic regression model were reported as unadjusted odds ratios (OR) with 95% confidence intervals (CI), and from the multivariate logistic regression analysis the adjusted OR with 95% CI were reported.

3.5.3 Study III

Corresponding ECG and Polar IBIs from each subject were aligned to enable pairwise comparisons and the difference between each ECG IBI and corresponding Polar IBI was calculated. A measurement error was considered when the difference between ECG and corresponding Polar IBI was more than 50 ms. When the difference was more than 50 ms, the IBIs were checked against the ECG tracings [122,128]. Interbeat intervals from ECG were slightly positively skewed in the group of 11 dogs. During the synchronization procedure, extra or missing IBIs from Polar resulted in empty cells in either ECG or Polar IBI series. Incomplete pairwise data, produced by Polar, was assumed to be missing at random. Three different methods for handling missing IBI data were used. Empty cells were kept blank, i.e. pairwise deletion, zero-values were added in the empty cells, i.e. worst-case analysis, and mean imputations. Mean imputation was defined as the mean of the two immediately preceding IBIs differences. The missing value was replaced by the sum of the estimated IBI difference and the observed ECG or Polar IBI [122]. The level of agreement between ECG and Polar data was assessed in Bland and Altman plots with 95% limits of agreement (LoA) [166] using the Bland–Altman method accounting for repeated measurements per subject. A multilevel model was fitted for the IBI measurements to obtain estimates of within-subject and between-rater variances [167,168]. Interbeat intervals by ECG and Polar (level 1) were nested within measurement methods, i.e. Polar or ECG, (level 2) and within the same dog (level 3). A three-level nested model with random intercepts was used. Intraclass correlation coefficients (ICC) were also calculated separately for each dog. Correlation between ECG and Polar within the group of dogs studied was calculated using ICC coefficients of a single measure and in absolute agreement with two-way random effects ($ICC_{2.1}$). Intraclass correlation coefficient >0.75 was classified as excellent [169].

3.5.4 Study IV

Paired t-test was used in all HRV parameters to determine the statistical significance of differences between the measurement methods. No corrections for multiple tests were performed. The correlations between the measurement methods and the relative reliabilities of Polar RS800CX were estimated by using Spearman rank correlation coefficient, and $ICC_{2.1}$ [169], with a 95% CI. Intraclass correlation coefficient >0.75 was classified as excellent [169]. Absolute reliabilities were investigated by calculating the SEM and SEM% [142,145] in Polar and ECG measurements, respectively. Estimates of SEM were represented in the same unit as the original measurement for each HRV parameter selected and were calculated according to [145]:

$$SEM = SD\sqrt{1 - ICC_{2.1}}$$

SEM% was defined as SEM% = SEM/mean x 100, whereby mean was the average of measures from Polar and ECG, respectively. Bland and Altman plots with 95% LoA and 95% CI of mean differences were constructed to examine the level of agreement between ECG and Polar HRV parameters. The presence of any systematic overestimation and underestimation of time- and frequency-based parameters was assessed, and the upper and lower LoA were calculated by the SD ± 1.96 of the mean difference between methods [170].

Table 2. *A summary of the statistical methods used in this thesis.*

Methods	**Study I**	**Study II**	**Study III**	**Study IV**
Descriptive analyses				
- Mean and standard deviation		X	X	X
- Interquartile range	X	X		
- Median	X	X		
- Frequencies and proportion	X	X	X	X
Interferential analyses				
- Spearman's rank correlation coefficient				X
- Paired t-test				X
- Mann-Whitney U test	X	X		
- Univariate logistic regression		X		
- Multivariate logistic regression		X		
- Pearson's Chi square test		X		
Psychometric analyses				
- Standard error of measurements				X
- Standard error of measurements (%)				X
- Bland-Altman analysis				X
- Intraclass correlation coefficient				X
- Multilevel model analysis			X	
- Exploratory factor analysis	X		X	
- Confirmatory factor analysis	X		X	
- Cronbach's α	X			

4 Results

4.1 Psychometric properties of the CBPI

Data from 82 adult dogs out of various breeds were included in *Study I*. Inadequate completion of the CBPI questionnaire was present in three OA cases. Those were handled as internal missing values and the total completion rate was 97.5%. There were significant differences between sound control dogs (n=21) and OA dogs (n=58) in terms of age and body condition score. OA dogs were older and had higher body conditions scores. Most OA dogs (79%) had ongoing antiinflammatory medication. Descriptive information about the breeds included in the cohort can be found in additional file 1 in *Study I* [15].

4.1.1 Construct validity; structural validity

In the CFA, both one- and two-factor models had similar goodness-of-fit values. The comparative fit index and normed fit index values were too low, the ratios Chi^2/df were small and the root mean square error of approximation were too high to be acceptable in all models. Altogether, this indicates that the proposed models could not be confirmed based on our data and we thereby do not show any estimated factor loadings and covariances. Analysis by bootstrap modeling and by Bayesian estimations differed somewhat from the maximum likelihood estimates. These results are shown in additional file 2 in *Study I* [15]. Exploratory factor analysis by principal component analysis showed a one-component structure with an eigenvalue of 6.7, in the total OA group (n=58). One component showed an eigenvalue of 0.99 and was extracted together with the first component. Those two components accounted for 76.8% of the total variance (66.9 and 9.9% respectively), suggesting an acceptable fit of a two-component structure. In the group of OA dogs with CBPI total sum ≥1 (n=49) two components with eigenvalues >1 were extracted. These components accounted for 60.9 and 11.9% of the total variance of the CBPI respectively. Together the components accounted for 72.8% of the total variance (Table 3).

4.1.2 Construct validity; hypothesis testing

Clinically sound dogs differed from OA dogs by showing significantly lower CBPI total sum, and significantly lower pain severity and pain interference with function sums.

4.1.3 Internal consistency and interpretability

Cronbach's α was 0.94 in the total CBPI, and 0.91 in the pain severity domain and 0.91 in the pain interference with function domain (n=58). There was a marked floor effect present in the pain severity sum and the pain interference with function sum (Table 4). In the group of owners rating presence of pain related to OA in their dogs (n=49), the pain severity average (min–max) was 2.5 (0.0–5.8) and the pain interference with function average (min–max) was 2.3 (0.3–7.7). Including the owners that did not rate presence of pain related to OA (n=58), the pain severity average (min–max) was 1.9 (0.0–5.8) and the pain interference with function average (min–max) was 2.1 (0.0–7.7).

Table 3. *Factor loadings in the exploratory factor analysis by principal component analysis with subsequent varimax rotation for the CBPI (two factors extracted) in all dogs with OA (n=58), and dogs with OA and CBPI ≥1 (n=49).*

	All OA dogs (n=58)		**OA dogs with CBPI ≥1 (n=49)**	
CBPI item	**Factor 1**	**Factor 2**	**Factor 1**	**Factor 2**
Pain at its worst	0.83	0.27	0.85	0.14
Pain at its least	0.87	0.22	0.85	0.21
Pain on average	0.90	0.38	0.91	0.34
Pain right now	0.82	0.38	0.80	0.37
General activity	0.66	0.58	0.66	0.53
Enjoyment of life	0.48	0.58	0.45	0.57
Ability to rise	0.59	0.57	0.57	0.52
Ability to walk	0.14	0.91	0.04	0.91
Ability to run	0.37	0.82	0.33	0.82
Ability to climb	0.45	0.69	0.42	0.66

CBPI, Canine Brief Pain Inventory; OA, osteoarthritis.

4.2 Owner-perceived chronic pain behavior in canine osteoarthritis

Data from 71 OA dogs out of various breeds were included in *Study II*. Inadequate completion of the CBPI questionnaire was present in two cases and of the HCPI questionnaire in two cases. Those were handled as internal missing values and were excluded from estimations based on pain interference score respectively total HCPI score.

4.2.1 Presence of owner-perceived chronic pain behavior

Total HCPI scores were higher in dogs with HCPI≥12 in all items except item three, i.e. vocalization (Figure 4A and 4B). Median values were the same in mood, vocalization, galloping and jumping items, whereas ranges were overall larger in dogs with owner-perceived chronic pain behaviors. Owners reported higher levels and proportions of chronic pain behavior particularly in items targeting physical activities, e.g. getting up, moving after rest and moving after major exercise (Figure 4A and 4B).

4.2.2 Differences between dogs with and without chronic pain

Dogs with and without owner-perceived chronic pain behavior did not differ in age, body weight, body condition score, sex or use of antiinflammatory medication. The CBPI pain interference with function score was significantly higher in dogs with HCPI≥12, compared to dogs with HCPI≤11 ($p=0.001$). The median pain interference scores were 2.50 and 0.67 respectively.

4.2.3 Association between chronic pain and explanatory variables

The value of the adjusted OR indicates that each unit increase in the CBPI score, is associated with 1.74 (95% CI 1.23-2.47) times higher odds for owners to report chronic pain behavior in their dogs. Table 5 gives crude and adjusted OR for the explanatory variables for chronic pain behavior in canine OA. Sex, body condition and use of antiinflammatory medication were not significantly associated with owner-perceived chronic pain behavior.

Table 4. *Pain severity (items 1-4) and pain interference with function (items 5-10) scores, percentage of minimum scoring and communalities for the CBPI items, pain severity sum, pain interference with function sum and the CBPI items totaled.*

	All OA dogs (n=58)			OA dogs with CBPI ≥1 (n=49)			Control dogs (n=21)		
CBPI item	**Median (min-max)**	**%Floor[3]**	**Communalities**	**Median (min-max)**	**%Floor[3]**	**Communalities**	**Median (min-max)**	**%Floor[3]**	**Communalities**
Pain at its worst[1]	3(0-8)	24.6	0.76	4(0-8)	12.2	0.74	0(0-1)	95.2	NA
Pain at its least[1]	0(0-5)	54.1	0.80	1(0-5)	46.9	0.76	0(0-0)	100	NA
Pain on average[1]	2(0-6)	31.7	0.95	2(0-6)	20.4	0.94	0(0-0)	100	NA
Pain right now[1]	1(0-6)	42.6	0.82	2(0-6)	32.7	0.78	0(0-0)	100	NA
General activity[2]	2(0-10)	34.4	0.77	3(0-10)	22.4	0.72	0(0-0)	100	NA
Enjoyment of life[2]	1(0-9)	44.1	0.57	1(0-9)	34.7	0.53	0(0-0)	100	NA
Ability to rise[2]	2(0-9)	28.3	0.68	3(0-9)	16.3	0.60	0(0-0)	100	NA
Ability to walk[2]	1.5(0-8)	31.7	0.84	2(0-8)	20.4	0.82	0(0-0)	100	NA
Ability to run[2]	2(0-10)	31.7	0.81	2(0-10)	20.4	0.78	0(0-0)	100	NA
Ability to climb[2]	1(0-10)	43.3	0.67	2(0-10)	32.7	0.62	0(0-0)	100	NA
PS sum	7.5(0-23)	25.9	NA	10(0-23)	12.2	NA	0(0-1)	95.2	NA
PI sum	12.5(0-46)	15.5	NA	14(2-46)	0	NA	0(0-0)	100	NA
CBPI total sum	19.5(0-66)	15.5	NA	24(2-66)	0	NA	0(0-1)	95.2	NA

Values are presented as median (minimum-maximum). Proportion (%) of scoring minimum value (floor) value. Communalities showing proportions of variance for each item that can be explained by the two components, pain severity and pain interference with function, in the exploratory factor analysis by principal component analysis.
[1] Range 0-10 (no pain, extreme pain), [2] Range 0-10 (does not interfere, interferes completely), [3] % scoring minimum value; CBPI, Canine Brief Pain Inventory; OA, osteoarthritis; PS, pain severity (item 1-4); PI, pain interference with function (item 5-10); NA, not applicable.

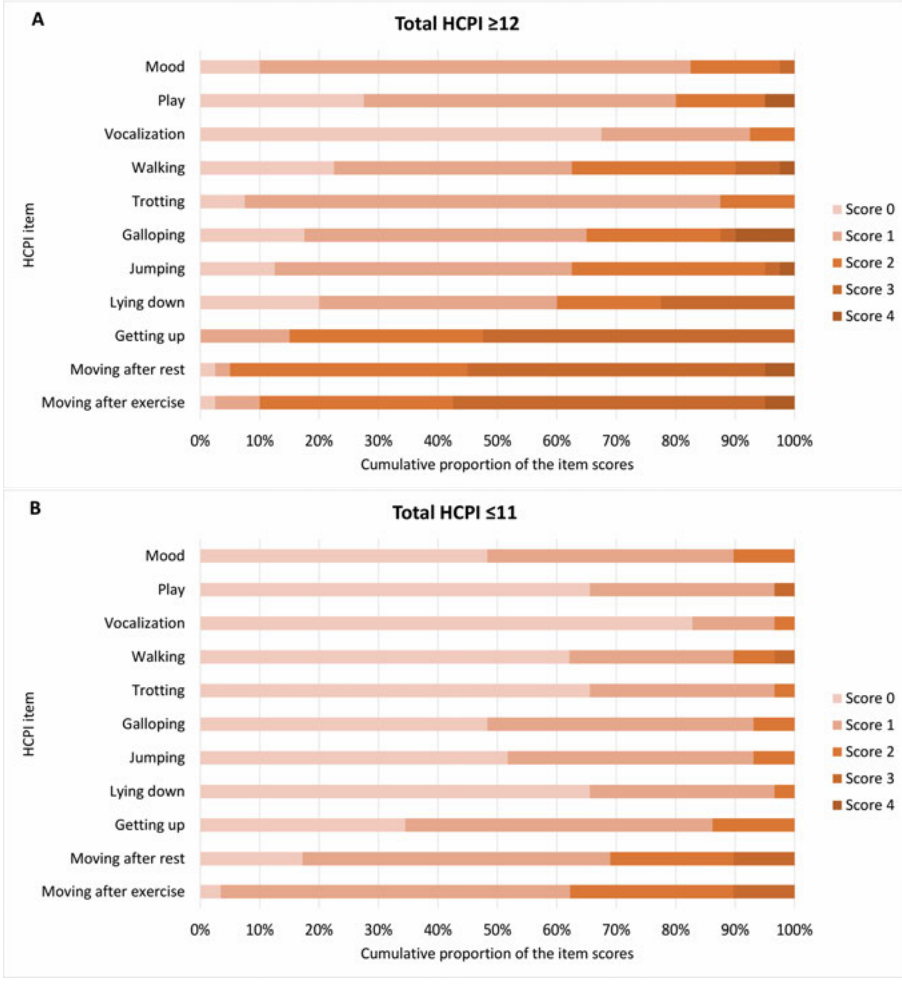

Figure 4A and 4B. *The cumulative proportions of owner reported pain behaviors associated to canine osteoarthritis, in each item of the Helsinki Chronic Pain Index (HCPI). Score of 0 and 1 indicate normal canine behavior, and score 2, 3 and 4 indicate increasingly severe chronic pain behaviors. Figure 4A; dogs with total HCPI≥12, 4B; dogs with total HCPI≤11.*

Table 5. *The explanatory variables for main outcome variable, chronic pain behavior, measured by Helsinki Chronic Pain Index, in a univariate and a multivariate logistic regression analysis based on a sample of dogs with pain related to OA, referred for animal physiotherapy.*

	Univariate logistic regression (n=69)		**Multivariate logistic regression** (n=67)	
Explanatory variable	**β-value**	**OR (95% CI)**	**β-value**	**OR (95% CI)**
Sex	-0.83	0.44 (0.16-1.16)	-0.85	0.43 (0.14-1.32)
Body condition	-0.65	0.52 (0.20-1.38)	-0.51	0.60 (0.20-1.85)
Antiinflammatory medications	0.05	1.05 (0.35-3.18)	0.19	1.21 (0.33-4.42)
Pain interference score[1]	0.57	1.77 (1.26-2.50)[*]	0.56	1.74 (1.23-2.47)[**]

OR; odds ratio, CI; confidence intervals;

[1] n=67

[*] P-value= 0.001; [**] P-value=0.002

Model Chi square value = 17.79, df= 7, P-value= 0.001. Nagelkerke R^2 = 0.31 in the multivariate logistic regression model.

4.3 Measuring interbeat intervals

In total, 4814 IBIs from ECG and 4794 IBIs from Polar RS800CX were analyzed. The ECG and Polar IBIs were synchronized to a total of 4851 pairs to allow statistical pairwise comparisons. A total of 595 errors were identified in the Polar data, representing involvement in 12.3% of the pairwise data set. Because the errors were not present in the ECG tracings, none of the artifacts in the Polar data were nonsinus in origin. The number of errors were unequally distributed among the 11 dogs (Table 6).

4.3.1 Relationship and relative reliability between ECG and Polar interbeat intervals

There was strong association between IBI data from ECG and Polar RS800CX (n=11). The estimated relative reliability showed strong to excellent correlation; the values of ICC were between 0.73 and 0.84 depending on how missing values were handled (Table 6). 95% CI for ICC varied moderately, ranging from 0.74–0.95, 0.62–0.82 and 0.72–0.90 indicating that the true difference between these measures was moderate. Table 6 shows within-subject mean ± SD IBI and individual relative reliability investigated by estimating ICC using pairwise deletion, inserted zero "0" values, and mean imputation, to address and replace missing IBI values.

The Bland and Altman plots of the differences between the ECG and Polar IBI data against their means illustrate the discrepancies between the devices in individual subjects and the group (n= 11) data, when missing values were pairwise deleted (Figure 5). Because the difference between methods increased with the magnitude of the measurements, another Bland and Altman analysis was performed on log (base 10) transformed IBI values. The mean difference between the methods was 0.8%. Lower LoA was 0.81 and upper LoA was 1.26, indicating that for about 95% of cases Polar measurement of IBI was between 19% lower and 26% higher than the ECG measurements of IBIs. Moreover, 93.2% of the values were within the LoA.

Despite overall high ICC values and moderate CI on group level, the relative reliability and agreement were not acceptable individually in three of the subjects. Accordingly, three dogs (subject 5, 10 and 11) were internally excluded as their error rates also were more than 5% [52,89] (Table 6). Consequently, the relationship between ECG and Polar IBI measurements in the subgroup (n= 8) was stronger and the relative reliability, estimated with ICC, was excellent with narrow 95% CI indicating the true differences between these measures were small (Table 6).

Bland and Altman analysis (n=8) showed that Polar was over- and underestimating IBIs compared to ECG. Mean difference in the Bland and Altman analysis, adjusted for multiple measurements per individual, was 1.8 ms.

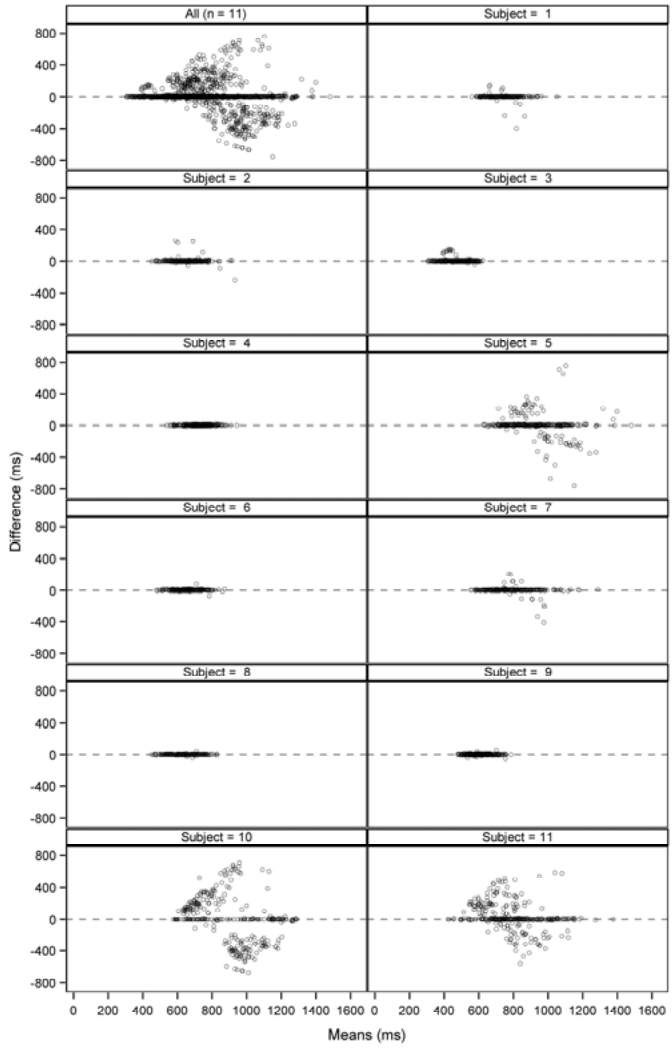

Figure 5. *Bland-Altman plot illustrating the agreement between ECG and Polar IBIs in the individual data and in all dogs (n=11). X-axis showing the mean of Polar and ECG (Polar+ECG / 2) is plotted against y-axis showing differences between the methods (Polar minus ECG). Dotted lines represent mean differences.*

Table 6. *Individual descriptive statistics (Mean IBI ± SD), individual and group intraclass correlation coefficient for IBI measures obtained by ECG and Polar. Missing IBI values were handled with blanks, zeros, and imputation. Error rate (percentage of IBI pairs involved in measurement errors) within individual and group IBI series obtained by Polar.*

Subject	**ECG mean IBI(SD)**	**Polar mean IBI(SD)**	**ICC blank**	**ICC zero**	**ICC imputation**	**Error rate %**
1	739.9(75.7)	739.5(72.2)	0.92	0.85	0.90	2.6
2	646.4(72.3)	649.3(69.0)	0.94	0.90	0.93	1.9
3	469.8(62.6)	473.9(60.4)	0.96	0.87	0.96	2.7
4	731.5(61.7)	733.4(61.8)	1.00	0.87	0.96	0.2
5	922.3(176.4)	922.0(153.2)	0.64	0.53	0.61	26.4
6	649.6(59.9)	651.5(59.5)	0.99	0.99	0.99	0.2
7	782.4(119.1)	782.1(113.5)	0.95	0.90	0.94	4.2
8	618.9(73.4)	620.2(73.6)	1.00	1.00	1.00	0.0
9	599.9(55.2)	601.4(55.0)	0.99	0.99	0.99	0.0
10	876.3(270.9)	878.0(192.8)	0.23	0.32	0.22	74.5
11	768.0(198.2)	807.7(156.9)	0.58	0.49	0.55	44.9
Total (n=11)*			0.84 95% CI: 0.74-0.95	0.73 95% CI: 0.62-0.82	0.82 95% CI: 0.72-0.90	12.3
Total (n=8)**			0.99 95% CI: 0.97-0.99	0.97 95% CI: 0.94-0.98	0.99 95% CI: 0.97-0.99	1.5

SD, standard deviation; CI, confidence intervals; IBI, interbeat intervals

* All 11 dogs (n=11). ** Subject 1, 2, 3, 4, 6, 7, 8, 9 in a subgroup and subject 5, 10 and 11 internally excluded (n=8).

Figure 6 shows a Bland and Altman plot on pairwise deleted missing data. The dotted lines on the scatter plot indicate the upper and lower LoA, stretching from 40.3 ms to 36.8 ms. Limits of agreement and number of measures within LoA varied depending on method used to handle missing IBI data (Table 7).

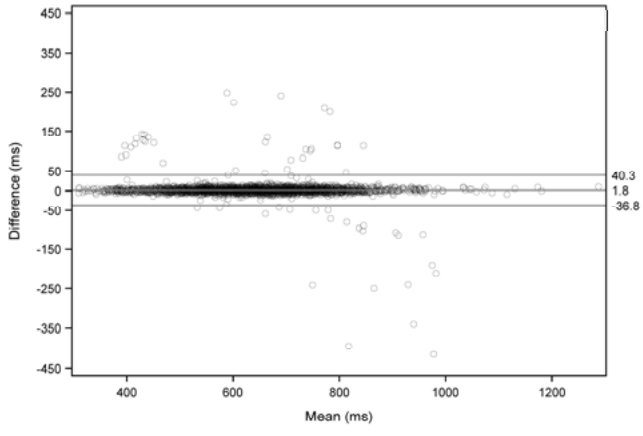

Figure 6. *Bland-Altman plot illustrating the agreement between ECG and Polar IBIs, based on subject 1, 2, 3, 4, 6, 7, 8 and 9 (n=8). X-axis showing the mean of ECG and Polar (Polar + ECG / 2) plotted against y-axis showing differences between the methods (Polar minus ECG). Lines represent mean differences and the upper and lower limits of agreement (mean ± 1.96 SD). Missing IBI values are handled as blanks in the data set.*

Table 7. *Mean differences (milliseconds) in IBI between Polar and ECG and 95% limits of agreement. The number of values within respectively outside 95% limits of agreement, also presented as proportions of all values (n=8).*

Missing data	**Mean difference**	**Limits of agreement**	**Number within limits (proportion)**	**Number outside limits (proportion)**
Blanks	1.8	-36.8 - 40.3	3692 (98.5%)	55 (1.5%)
Zeros	1.8	-58.9 - 62.5	3703 (98.6%)	53 (1.4%)
Imputation	1.8	-36.8 - 40.3	3693 (98.3%)	63 (1.7%)
Values are presented as mean, limits of agreement, amount and proportion.				

4.4 Analyzing heart rate variability

Eight dogs completed the pilot study and provided data from the recording of the IBI series. The time-based parameters obtained from ECG and Polar data indicated there was a difference between ECG and Polar in SDNN ($p=0.035$) and RMSSD ($p=0.034$). There were no differences in the frequency-based parameters from the HRV analysis. Summary statistics of time- and frequency-based parameters of HRV analysis from Polar and ECG are presented in Table 8.

4.4.1 Associations and agreement between HRV parameters from Polar and ECG

The correlations between Polar and ECG varied among HRV parameters, although Spearman's rank correlation coefficient and $ICC_{2.1}$ showed overall strong correlations, and narrow 95% CI in all time- and frequency-based parameters indicating that the true difference between these measures was small (Table 9). Mean differences in Bland-Altman analysis showed that Polar was both overestimating and underestimating the HRV parameters, compared to ECG. In the time-based HRV parameters, SDNN and RMSSD, differences between Polar and ECG were within LoA. However, in the frequency-based parameters the Bland and Altman plots indicated that the majority, but less than 95% of the differences were within LoA in LF, LF n.u. and HF n.u.

4.4.2 Within-group variation in Polar and ECG measurements

Percentage measurement reliability varied between 2.8% and 11.6% in ECG and between 2.6% and 11.8% in Polar, indicating the levels of accuracy varied between low and high among HRV variables. The absolute reliabilities of each HRV parameter in Polar and ECG, estimated by the SEM and SEM%, are shown in Table 9. There were large within-group variations observed in both time- and frequency-based HRV parameters in both measurement methods.

Table 8. *Summary statistics of time- and frequency-based parameters of heart rate variability analysis from ECG and Polar HRV data (n=8).*

HRV parameter	Polar, mean(SD)	ECG, mean(SD)	p-value
SDNN (ms)	70.5(18.4)	72.5(20.1)	0.035*
RMSSD (ms)	54.4(32.9)	58.6(37.1)	0.034*
LF(ms^2)	1411.1(1045.2)	1443.4(1028.5)	0.298
HF(ms^2)	1486.8(1758.8)	1653.3(1918.2)	0.061
LF n.u.	55.9(18.5)	54.4(18.9)	0.299
HF n.u.	44.1(18.5)	45.6(18.9)	0.223
LF/HF	1.8(1.5)	1.7(1.5)	0.223

HF, power in the high frequency range; HF n.u. high frequency power in normalized units; LF, power in the low frequency range; LF n.u., low frequency power in normalized units; LF/HF, ratio low frequency power/high frequency power; SD, standard deviation; SDNN, mean of standard deviation of normal-to-normal intervals; RMSSD, root mean square of successive differences. Significance of the difference between Polar and ECG, $p<0.05$. * represents significant difference in comparison.

Table 9. *Reliability estimates of Polar, compared to ECG, measuring time- and frequency-based heart rate variability parameters in dogs during stationary standing position.*

HRV parameter	Spearman's ρ	$ICC_{2.1}$ (CI)	SEM ECG	SEM% ECG	SEM Polar	SEM% Polar
SDNN	0.95	0.99(0.90-1.00)	2.0	2.8	1.8	2.6
RMSSD	0.98	0.99(0.85-1.00)	4.5	7.7	4.0	7.4
LF(ms^2)	0.93	1.00(0.99-1.00)	56.3	3.9	57.2	4.1
HF(ms^2)	0.95	0.99(0.93-1.00)	191.8	11.6	175.9	11.8
LF n.u.	0.93	0.98(0.93-1.00)	2.4	4.4	2.3	4.1
HF n.u.	0.93	0.98(0.93-1.00)	2.4	5.3	2.3	5.2
LF/HF	0.93	0.99(0.96-1.00)	0.2	9.0	0.2	8.6

CI, confidence interval (lower and upper); HF, power in the high frequency range; HF n.u., high frequency power in normalized units; ICC, intraclass correlation coefficient; LF, power in the low frequency range; LF n.u., low frequency power in normalized units; LF/HF, ratio low frequency power/high frequency power; RMSSD, root mean square of successive differences (ms); SDNN, mean of standard deviation of normal-to-normal intervals (ms); SEM, standard error of measurement; SEM%, standard error of the measurement expressed as a percentage.

Discussion

The general aim of this thesis was to psychometrically evaluate measurement properties in clinically applicable assessment methods related to pain in naturally occurring canine OA. In the studies included in this thesis, a potential biophysiological marker of chronic pain, i.e. HRV, was explored in clinically sound dogs and two observational measures, more specifically two owner-reported questionnaires, the CBPI and the HCPI, were translated. Further, the CBPI was psychometrically tested in dogs diagnosed with OA and referred for animal physiotherapy. This thesis has a methodological approach and contributes with novel and confirmatory knowledge with assessment methods that are used in preclinical trials, clinical veterinary research and in canine behavioral studies, the CBPI and the Polar heart rate monitor. Owner-reported perceptions of pain severity, interference of pain with function and chronic pain behavior were studied in – *Study I* and *II* – and the Polar heart rate monitor measuring IBI series for HRV analysis was studied in – *Study III* and *IV*. In extension, the presence of chronic pain behavior and disability in canine OA was studied in *Study I* and *II*. The main findings are summarized in the text below.

The translated Swedish versions of the CBPI and the HCPI are valid to use in the original samples and in contexts similar to this thesis [62,81]. The original two-factor structure in the CBPI is not ideally suited to measure owner-perceived pain related to OA in a diverse sample of dogs referred for animal physiotherapy. Instead, the pain interference with function domain can be used alone. Owners reported higher proportions of chronic pain behavior in items targeting physical functionality, e.g. getting up, moving after rest and moving after major exercise. Despite radiographic findings and clinical signs observed prior to inclusion, some dogs did not show owner-perceived behavioural signs of chronic pain. Owner observations were not influenced by the ongoing anti-inflammatory medications in their dogs. Only small amounts of artifacts could be accepted for valid and reliable IBI measures by Polar RS800CX heart rate monitor. Polar RS800CX heart rate monitor has shortcomings in registering sequences of large variability between IBIs. Small numbers of erroneous IBI segments from Polar negatively impact the validity and reliability properties in subsequent HRV analysis. An additional finding was that the within-group variation was large in some of the HRV parameters, indicating difficulties detecting changes in group level data.

5.1 Translation of owner-perceived questionnaires

For *Study I* and *II*, high-quality linguistic translations of the CBPI and the HCPI questionnaires were conducted according to the standard procedure for translation and back-translation of instruments designed for self-reported outcome. Semantic equivalents were found in Swedish and the conceptual meaning in the translated versions of the questionnaires could be kept and therefore we do not relate the findings in this thesis to the translation processes. The translated versions of the CBPI and the HCPI are considered valid to use in contexts similar to the original and to those presented in this thesis.

5.2 Psychometric properties of the CBPI (Study I)

The results from our study supplement the existing knowledge with the CBPI by confirming good to excellent internal consistency, the ability to discriminate OA dogs from clinically sound dogs and the number of components extracted in EFA. The number of components retained, and the eigenvalues of each component, were similar in the group of dogs with CBPI ≥1 and the group of dogs studied by Brown et al. [62] during the original development and psychometric testing of CBPI. The internal consistencies were also similar. In comparison, Cronbach's α for the total CBPI sum was 0.91 in the present study and 0.92 in the study conducted by Brown et al. [62]. Cronbach's α for severity of pain and pain interference with function were 0.91 and 0.91 respectively in our study, and 0.93 and 0.89 in the study by Brown et al [62].

The fit indices achieved in the CFA in *Study I* were not acceptable and neither the one-factor nor the two-factor models proposed could be confirmed. The hypothesis of the presented two-factor representation in the CBPI was rejected as causal structure underlying the construct. In the subsequent principal component analysis, three of the CBPI items, i.e. general activity, enjoyment of life and ability to rise, loaded equally on the two extracted factors. This indicates that the items were correlated with both the pain severity and the pain interference factor. That factor loadings were equal in several items, and that the one-factor and two-factor models did not allow for dual loading, may explain why the fit indices were not acceptable. Analyzing ordinal scaled items as they were on a continuous scale may also affect the results. The analysis by bootstrap modeling and by Bayesian estimations differed somewhat from the maximum likelihood estimates, which supports the notion that statistical difficulties may arise when the assumption of normal distribution is accepted in ordinal scaled items.

Like the owners rating pain intensity in their dogs using a visual analog scale [130], the dog owners in *Study I* may have had a lack of recognition of behavioral signs related to OA pain in their dogs. This may explain the high proportion of floor effects in the pain severity items. The responsiveness of

the CBPI may be reduced because many dogs would not be able to change their item score despite the likelihood of clinical improvement. However, the sum of pain interference with six daily activities, i.e. general activity, enjoyment of life, rising to standing, walking, running, and climbing, showed no floor effect in the group of dogs rated CBPI >1. Therefore, pain interference items addressing the dogs' ability to move, to perform activities of daily living and to participate in various activities in various environments may be more sensitive to change in OA dogs undergoing animal physiotherapy.

5.3 Owner-perceived chronic pain behavior in canine OA (Study II)

Most OA dogs presenting with chronic pain behaviour in *Study II* were overweight and had higher body condition score compared to OA dogs without chronic pain behaviour. There was no significant association between body condition and the outcome variable i.e. chronic pain behaviour measured by the HCPI. German et al. [171] showed that weight loss in OA dogs with excessive body weight increases the vitality component and decreases the emotional component of health-related quality of life. However, the pain component was not influenced by weight loss in their study, which is supported by our findings. In line with previous studies on canine osteochondrosis, male sex was more frequently represented in dogs with total HCPI ≥12 [172]. Osteochondrosis affects young dogs and is a major cause of secondary OA. One reason male dogs here present with owner-perceived chronic pain behavior is that they may have endured joint pathology since they were puppies and the progress of OA may be more severe. However, there was no significant association between sex and chronic pain behavior, and the duration of the clinical signs or the stage of OA were not accounted for in this thesis. In *Study I* we raised a concern that dog owners may be attributed to a response shift in the internal standards because they were aware their dogs were undergoing pain management, which may influence their ability to reliably rate pain severity and interference of pain on function in daily living. In contrast to our concern, the findings in *Study II* indicate that the owner-reported ratings of chronic pain behaviour were not associated with the antiinflammatory medication. Instead, the results from *Study II* indicate that the owner-perceived rating of pain interference is significantly associated with higher odds of the outcome, i.e. chronic pain behaviour measured by HCPI. The OR and the β value of the CBPI pain interference score show that the odds of reporting chronic pain behaviour if the CBPI pain interference score increases with two units is three times higher. The 95% CI were narrow for all explanatory variables, indicating high precision of the OR. According to the user guide for the CBPI the criteria for successful treatment of an individual patient are predefined as a reduction ≥ 1 in

pain interference score and ≥ 2 in pain severity score. The average pain severity, i.e. 2.5, and average pain interference, i.e. 2.3, presented in *Study I* are in consistence with findings previously presented in dogs undergoing pain management [135].

5.4 Impact of pain on body function and activities (Study I and II)

The findings from this thesis add knowledge to pain in canine OA and the disabilities that follow with the disease. Most dog owners in *Study I* and *II* reported that their dogs presented with pain and disability related to OA, and that their dogs were affected by body function impairments and activity limitations. These findings are consistent with previous reports on the efficacy of antiinflammatory medications in dogs with OA, showing that pain control is not complete with one modality only, i.e. antiinflammatory medication. Although 79% of the OA dogs had ongoing antiinflammatory medication the owners reported presence of pain and pain-related disability. Our findings in *Study I* and *II* showed that pain interferes with general activity, enjoyment of life and daily activities. Dogs with HCPI ≥12 were more affected by pain-related disability, particularly in physical activities e.g. getting up, moving after rest or major exercise. Notably, some owners do not perceive pain or pain behavior in their dogs despite radiographic findings and clinical signs observed prior to inclusion in *Study I* and *II*. Like human OA, there may be a poor concordance between the detectable pathology of canine OA and pain experienced by the dog [16,173]. Pain in dogs is a phenomenon that is difficult to quantify. The clinical picture of a dog with persistent OA pain or chronic pain behavior associated with OA includes changes in several dimensions and may be evaluated by deconstruction of the pain behavior. In contrast to the CBPI, the HCPI is a pain questionnaire that allows the owners to describe the behavior of the dog rather than to rate the level of pain severity or the level of interference of pain on function. A description of each canine chronic pain behavior under investigation may contribute to make the ratings of pain-related disability more defined, which may overcome part of the subjectivity in rating a sensory and emotional pain experience of an animal [174,175]. While several predictors of human OA pain have been recognized, there remains no consensus among animal clinicians and researchers on how sensory, cognitive, emotional, social, and behavioral components interact to cause pain in canine OA [176].

5.5 Measuring interbeat intervals (Study III)

Despite a highly standardized protocol and the fact that the dogs maintained a standing position in this thesis, there are various sources of artifacts that may interfere with the electrical signals and make it difficult for the devices to recognize IBIs. However, in *Study III* there were no corresponding artifacts or nonsinus beats in the ECG tracings and Polar showed no continuous sequences of unregistered IBIs. There were overall high ICC values and moderate CIs at group level, and yet the relative reliability and agreement were not acceptable individually in three of the dogs, due to considerable number of errors. Internally excluding three subjects with error rates >5% made obvious differences to the CIs of the ICC and the LoA in Bland-Altman analysis. Polar RS800CX heart rate monitor was valid and reliable and may be used interchangeably with ECG in the group of dogs studied, when the recorded IBI series did not contain more than 5% measurement errors. It is essential for the validity of Polar IBI data that the quality of recorded IBI series is high and to a large extent free from errors. Altogether there seems to be some shortcomings of Polar RS800CX to reliably measure IBIs in all dogs in *Study III*. The exact cause of the errors produced by Polar, particularly in three of the subjects, was not possible to explain. One assumption is that Polar may register the depolarization illustrated in the ECG tracing as a P-peak, instead of or followed by the R-peak, which may account for some errors. We agree with the description stated by Jonckheer-Sheehy et al. [122] that Polar repeatedly showed sequences of rather invariable IBIs compared to corresponding sequences with considerable variability in ECG. It therefore should be mentioned that Polar may have a shortcoming with respiratory sinus arrhythmia in dogs, which may influence the reliability particularly in sound and fit dogs.

5.6 Analyzing heart rate variability (Study IV)

Time- and frequency-based parameters in HRV analysis are used as biophysiological markers, indicating modulations and activity in the ANS, in dogs suffering from chronic stress and during aversive emotional states [57,87,106,107]. Performing adequate HRV analysis requires a series of normal-to-normal IBIs. Preferably only segments of IBIs that are completely free from error and/or nonsinus beats should be included in an HRV analysis, because even small errors in IBI data may bias the outcome of time- and frequency-based parameters. In *Study IV*, only IBI series containing less than 5% of erroneous data were used, and yet the small error negatively impacted the criterion validity and reliability properties of Polar RS800CX HRV parameters against ECG. Absolute and relative reliabilities of Polar RS800CX were estimated on HRV time- and frequency-based parameters previously used within canine behavioral research [96,101-105]. Heart rate variability parameters derived from Polar

and ECG were strongly associated. However, our findings indicate differences in two of the time based HRV parameters recorded by Polar, i.e. SDNN and RMSSD. Estimations of the absolute reliabilities in *Study IV* demonstrated that SEM and SEM% values in the data obtained with the Polar RS800CX were close to the measurement errors obtained by ECG. Rather similar and high SEM and SEM% in Polar and ECG are possibly due to the large within-group variations in both measurement methods. Hence, the interpretation of future research results will be challenged by the presence of individual variations that needs to be considered when implementing canine HRV studies.

5.7 Methodological considerations

Some methodological issues are important to discuss when considering the results and conclusions of this thesis. The internal, external, construct and data-evaluation validity needs to be addressed. One strength of this thesis was a highly standardized research protocol in *Study III* and *IV* contributing to a complete data set without technical artifacts. Few dog owners declined to participate in *Study I* and *II*, and only a few questionnaires were internally excluded due to incomplete completion of the questionnaires.

In *Study I*, age and body condition score differed between OA dogs and clinically sound dogs in a control group. Selection bias may potentially threaten the internal validity because extreme groups were used to assess the ability of CBPI to discriminate OA dogs from sound dogs. The potential relevance of the differences in age and body condition was considered, and we found that the descriptive characteristics of the dogs did not explain the results. Some other characteristics, e.g. within the owner, should be explored further. For example, the gender or experience of the owner may influence the pain rating in their dog. Several plausible moderating factors, e.g. owners' attachment to the dog, may have a moderating effect in pain assessment by proxy. In nonverbal humans, for example in noncommunicative humans with dementia, there are specific conceptual models of pain assessment by proxy. The external rater has been added to Loeser's conceptual model of pain, accounting for the unique features of pain assessment in persons with dementia [177]. Without an adequate conceptual model, research and clinical advancement in pain assessment in veterinary medicine and animal rehabilitation is restricted and instruments tend to focus on a limited part of the pain experience. To increase the understanding of how different measures of pain are related, a multidimensional understanding of pain needs to be further implemented. A multidimensional conceptual model of pain assessment in animals, based on the concepts of current pain theory, may serve as a guide for the development of a pain assessment strategy for animals undergoing rehabilitation.

Although *Study I* and *II* did not involve intervention, the dog owners may have been subjected to unintentional expectancy effects in their responses in

the questionnaires. Some experimenter-subject contact occurred because the main author of all studies in this thesis was also the animal physiotherapist responsible at the veterinary clinic. To handle this threat to construct validity, the owners were instructed according to the user guides available for each of the owner-reported questionnaires.

A limitation in *Study II* was we did not control for either breed or personality traits of the dogs. In the HCPI questionnaire one mood item targets play behavior, which may be associated with breed and personality. The multivariate logistic regression model in *Study II* did not fully explain the variance in the outcome variable, i.e. chronic pain behavior related to OA. Three explanatory variables in the model were not significantly associated with the outcome. Therefore, we encourage further clinical research on chronic pain behavior and functionality in canine OA. Correlative observational studies with larger sample sizes, longitudinal designs and several additional explanatory variables are needed so clinicians can tailor more effective approaches to pain management in dogs.

The shortcomings of Polar RS800CX to reliably measure IBIs in *Study III*, and the large with-in group variations generating high SEM% in *Study IV*, were interpreted as a threat to internal validity in *Study II*. Therefore, HRV parameters by Polar RS800CX was excluded from the study design in *Study II* aiming to assess chronic pain and explanatory variables associated with chronic pain in canine OA.

A strength in *Study III* was that we were able to present varying results depending on which way missing data were handled. There were also some possible limitations in *Study III*. First, the sample size was small, even if repeated measurements within each subject generated large number of pairwise data. The ability to generalize our results from the reliability analysis would increase with larger sample size. Second, it would have been possible to make more assumptions about variables influencing the results if we had controlled for breathing frequency, respiratory sinus arrhythmia and level of fitness within the study group.

In *Study IV*, we were aware of the increased probability of statistical type 1 error with each additional test performed. Rather than to compensate for multiple tests by dividing alpha, we chose to not reduce the power of each test by adjusting the alpha value.

Considering external validity of the results and conclusions of this thesis, the dogs diagnosed with OA was recruited from one veterinary clinic, which may influence the generalizability of the findings in *Study I* and *II*. Replication of studies targeting canine OA pain in larger samples are needed. There is also a concern about the sample of convenience in *Study I* and *II.* The dogs were referred for animal physiotherapy and there may be a reactivity to the experimental arrangements, i.e. the dog owners were aware that they were participating in a study involving physiotherapy and physical disabilities. Animal physiotherapy interventions target functionality and therefore the level of

chronic pain behavior in *Study II* may not be generalized to all dogs with OA. Results of *Study I* and *II* in this thesis can be generalized for dogs with a wide range of age (1 to 12 years), body weight (9 to 56 kg), body condition score (3-8), CBPI severity sum (0 to 23), CBPI pain interference sum (0 to 46) and HCPI score (3 to 25). Although dogs represented 19 breeds and mixed-breeds in *Study I*, and reflected the population of orthopedic patients in one veterinary clinic, this may not represent the breed distribution at other veterinary hospitals or practices. The results of *Study III* and *IV* in this thesis are valid for mid-size to large (20-40 kg), clinically sound dogs, undergoing measurements in the environment provided in this thesis.

6 Conclusions

- The quality of our comprehensive Swedish translation of the CBPI and the HCPI were high and the translated versions are valid for use in the Swedish language.
- The psychometric properties of the CBPI were satisfying, even in a heterogenous sample of OA dogs referred for physiotherapy. The original two-factor structure in the CBPI is not ideally suited to measure pain related to OA in a diverse sample. The pain interference with function items of the CBPI can be used separately. There is a potential floor effect in the CBPI pain severity scores and the interpretability of the results may be affected.
- Owners of dogs with chronic pain related to naturally occurring OA, referred for animal physiotherapy, reported that their dogs were affected by pain-related disabilities, particularly in physical activities e.g. getting up, moving after rest or major exercise. The observations and ratings performed by dog owners were not associated with ongoing antiinflammatory medication in their dogs. Instead, their perception of pain interfering with functionality was significantly associated with higher odds of chronic pain behavior.
- Polar RS800CX heart rate monitor systematically biased recorded IBI series and it was essential to detect measurement errors. For Polar RS800CX heart rate monitor to be used interchangeably with ECG, less than 5% of artifacts could be accepted.
- Polar RS800CX heart rate monitor showed acceptable relative reliability, in measuring time- and frequency-based HRV parameters in dogs with less than 5% of artifacts in their IBI series. Small amounts of erroneous IBI segments from Polar negatively impact the measurement properties of the time-based SDNN and RMSSD parameters Polar RS800CX.

6.1 Implications for clinical practice

Animal health care professionals are recommended to use the translated version of the CBPI and the HCPI in dogs in their early assessment of dogs with naturally occurring OA. Dogs with clinical signs and radiographically confirmed OA present with behavioral signs and disabilities, despite undergoing antiinflammatory medication. To reliably assess and reassess bodily functional impairments, activity limitations and restricted participation related to pain in canine OA owner-reported measures should be added in clinical practice to evaluate rehabilitation interventions.

Heart rate variability parameters may be potential biophysiological markers of activity in the ANS in dogs undergoing interventions intended to reduce pain related to canine OA. Due to large within-group variability, changes in HRV parameters may be difficult to monitor. The measurement needs to be strictly standardized and even small erroneous IBI needs to be detected. The Polar RS800CX may systematically bias recorded IBI series in sound dogs during stationary conditions, therefore ECG is recommended prior to the Polar RS800CX heart rate monitor.

6.2 Implications for future research

The translations of two owner-reported pain questionnaires used in this thesis may encourage study designs exploring assessment of cross-cultural differences among dog owners in different countries. However, further research needs to be conducted to determine whether the original psychometric results from CBPI can be replicated across different target groups and particularly with larger sample size. The absolute reliability estimated from Polar and ECG showed that it may be difficult to monitor small changes in some of the canine HRV parameters in group level data because of large within-group variations. Subject heterogeneity is a potential threat to data-evaluation validity in future research studies measuring canine HRV, and researchers should consider all sources of variation that might be valuable to control prior to implementing their study design. Study designs based on group data may need large sample sizes for detection of statistical differences e.g. in clinical intervention studies. To increase the understanding of how different measures of chronic pain are tied, a multidimensional conceptual model of pain, and pain assessment by proxy, need to be further implemented in musculoskeletal assessment and research in dogs.

7 Svensk sammanfattning (Swedish summary)

Vi behöver fler giltiga och pålitliga metoder för att undersöka smärta och för att följa upp rehabiliterande åtgärder för hundar med artros. Artrossmärta som inte upptäcks eller inte klingar av riskerar att utvecklas till kronisk smärta. Kronisk smärta är mer svårhanterad än akut smärta och riskerar att påverka hundars livskvalité på ett negativt sätt.

Syftet med den här avhandlingen var att utvärdera mätegenskaper hos undersökningsmetoder relaterade till smärta hos hundar med naturligt uppkommen artros. Ett annat syfte var att beskriva beteenden hos hundar med och utan kronisk smärta till följd av artros. Mätegenskaperna hos metoder som representerar olika delar av hundens smärtupplevelse studerades. En smärtenkät, i vilken hundägare skattar artrossmärtans intensitet och smärtans påverkan på funktion i dagliga livet - Canine Brief Pain Inventory (CBPI); och en metod framtagen för analys av hjärtfrekvensvariabilitet med hjälp av en pulsmätare från Polar, utvärderades. Även smärtenkäten Helsinki Chronic Pain Index (HCPI) översattes till svenska och användes i avhandlingen.

Delarbete I var en tvärsnittsstudie bestående av två grupper av hundar. Giltigheten hos CBPI-formuläret undersöktes genom att enkätfrågorna besvarades av ägare till hundar med artros som blivit remitterade för fysioterapi (n=61) och av ägare till hundar som var friska (n=21). Resultatet visade att 26% av hundägarna skattade att deras hundar inte hade någon smärta i frågorna om smärtintensitet. Flera av frågorna i CBPI formuläret hade samband med både smärtans intensitet och smärtans påverkan på hundarnas funktion, vilket gjorde att det inte verkade lämpligt att dela upp formuläret i två delar för hundarna som ingick i studien. Frågorna som mäter hur smärta påverkar hundars funktion i dagliga livet kan vara mer pålitliga att använda vid undersökning av artrossmärta.

I delarbete II undersöktes smärtbeteenden hos hundar med artros (n=71) med HCPI samt samband mellan olika faktorer som kan förklara förekomsten av kronisk smärta hos hundarna. Resultaten visade att hundar med kronisk smärta är mer begränsade i fysisk aktivitet, exempelvis när de ska resa sig upp, röra sig efter att ha vilat respektive ansträngt sig kraftigt. En mindre andel hundar visade inga eller få smärtbeteenden. Det fanns inget samband mellan ägarnas skattning av hundarnas smärtbeteende och behandling med antiinflammatoriskt läkemedel.

I delarbete III och IV undersöktes giltigheten och pålitligheten hos Polars pulsmätare RS800CX med elektrokardiogram (EKG) vid mätning av R-till-R

intervall samt tids- och frekvensbaserade hjärtfrekvensvariabilitets mått på friska hundar (n=11) som stod stilla under fem minuter. Resultaten visade att det förekom mätfel hos Polars pulsmätare, speciellt hos tre av hundarna. Sammanlagt kunde 595 (12.3%) mätfel hittas bland R-till-R intervallen trots att inga tekniska problem uppstod. Sambanden var starka mellan Polar och EKG-mätningar. Hundarnas hjärtfrekvensvariabilitet varierade stort mellan individerna och det fanns skillnader mellan de tidsbaserade variablerna från Polar och EKG. För att Polars pulsmätare ska kunna användas istället för EKG bör det inte förekomma mer än 5% mätfel.

Slutsatserna i den här avhandlingen är: 1) De svenska översättningarna av CBPI och HCPI är lämpliga att användas; 2) Mätegenskaperna hos CBPI var tillfredsställande. Det fanns en golveffekt hos CBPI när hundägarna skattade smärtintensitet, vilket leder till svårigheter att tolka svaret på de frågorna. Uppdelning av CBPI-frågorna i två faktorer var inte idealisk i den här studiegruppen och frågorna som handlar om i vilken utsträckning smärta påverkar hundarnas funktion i dagliga livet kan användas separat; 3) Ägare till artroshundar remitterade för fysioterapi upplevde att hundarna hade smärtrelaterade funktionsnedsättningar och aktivitetsbegränsningar exempelvis när hundarna skulle resa sig och röra sig efter vila respektive efter kraftig ansträngning. Ägares upplevelse av hundars smärtbeteenden hade inget samband med eventuell medicinering med antiinflammatoriska läkemedel; 4) Polar RS800CX visade sig systematiskt mäta fel vid registrering av R-till-R intervall. Det är avgörande för Polars pålitlighet att mindre än 5% mätfel förekommer; 5) Polars pålitlighet vid analys av hjärtfrekvensvariabilitet är acceptabel om R-till-R intervallen innehåller mindre än 5% mätfel. Även få mätfel kan påverka mätegenskaperna negativt. Den här avhandlingen bidrar med ökade kunskaper om undersökningsmetoder som är relaterade olika delar av smärtupplevelsen hos artroshundar, vilket möjliggör förbättrad klinisk hantering av smärtproblematik.

8 Acknowledgments

Jag vill framföra ett stort och varmt tack till alla personer som varit betydelsefulla för mig under min forskarutbildning och i arbetet med denna avhandling. Ett stort tack till alla hundar och deras människor som deltagit i studierna.

Jag vill rikta ett särskilt tack till:

Ni som stöttat och trott på mig från allra första början; Håkan Kasström, R.I.P. Marianne Mellgren och Birgitta Lindmark.

Min huvudhandledare Karin Hellström, för att du gav mig möjligheten och för att du på ett uppmuntrande och tålmodigt sätt har lotsat mig på fria tyglar igenom hela forskarutbildningen. Jag är så glad över att du hela tiden har trott på mig och att jag har fått ta del av din kompetens och klokhet.

Min handledare Lena Zetterberg, för din förmåga att konstruktivt ifrågasätta och uttrycka funderingar. Det har inspirerat mig och i mitt fall har lett till att jag och mina arbeten har utvecklats på ett positivt sätt.

Min handledare Rita Sjöström, för att du har följt mig sedan magisterutbildningen, och för att du på ett generöst och uppmuntrande sätt har lotsat mig under forskarutbildningen. Du har genom åren också blivit en kär vän.

Min handledare Pia Gustås, för ditt engagemang och dina kloka reflektioner under hela forskarutbildningen. Jag uppskattar att du är tillgänglig för frågor och funderingar och att du kan konsten att lyfta och diskutera viktiga delar i vår forskning.

Jag vill också tacka:

Medförfattarna till delarbetena: Hans Högberg, Erik Ahlgren och Laurie Edge-Hughes. Tack för ett gott samarbete och för att ni på olika sätt har delat med er av er tid, kunskap och bidragit med många värdefulla synpunkter.

Min rotekamrat, Cajsa Ericson, för långa och givande diskussioner när som helst på dygnet. Vi har delat stort och smått, glädjeämnen och sorger, vilket har varit ovärderligt.

Doktorandgruppen, Institutionen för neurovetenskap, Fysioterapi, för intressanta och givande diskussioner och trevlig samvaro både på och utanför forskarutbildningen: Ylva Åkerblom, Regina Bendrik, Hanna Ljungberg, Hedvig Zetterberg och Sofia Wagner.

Tack även till f.d. doktorander: Susanna Tuvemo Johnson, Christina Emilson, Søren Spörndly-Nees, Carina Hagman, Åsa Revenäs, Henrik Johansson, Birgit Vahlberg, Mikael Andersson, Sara Holm.

Institutionen för neurovetenskap, Uppsala universitet, under ledning av tidigare biträdande prefekt Cathrin Martin och studierektor Lena Zetterberg, för att jag har fått möjligheten att genomföra min forskarutbildning i en stimulerande miljö. Tack också till seniora forskare för uppmuntran och kloka synpunkter längs vägen.

Alla fantastiska och generösa arbetskamrater på Evidensia Djurkliniken Gefle och Sandviken. Ni alla bidrar verkligen till ett positivt arbetsklimat!

Mina kära föräldrar Ingrid, Cay och StigÅ för att ni har förmedlat och uppmuntrat min inre drivkraft och ständiga nyfikenhet.

Min älskade son Lukas, och min sambo Staffan och hans barn Emma och Pontus, för att ni finns och är fantastiska och kärleksfulla människor som gett mig viktiga perspektiv på tillvaron.

Min faster Ulla och farbror Stig, mina kusiner Lova och Erik och deras familjer, min bror Johan och hans familj, som representerar en stabil grund att stå på och påminner mig om vem jag är och var jag kommer ifrån.

Mina fantastiska vänner, tack för all stöttning.

Denna avhandling har delfinansierats med anslag från:
AGRIA och Svenska Kennelklubbens gemensamma forskningsfond
Jan Skogsborgs Stiftelse för forskning och fördjupad utbildning rörande hundsjukdomar
Stiftelsen Sveland
Minnesfonden Fysioterapeuterna

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