Development of novel thermal imaging techniques for pain detection in wild animals

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to my loved ones who understood even when I couldn't find the words

Abstract

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Abstract: Pain is a subjective feeling often difficult to interpret or study and thus, pain of those unable to communicate their pain is difficult to recognize. According to the new definition of pain by IASP (Raja et al 2020), verbal description is only one of the many behaviours that can be used to express pain, and the inability to communicate pain does not negate the possibility of experiencing it. This addition to the definition points out that non-human animals, too, even if they cannot express it in words, are capable of both experiencing and communicating pain. Can we as humans interpret a state of pain in an animal in a trustworthy way – and in a manner that would be respectful and non-invasive to the animal?

Infrared thermography (IRT) is a technology based on using infrared radiation instead of normal light to form images. These images can be used to quantify the surface temperature of an object with high resolution. The intensity of the radiation emitted by the object being imaged depends on the surface temperature and for this reason thermal imaging enables detecting and measuring changes of surface temperature. Pain and stress might manifest physiologically as activation of the autonomic nervous system, which in turn might result in changes in surface temperatures of the body. These changes might be detectable with a thermal camera. If we could establish a link between certain intricate temperature changes of the head area to certain type of activation of the sympathetic nervous system resulting from pain, thermal imaging could have the potential to detect this.

In this study I investigated if there were detectable temperature changes in animal patients before and after a standard examination conducted to each patient admitted to the Wildlife Hospital of Helsinki Zoo, where my data was gathered. Another question was whether the patients that had pain differed in their temperature changes as compared to other patients. The question at the heart of my research was whether there would be a change in peripheral facial temperatures of patients before and after the examination. Another question was whether thermal patterns would be different for pain- and non-pain patients. I found that for some parameters, the temperature differences between pain- and non-pain patients were indeed different, for example the crown temperature of birds seemed to change with examination for patients without pain but not for patients with pain. A more prominent finding was that temperatures decrease across many parameters after an examination as compared to prior to it, across all or many patient groups. My research does not univocally show that thermal imaging could be used to detect pain; rather it affirms the thought that the measurement of changes in peripheral temperatures could be a potential window to non-invasively detect some changes of activation of the sympathetic nervous system in animals.

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Tiivistelmä: Kipu on subjektiivinen tunne, jota on usein vaikea tulkita tai tutkia. Niiden kipu, jotka eivät voi sitä kommunikoida, on usein vaikeaa tunnistaa. IASP:n vuonna 2020 päivitetyn kivun määritelmän mukaan verbaalinen kuvailu on vain yksi tavoista ilmaista kipua. Kyvyttömyys ilmaista kipua verbaalisesta ei tarkoita, ettei sitä olisi. Tämä lisäys kivun määritelmään kertoo siitä, että esimerkiksi muut eläimet kuin ihmiset voivat sekä kokea että kommunikoida kipua. Kuinka ihmiset voivat tulkita eläinten kipua luotettavalla tavalla, ja niin että se olisi kunnioittavaa ja non-invasiivistä eläimelle?

Lämpökuvantaminen on teknologia, jossa kuva muodostuu kappaleen lähettämän infrapunasäteilyn vaikutuksesta. Näistä kuvista voidaan irrottaa lämpötila-arvoja. Säteilyn intensiivisyys riippuu kuvattavan kohteen pintalämpötilasta, joten lämpökuvantamista voidaan käyttää mittaamaan muutoksia kyseisessä pintalämpötilassa. Kipu ja stressi voivat aiheuttaa autonomisen hermoston aktivaatiota, mikä vuorostaan voi vaikuttaa kehon pintalämpötiloihin. Lämpökuvantamisella pystytään havainnoimaan näitä hienovaraisia pintalämpötilojen vaihteluita.

Lämpökuvantamalla voitaisiin mahdollisesti tulevaisuudessa tutkia esimerkiksi kipuun liittyviä ilmiöitä, jos ne aiheuttavat vaihtelua ihon pintalämpötiloissa. Tässä tutkimuksessa selvitin, että oliko tutkimallani villieläinotannalla havaittavissa pintalämpötilavaihteluja kasvojen alueella, voisivatko ne johtua kivusta, ja voisiko näiden perusteella lämpökuvantamista käyttää kiputilojen havainnointiin. Spesifimmin tutkin sitä, että muuttuuko potilaiden päänalueen pintalämpötilat alkututkimuksen aikana, joka suoritetaan aina uuden potilaan saapuessa Korkeasaaren Villieläinsairaalaan, jossa keräsin datani. Toinen kysymys oli, että ovatko nämä erot erilaisia niillä potilailla, joilla on todettu kiputila, verrattuna niihin, joilla ei ole todettua kiputilaa. Tutkimuksen tarkoitus oli myös kehittää tämän teknologian käyttöä villieläinten kohdalla. Tutkimukseni osoitti, että joillakin parametreilla (esim. pään lämpötila linnuilla) erot kipu- ja ei-kipu-potilailla olivat erilaisia. Kuitenkin merkittävämpi havainto oli se, että useiden parametrien kohdalla ja useiden potilasluokkien kohdalla pintalämpötilat laskivat alkututkimuksen jälkeen, mahdollisesti johtuen stressiin liittyvästä sympaattisen hermoston aktivaatiosta. Tutkimukseni ei yksiselitteisesti osoita sitä, että lämpökuvannusta voisi käyttää kivun havainnointiin. Sen sijaan se antaa vahvistusta ajatukselle, että pintalämpötilaerot voisivat olla potentiaalinen mittauskohde non-invasiiviselle autonomisen hermoston aktivaatiovaihtelun havainnoinnille.

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1. Introduction

"Physical pain has no voice, but when it at last finds a voice,
it begins to tell a story."

The Body in Pain, Elaine Scarry (1985)

Pain is a subjective feeling often difficult to interpret or study. Pain of those unable to communicate it is often difficult to recognize. According to the new definition of pain by International Association for the study of pain, **IASP** (Raja et al 2020), verbal description is among the many behaviours that can be used to express pain, and the inability to communicate pain does not negate the possibility of a pain experience. This underlines that non-human animals are capable of both experiencing and communicating pain. How and by what means are we as humans able to interpret a state of pain in an animal in a trustworthy, respectful and non-invasive manner? Historically, the techniques to interpret pain in animals has heavily relayed on interpreting behaviour, posture, facial and vocal expression, and on hormonal measurements from blood or feces and urine. There is a need for more pragmatic techniques.

The link between the autonomic nervous system and emotion is well established, but the actual underlying mechanisms are still under debate (Kreibig 2010). Pain among other intense feelings activates the sympathetic nervous system (**SNS**) which opens potential to detect pain if we can detect the activation of the nervous system. The type of pain – acute or chronic – might further affect how this activation appears. One potential way to detect the activation is through the effects it has on surface temperatures of the facial region that pertain to changes in circulation and brain activity. In this thesis I examine potential for developing infrared thermography (**IRT**) methodology for non-invasive detection of animal emotional states, focusing on pain and its relation to the activation of SNS. Current studies indicate that SNS-related physiological phenomena related to changes of surface temperature seem to be similar across at least mammals and birds, but there might be differences related to e.g., anatomy of the head and different thermal regulation systems among species.

The purpose of this thesis is to investigate the potential of the thermal imaging technology to develop methodology for pain detection on wild animals by doing basic research about what kind of detectable temperature differences are possibly related to pain. I have done this by collecting numerical data of the surface temperature distribution in the head area from the thermal imagery collected from multiple species of wild vertebrates, diagnosed either with painful conditions or not, that are taken in as patients at the Wildlife hospital associated with the Korkeasaari zoo in Helsinki. This basic research also includes investigation of other factors that might affect the surface temperatures.

In this introduction I summarise what is known about pain and how it has been researched in non-human animals, the current fallbacks in its measurement and the potential of thermal imaging technology as a means for non-invasive detection of pain. After that I will summarise my research hypotheses and aims for the thesis.

1.1. Pain

1.1.1. Definition of pain

The definition of pain by IASP was renewed in 2020. Pain in humans is defined as: "An unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage." Six key notes were also added, last of them being "Verbal description is only one of several behaviors to express pain; inability to communicate does not negate the possibility that a human or a nonhuman animal experiences pain." (IASP 2020; see Text boxes 1 & 2 below).

Text box 1. IASP definition of pain (1979).

Pain

An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage.

Note

Pain is always subjective. Each individual learns the application of the word through experiences related to injury in early life. Biologists recognize that those stimuli which cause pain are liable to damage tissue. Accordingly, pain is that experience which we associate with actual or potential tissue damage. It is unquestionably a sensation in a part or parts of the body but it is also always unpleasant and therefore also an emotional experience. Experiences which resemble pain, eg, pricking, but are not unpleasant, should not be called pain. Unpleasant abnormal experiences (dysaesthesiae) may also be pain but are not necessarily so because, subjectively, they may not have the usual sensory qualities of pain.

Many people report pain in the absence of tissue damage or any likely pathophysiological cause; usually this happens for psychological reasons. There is no way to distinguish their experience from that due to tissue damage if we take the subjective report. If they regard their experience as pain and if they report it in the same ways as pain caused by tissue damage, it should be accepted as pain. This definition avoids tying pain to the stimulus. Activity induced in the nociceptor and nociceptive pathways by a noxious stimulus is not pain, which is always a psychological state, even though we may well appreciate that pain most often has a proximate physical cause.

Text box 2. Revised IASP definition of pain (2020).

Pain

An unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage.

Notes

- Pain is always a personal experience that is influenced to varying degrees by biological, psychological, and social factors.
- Pain and nociception are different phenomena. Pain cannot be inferred solely from activity in sensory neurons.
- Through their life experiences, individuals learn the concept of pain.
- A person's report of an experience as pain should be respected.*
- Although pain usually serves an adaptive role, it may have adverse effects on function and social and psychological well-being.
- Verbal description is only one of several behaviors to express pain; inability to communicate does not negate the possibility that a human or a nonhuman animal experiences pain.

Etymology

Middle English, from Anglo-French peine (pain, suffering), from Latin poena (penalty, punishment), in turn from Greek poinē (payment, penalty, recompense).

*The Declaration of Montréal, a document developed during the First International Pain Summit on September 3, 2010, states that "Access to pain management is a fundamental human right."

Text boxes 1 & 2: Text boxes from Raja et al., 2020: The revised International Association for the Study of Pain definition of pain. Boxes show the previous and the current definitions of pain.

Animal pain often goes unnoticed for us. For many species pain-related behaviour might resemble their normal behaviour (Viñuela-Fernandéz et al. 2007). There is no universal definition of pain for animals (Viñuela-Fernández et al. 2007): yet, the current IASP definition of pain can be thought to include non-human animals, too. The nervous system and pain-relying pathways of other animals are very similar to humans in many species, and actions that are painful to humans have been recognized to be painful to other mammals too (Sneddon et al. 2014).

1.1.2 Nociception & physiology of pain

In this thesis, I assume that the following physiological patterns broadly apply to the taxa relevant to my study, mammals and birds, although both species-specific and individual-specific differences might arise (But see Sneddon et al. 2014).

The physical sense of pain is called **nociception**, from Latin *nocere*, "to harm or hurt". It is important to differentiate between nociception and pain – stimuli that can be detected by nociception are not necessary for pain to exist. Pain is not a sensory but a neurological phenomenon.

The sensors sensing nociception, nociceptors, are free nerve endings. They can detect injurious stimuli that could cause tissue damage (Lynn 1994). The fibers respond to noxious stimuli by generating action potentials which then travel through the afferent axons to the dorsal horn of the spinal cord (Viñuela-Fernandéz et al. 2007). A possibly harmful stimulus causes the activation of the nociceptive pathway (transduction): stimulus is transduced into a nerve impulse, which travels from the periphery, through the spinal cord to the brain (Kalso et al. 2018).

Pain causes the activation of the sympathetic nervous system (**SNS**). This affects the release of glucocorticoid hormones, such as corticol. Glucocorticoid hormones are released from the adrenal cortex, which is controlled by the hormone corticoliberin (**ACTH-RH**) that is released from hypothalamus, which controls the release of adenocorticotropin (**ACTH**) from the pituitary. This connection that is responsible for many stress-related reactions, is called the hypothalamic-pituitary-adrenal-axis, or the **HPA-axis** (Sjaastad et al. 2010). The hormones related to the function of sympathetic nervous system and neurotransmitters have been used as biological markers for pain. Measuring these markers is often invasive (Stubsjøen et al. 2009). Corticol levels, the amount of blood cells and other pain-related neurotransmitters and proteins, such as substance P, can be measured to detect the physiological changes caused by pain or inflammation. This has been studied for example in calves (Brown et al. 2015; Mirra et al. 2018).

1.1.3. Recognizing pain in animals

According to Weary & Niels (2006), research on pain assessment in animals has largely followed one of three approaches: measures of general body functioning (food and water intake, weight gain), measures of biomarkers (e.g., plasma cortisol concentrations) and measures of behaviour (vocalizations, grimace- or other position-related scales) (Weary & Niel 2006).

Biomarker-related measures have been considered useful by some in assessing pain especially in animals that are unlikely to show behavioural responses to pain (Viñuela-Fernández 2007). These measures have multiple limitations and issues. Most of them require animal restraint and/or tissue sampling. This might be either stressful or painful to the animal, which can affect the results (Weary & Niel 2006). The same issues are present in measuring body functions to some extent. Behavioural measures always bear the risk of being too subjective to be scientifically reliable.

By developing minimally invasive techniques to study pain, we increase our possibilities to both study and enhance animal welfare, which is inherently valuable. Thermal imaging is non-invasive and harm free to the animal. For this reason, its potential as a pain detection technique should be surveyed.

1.2. Thermal Imaging

Thermal imaging (infrared thermography; IRT) uses infrared radiation instead of normal light to create images. The surface temperature of the object defines the intensity of the radiation it emits. Technology behind thermal imaging uses this to detect and measure changes of surface temperature, through measuring emitted radiation. Depicting the surface temperature with thermal imaging has been used in medical (e.g., Ring 2006) and veterinary (e.g., Vainionpää et al. 2012 (1,2); Rekant et al. 2016; McCafferty 2007) contexts in different ways: e.g., in detecting inflammation based on observing visual cues from the images or extracting numerical data from the images. Potential of using the extraction of numeric data from thermographs for animal welfare have been explored in various contexts (see e.g., Stewart et al. 2005 for a review of thermal imaging used as a tool to assess animal welfare; Zhang 2020 for using it for livestock monitoring), and in this study, the latter method of extracting numerical data from the images is investigated.

1.2.1. Physics

All matter emits thermal radiation when its temperature differs from absolute zero. This is due to all objects consisting of matter in random motion, which contains kinetic heat. This motion leads to colliding particles, which change their energy states, resulting to a release in electromagnetic radiation (Tattersal 2016). Thermal radiation is therefore electromagnetic radiation, caused by the thermal motion of particles, and infrared thermography measures radiated electromagnetic energy. Electromagnetic radiation is a stream of photons, massless particles, moving at the speed of light in a wave-like pattern. Those of the photons that have the highest energy are correspondent to the shortest wavelengths (Stewart et al. 2005). The mid- to long wave infrared radiation wavelengths are not visible, but a thermal camera can detect these and convert them to temperature values (Tattersal 2016).

1.2.2. IRT & Animals

IRT can be used to measure surface temperatures from a distance, and thus it enables non-invasive studies even in the natural habitat of animals. A promising attribute of IRT is the possibility to study physiological processes related to emotional and pain states that are not visible through behaviour, such as when the animal is still. The differences within the temperature distribution of the head and face might indicate changes of brain- and SNS activation, and thus could potentially indicate something about the experiences of the animal. So far, the basic research with IRT in researching emotional states has been limited to study mainly domestic or captive animals, including the studies concerning the experience of negative states such as pain, fear, or stress (e.g., cow: Stewart et al. 2007 & 2008, primates: Dezecache et al. 2017; Kuraoka & Nakamura 2011, chicken: Edgar et al. 2011 & 2013). Thus, a study concerning pain in wild animals is a new opening in the field.

The main challenge in using IRT is to separate noise from signal, i.e., differentiate between temperature changes that relate to emotions and those that do not. The surface temperature of animals changes subtly depending on factors such as blood circulation, the activation of motor- and sensory nerves, muscle work, breathing and metabolic processes. Metabolism and the oestrous cycle can change the core temperature (McCafferty 2007). Metabolism affects the heat dissipation from the body, which might affect the surface temperature of the body as the heat leaves an animal's body: all metabolic processes produce heat as a by-product (Hill 2017). The activation of SNS gives rise to vasoconstriction in the peripheral circulation, often resulting in lowering of the surface temperature in that area, whereas the activation of the parasympathetic nervous system, **PNS**, gives rise to vasodilation, often resulting in a rise in surface temperature. It is known that chronic stress affects the capability of the PNS to regulate the SNS (Gormally and Romero 2020). As a study about wild blue tits pointed out, body surface temperature might be an integration of multiple different aspects of the physiological state (Jerem et al. 2018).

Animals have multiple ways to exchange heat with the environment: conduction, convection, evaporation, and thermal radiation. During a clear night sky, animals emit radiation to the sky and the sky emits a weak radiation back to the animal: thus, animals lose heat to a clear sky. Homeotherms, that species in our study mainly are, do not have an absolute constant deep-body

temperature; it varies 1-2 degrees during the day, and seasonal or underlying circumstances might affect these. Each animal also has a thermoneutral zone, in which the resting metabolic rate is constant. The thermoneutral zone varies across species and climate. When staying in the thermoneutral zone, the evaporative heat transfer is minor. (Hill 2017).

1.2.3. IRT & Emotions

Using thermal imaging to detect emotional states based on changes in the surface temperature of face, both in humans and other animals, is a new field in need of much basic research. With a field so novel, some elementary questions need consideration in planning each research, including which temperature distributions are reliable indicators of the phenomena being studied, what are the informative regions for the phenomena being studied and whether the timescale of the change also bears information of the underlying processes. Even though the temperature changes can be observed throughout the body, the face is especially informative due to both the processing of emotions and because of anatomy.

An important finding is that it might not be possible to differentiate between a negative and a positive state based on the activation of sympathetic nervous system: both kind of states might amount to emotional arousal, and it is not known whether these can be differentiated based on the activation of the nervous system or other means detectable by IRT (e.g., Moe et al 2012) – peripheral temperatures may indeed be similarly affected by both positive and negative emotional states (Proctor et al. 2015).

Chronic stress makes animals interpret ambiguous stimuli negatively, and wellbeing makes them interpret it positively (Mendl et al 2009). The short-term effects of changes in emotional states have been studied with multiple species relating to both negative emotions such as fear (cow: Stewart et al. 2008, sheep: Cannas et al. 2018), stress (bird: Jerem et al. 2015, rabbit: Lydwig et al. 2003, dog: Riemer et al. 2016, cow: Stewart et al. 2007, sheep: Cannas et al. 2018, cat: Foster et al. 2017) and pain (calf: Stewart et al. 2010, sheep: Stubsjøen et al. 2009). Negative emotions might be hard to distinguish from each other, and sometimes the study question has been posited relating to the reaction to negative stimuli instead of trying to distinguish the exact emotion in question (chimp: Dezecache et al. 2017, macaque: Kuraoka & Nakamura 2011, chicken; Edgar et al. 2011, & 2013, marmoset: Ermatinget et al. 2019). Often the head area chosen for analysis has

depended on the species, but often either eye, nose or ear is chosen, and in some studies e.g., comb surface temperature for chickens (e.g., Edgar et al 2013).

1.2.4. Error sources

There are two main sources of error when collecting and interpreting data from thermal images. First, there are errors related to the technique itself. Multiple factors might make the camera interpret the temperature inaccurately. These include the evaporative cooling of wet surfaces (e.g., if the animal is wet for some reason), the factors related to the atmosphere (e.g., wind), and wrong settings in the camera.

Second, there are errors related to the interpretation of thermal patterns. Even if the camera measures the temperature changes correctly, they might be related to different things than the ones they are interpreted as. For example, vasoconstriction that causes changes in the temperature might be interpreted as being related to the activation of SNS, when it might be due to thermal regulation due to change of weather. I will return to these error sources in the Methods -part to explain how these are considered in our study, and in the Discussion to discuss how these error sources might have affected our data & how they could be considered even better in further research.

2. Aims of the study & hypotheses

The main question that is addressed in this study is: can we use thermal imaging to detect whether animals are experiencing pain or not, based on differences in surface temperatures of the head area between pain and non-pain patients?

The working hypothesis of the study was that the experience of pain will change the surface temperature of certain skin areas of the head (depending on the species), and that this can be detected with a thermal camera.

Specifically, this study aims to measure 1. whether there is a change of surface temperature in the designated areas between before and after the examination and 2. whether pain-patients differ in their surface temperature to non-pain patients, and if they do, what is this difference like.

3. Materials and methods

The project was conducted in the Wildlife Hospital of Helsinki Zoo as a part of a larger project conducted at the Helsinki Zoo, where IRT techniques are developed for the detection of different emotional states across species. We included patients with acute pain states (e.g., bone fracture) detected by veterinarian, and patients with no detectable pain states (e.g., famished, or dehydrated patients) to be used as controls. The species included in the study were mammal or bird species. A table of the species in the study can be found in the next chapter (see Table 1).

3.1. Animals

The animals that were brought in included individuals across vastly different species. e.g., *Erinaceus europaeus* (European hedgehog), *Sciurus vulgaris* (Red squirrel), and different species of *Laridae spp*. (gulls). Due to especially hot weather during part of the data collection period, orphaned juvenile *Apus apus* (swifts) were the most common patient. There were in total 120 patients, 89 birds and 31 mammals. The patients were classified with the following criteria:

1. **Pain.** Bone fracture, swollen joint, open wound, inner bleeding, eye injury or swallowed fishhook. Also, other states that are diagnosed as "pain" by the nurse in the examination.
2. **No pain.** Those patients whose diagnosis was "all ok" or "orphaned" without a physiological problem. Patients that had fly eggs without a wound are in this class.
3. **Pain status unclear.** NOT hypothermic or dehydrated.
4. **Pain status unclear.** Either hypothermic or dehydrated.

Birds consisted of 20 pain, 30 non-pain, 32 unclear without dehydration or hypothermic, and 7 patients that were unclear with dehydration or hypothermic. Mammals consisted of 8 pain, 8 non-pain, 12 unclear without dehydration or hypothermic, 3 unclear with dehydration or hypothermic. Of all these patients, 85 were included in the final statistical analysis, as shown in the table below (Table 1). Both groups of unclear patients were grouped together in the final analysis. Patients were classified according to age in two groups, adults and juveniles, but this is not shown on the Table 1 below as this information is not directly relevant to the questions of this study; however, the age is discussed later in the thesis in contexts where it was relevant.

Birds 64	Mammals 21
Pain 20	Pain 7
No pain 16	No pain 2
Unclear 28	Unclear 12
Apus apus 29	Sciurus vulgaris 11
Laridae (different species) 11	Erinaceus europaeus 9
Columba palumbus 4	Oryctolagus cuniculus 1
Passer domesticus 3	
Turdus pilaris 3	
Erithacus rubecula 2	
Turdus merula 1	
Mergus merganser 2	
Dendrocopos leucotos 1	
Ficedula hypoleuca 1	
Parus major 1	
Accipiter gentilis 1	
Delichon urbicum 1	
Branta leucopsis 1	
Turdus Philomelos 1	

Table 1: A list of all patients in the study.

My initial idea was to form pairs of pain- and non-pain patients of the same species and run the statistical analysis as a paired comparison. However, this turned out to be impossible due to the substantial number of species admitted as patients, and the large variation in the quality of the videos. Thus, the plan was changed to analyzing animals as comparable groups, within each species group. Groups were made up of mammals and birds, that are further separated into pain, non-pain, and unclear patients. Mammals and birds are analyzed separately from each other.

The reason mammals and birds are separated as groups is the differentiating physiologies between these two classes and how these differences in turn may affect the thermal patterns being imaged with IRT. For example, birds have a thinner skull than mammals, and thus surface temperature differences can be detected through the skull, from the temples, whereas mammals have a thicker skull, in which case eyes are used as a source for data. For this reason, in the final analysis the data collected from the eye area of the mammals is from the eyes, whereas for birds the designated area is the area around the eyes.

The permit to carry out this study was obtained from the Office of the Regional Government of Southern Finland, in agreement with the ethical guidelines of the European convention.

3.2. Thermal imaging protocol

The recording was done with a FLIR E60 thermal camera (FLIR Systems AB, Danderyd, Sweden). The situation where animals were imaged was always the first examination that is conducted as soon as possible when the animal arrives at the hospital. Patients are delivered to the hospital in closed cardboard boxes (e.g., shoeboxes), typically either by rescue services or individual people who have found them. When the animals are being examined upon arrival, the nurse is holding the animal, and after the examination, the animal is placed back in the box. The whole examination situation lasts approximately 2-3 minutes. After the examination the nurse makes a diagnosis of the patient, sometimes after consulting a veterinarian. This diagnosis was written down in a script and based on this the patients were later sorted out in pain, non-pain, and uncertain patients, as mentioned above.

The hand-held infrared camera was connected via USB cable to a laptop. Before recording, the camera settings were adjusted via FLIR+ Tools on laptop according to the following measures: the distance from the object being imaged (0.5 meters), ambient temperature (°C) and relative humidity (%) inside the sampling area were recorded in the beginning of the protocol and used to calibrate the camera for the conditions of the atmosphere. Since the recording was conducted indoors, there was no need to take the speed of the wind into account.

Recording begun when the animal was still in the box and was continued when the nurse was holding the animal. The purpose was to get the following types of data from the patient: 1. from above the head when they are in the box, both before and after the examination and 2. during the examination symmetrical data of the face, from forward and from both sides of the face that are comparable (from the same angle). The latter was not used in this research.

3.3. Data extraction with FLIR tools

The extraction of data from raw IRT videos was done using FLIR Tools+. After collection, the videos were uploaded to FLIR Tools analytical software.

The criteria for including videos for data extraction were 1**.** focus: The beak/nose should have visible outlines, and the location of the eyes should be visible. 2. symmetry in the before and after images. Head should be as symmetrically posited as possible. The images from each side should be as directly from that side as possible.

Images of five different time points were saved for each patient. Usually, five images were extracted from each patient in the examination for two separate research questions. In the following cases two images for each time point were extracted: 1. if eyes were fully visible in one screen but the beak/nose were fully visible in another or 2. if there is no symmetrical enough screen, but two screens in which the other is slightly to the left and the other slightly to the right.

First, two images were extracted when the patient was in the box, before lifting and after lifting. The effects possibly visible in these images are interpreted to hypothetically be related to SNS function. These were the images used for final analysis.

Second, three images were extracted during the examination: one from each side, and one from up front of the face. These images were not used in the final analysis but will be used for the follow up research.

From the collected images, temperature values were extracted for the following parameters:

Eye temperature. For birds, the maximum temperature of the eye area. For mammals, the maximum temperature of the eye.

Snout or beak temperature. The average for both mammals and birds.

Crown of the head. The average for both mammals and birds.

A specified protocol to define the exact points where the temperature values were collected was produced and used for each designated area. These protocols were different for birds and mammals, considering differences in facial morphology. Below is an image (Image 1) giving an example of this.

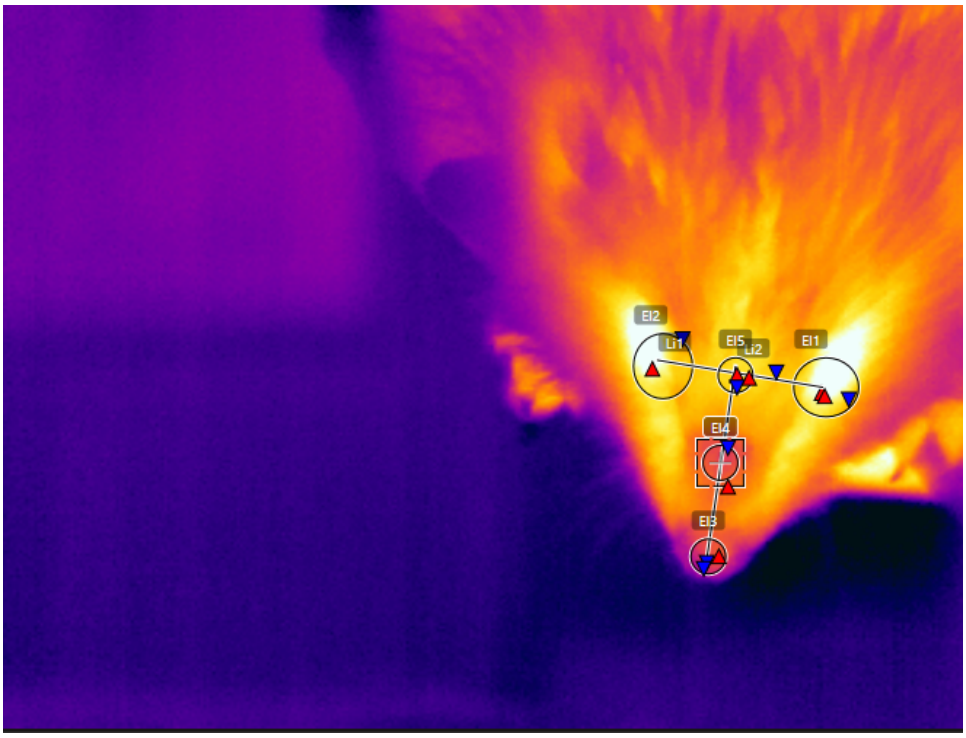

Image 1: Image of a hedgehog showing the points where temperature was measured from a picture taken directly from the front. This image shows all the temperature points in the study: snout temperature, center of the head (the point between the eyes), and the eye temperature of both eyes.

3.4. Statistical analysis

The data were analyzed using general linear models (GLM) as implemented in the software R (R Core Team 2021) using package lme4 (Bates et al. 2015). Based on the quantitative and qualitative differences in both the data and participating individuals and species, the birds and mammals were analyzed as separate categories and were not compared with each other. Only the data collected from images before and after the examination were analyzed for this research. Before and after data was hypothesized to possibly show effects related to the function of the ANS.

To answer the question whether there were differences between pain and non-pain patients I constructed the following models.

Model 1.1: This model was constructed for birds. The response variable was the mean temperature as measured from the crown of the head. Predictive variables were time (before or after examination) and pain status (pain, no pain, unclear) and age. Individual identity as well as species were fitted as a random variable to account for correlated observations.

Model 1.2: This model was constructed for mammals. The response variable was the mean temperature as measured from the crown of the head. Predictive variables were the species, time and pain status, and age as above. Individual identity was fitted as a random variable to account for correlated observations.

Model 2.1: This model was constructed for birds. The response variable was the mean temperature as measured from the axis of the beak. Unlike for the variables used in other models, the beak temperature was measured with a line instead of a circle, due to the whole beak instead of only the tip of it being relevant area to our study. Predictive variables were time, pain status, and age. Individual and species identity were fitted as a random variable to account for correlated observations.

Model 2.2: This model was constructed for mammals. The response variable was the mean temperature as measured from the tip of the snout (differently from the beak temperature in Model 2.1). Predictive variables were time, pain status, age, and species. Individual identity was fitted as a random variable to account for correlated observations.

Model 3.1: This model was constructed for birds. The response variable was the maximum temperature as measured from the eye area. Predictive variables were time, pain status and age. Individual and species identity were fitted as a random variable to account for correlated observations.

Model 3.2: This model was constructed for mammals. The response variable was the maximum temperature as measured from the eye. Predictive variables were time, pain status, age, and species. Individual identity was fitted as a random variable to account for correlated observations.

For the models constructed for mammals, species was a predictive variable, as individuals from only three species were included and fitting species identity as a random term would have caused problems in the model. For birds, species was fitted as a random variable, due to the larger number (N=15) of bird species admitted as patients. To test whether the effect of pain status depended on time, I initially fitted an interaction between time and pain status in all models; where nonsignificant, this was removed to allow for significance testing of the main terms.

The significance of the terms was determined using likelihood ratio tests (LRT) which compare the model to that where the term in question has been removed. I used $p = 0.05$ as the significance threshold.

4. Results

4.1. Crown of the head

4.1.1. Crown temperature of birds

For this, I used Model 1.1. When looking at the mean temperature of the crown of the head for birds, I found that there was a statistically significant interaction between pain and time (x^2 = 7.5672, p = 0.02274; Table 2).

Head, birds, time * pain				
fixed effects	β ± s.e.	x^2	p-value	
(intercept)	30.327 ± 1.403			
age (young)	0.612 ± 1.294			
time (after)	-0.586 ± 0.121			
pain status (pain)	-0.285 ± 1.598			
pain status (NA)	-0.685 ± 1.301			
time (after) : pain status (pain)	0.422 ± 0.198			
time (after) : pain status (NA)	-0.12 ± 0.176			
age		0.139	0.709	non-sig
time : pain status		7.567	0.0227	significant
Number of obs: 142, groups: ID, 46; species, 12				

Table 2: Results from the GLM investigating head temperature of birds as measured from the crown of the head. There is a significant interaction between time (before or after examination) and pain status.

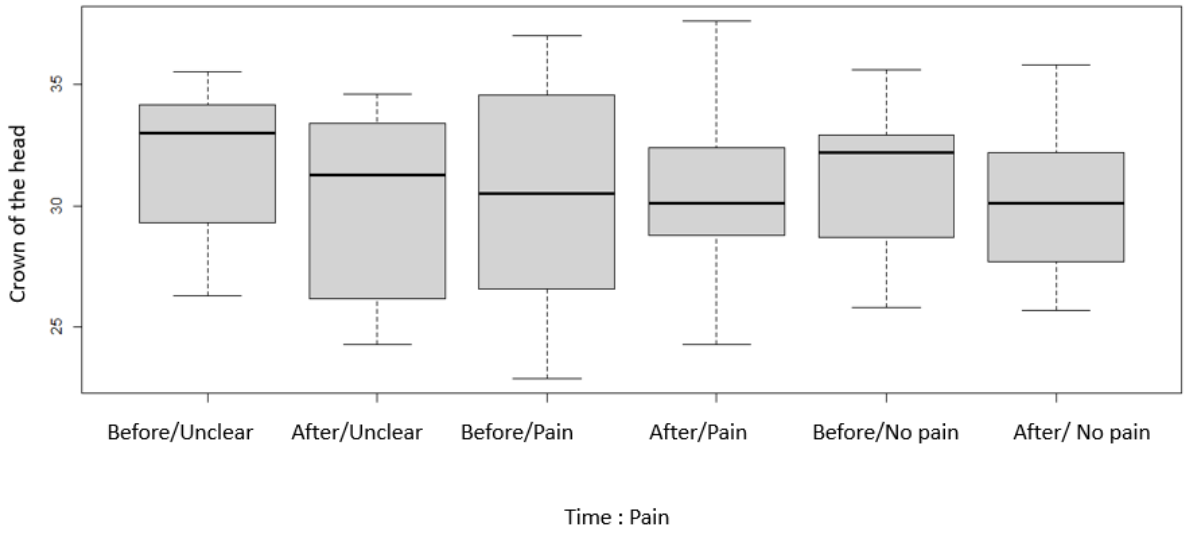

Figure 1. A boxplot of the raw data of the crown temperatures of birds with the interaction of time and pain. The Y axis is the temperature in Celsius degrees.

To find out which pairwise differences between pain status and time points were significant, the different levels were compared using a post-hoc test, and I found out that for both unclear (β =-0.550 ± 0.110, $p < 0.001$) and non-pain (β = -0.705 ± 0.121, $p < 0.001$) patients there was a significant difference between the temperatures of head before and after the examination; for pain patients, the difference was not significant (β = -0.206 ± 0.121, p =1).

These differences look similar on the Figure 1 above, which is a plot drawn from the raw data and thus differs from how data is analyzed through my model. For the patients with pain, the median temperature of all patients before and after changed the least as compared to the groups of other patients. For both unclear and non-pain patients, the median temperature of all patients after the examination was lower than it was before the examination. This is not directly comparable to the results of the model but demonstrates the same phenomenon as the results from the model.

4.1.2. Crown temperature of mammals

For this, I used Model 1.2. Looking at the mean temperatures of crown of the head temperature of mammals, the interaction of pain and time was nonsignificant, and I removed this from the final model, to be able to test the significance of the main terms. As visible from the Table 3 below, time (4.7, p = 0.03) and age (x^2 = 4.194, p = 0.06) were significant factors, and pain status (x^2= 4.779, p = 0.0916) was near significant. Species was nonsignificant.

Head, mammals, no interactions				
fixed effects	β ± s.e.	x^2	p-value	
(intercept)	34.4 ± 2.014			
age (young)	-3.727 ± 2.584			
time (after)	-0.273 ± 0.359			
pain status (pain)	-2.219 ± 1.543			
pain status (NA)	0.463 ± 2.294			
species (squirrel)	3.163 ± 3.316			
species (hedgehog)	0.246 ± 2.585			
pain		4.779	0.0916	near sig
time		4.7	0.0301	significant
age		4.194	0.0405	significant
species		3.26	0.195	non-sig
Number of obs: 28, groups: ID, 10				

Table 3: Results from the GLM investigating the head temperature of mammals without interaction between time and pain status. Note that species here is a fixed parameter. The intercept species in question is European rabbit.

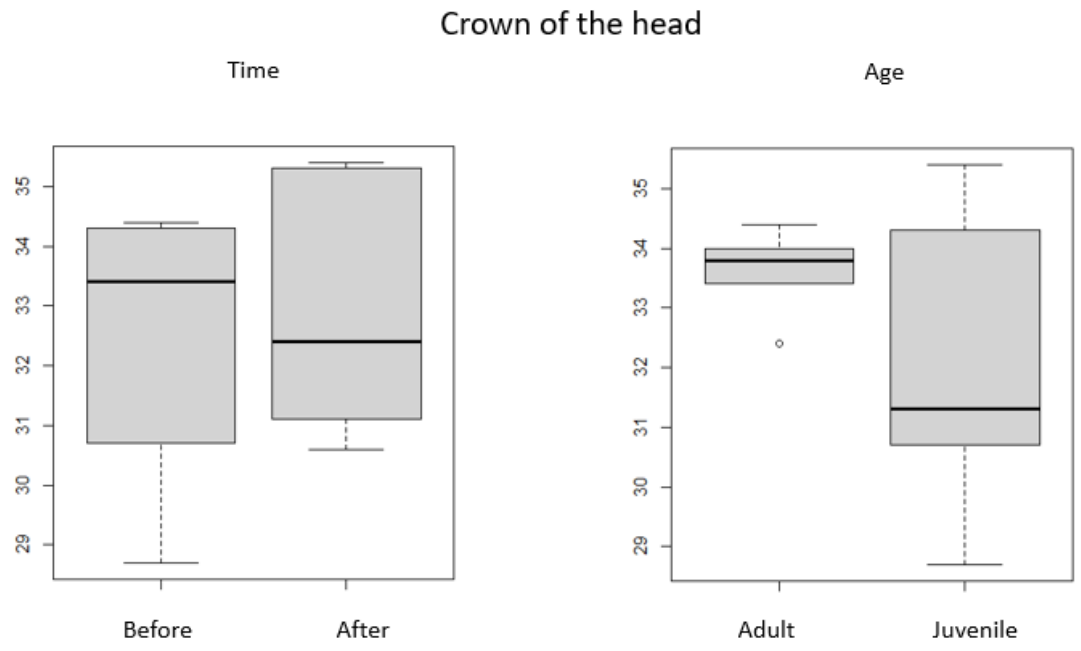

Figure 2. Box plots drawn from the raw data of mammals, from the crown of the head temperature. Left is a plot of time, and right is a plot of age.

Raw data box plots in Figure 2 give interesting further insights. On the plot of the temperature of all patients before and after the examination, the median of the crown temperature was higher prior to examination, whereas after examination it had lowered. On the plot that shows the difference between the two age groups, adult and juvenile, it shows that adults have less variation and a *higher* median, whereas juveniles have a lot of variation and lower median.

4.2. Snout & beak

4.2.1. Beak temperature of birds

For this, I used model 2.1. For birds, I analyzed beak temperatures as above; the interaction of time and pain was significant (x^2= 18.723, p <0.001; Table 4).

Beak, birds, time * pain fixed effects	β ± s.e.	x^2	p-value	
(intercept)	28.046 ± 1.815			
Pain status (pain)	1.392 ± 1.996			
Pain status (NA)	-0.380 ± 1.579			
Time (after)	-0.040 ± 0.232			
Age (young)	-1.517 ± 1.526			
Pain status (pain) : time (after)	-1.58 ± 0.380			
Pain status (NA) : time (after)	-1.109 ± 0.337			
age		1.084	0.298	non-sig
time : pain status		18.723	< 0.001	significant
Number of obs: 142, groups: ID, 47; species, 12				

Table 4: Results from the GLM investigating the beak temperature of birds. There is a significant interaction between time (before and after) and pain status.

To find out which pairwise differences were significant, I used a post-hoc test as above. The difference between the temperature after and before examination was significant for both pain patients (β = -1.618 ± 0.299, p <0.001) and non-pain patients (β = -1.149 ± 0.244, p <0.001).

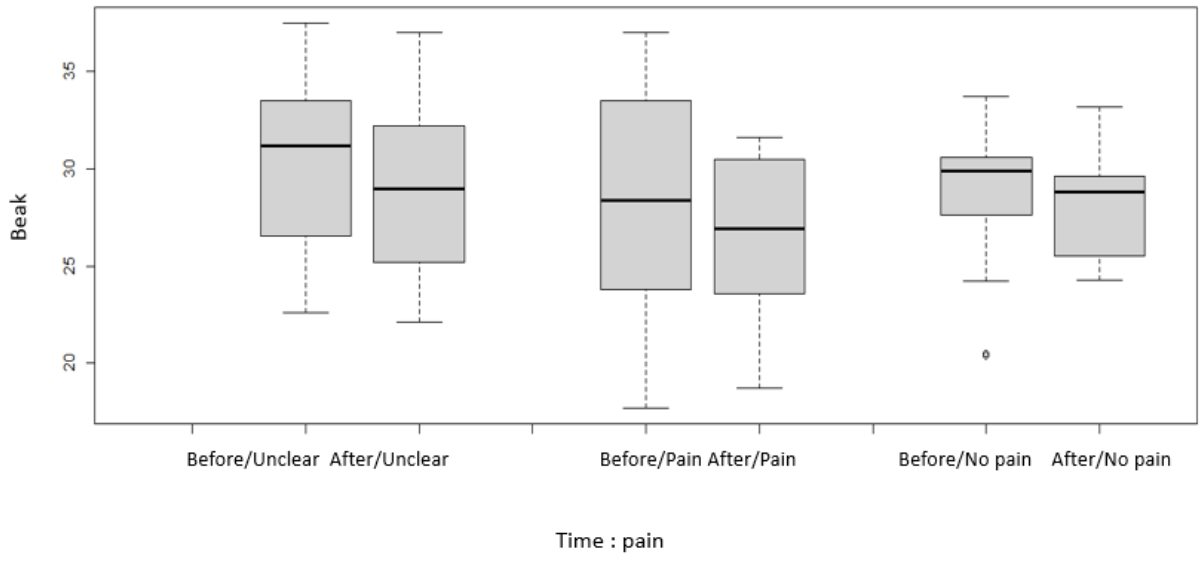

Figure 3. A boxplot of the raw data of the beak temperatures of birds with the interaction of time and pain. The Y axis is the temperature in Celsius degrees.

Based on the Figure 3 above it would seem like there is also a difference between the temperatures of unclear patients. This is due to the plot being drawn directly from the raw data which only gives a median value of all temperature values. The model considers several factors simultaneously, as well as corrects for correlated values within species and individuals; Thus, this visually clear difference is nevertheless statistically nonsignificant, due to the model analyzing the data in a more complex way than what can be seen in a raw data boxplot.

4.2.2. Snout temperature of mammals

For this, I used Model 2.2. Including the interaction between time and pain, all factors were significant (see Table 5 below), the interaction between time and pain status explaining most of the variation in the data (x^2=33.209, p<0.001; see Table 5). I used the Tukey post-hoc test and found that the significant differences in the after and before temperatures were for pain patients (β= -0.531± 0.171, p = 0.165) and unclear patients (β= 2.595± 0.296, p <0.001). Note that the baseline value on these calculations was the "after"-temperature, meaning that the after temperature for unclear-patients was higher than the before temperature, so there was a rise in temperature. Whereas, for the pain patients, the after temperature was lower than the before-temperature, so there was a decrease in temperature.

Snout, mammals, time * pain						
fixed effects	β ± s.e.			x^2	p-value	
(intercept)	36.3 ±3.154					
Pain status (pain)	0.912 ± 2.431					
Pain status (NA)	2.569 ± 3.598					
Time (after)	2.601 ± 0.296					
species (squirrel)	-0.055 ± 5.192					
species (hedgehog)	-5.898 ± 4.051					
Age (young)	-8.014 ± 4.047					
Pain status (pain) : time (after)	-3.135 ± 0.342					
age				6.837	0.008	significan
species				7.039	0.029	significan
time : pain status				33.208	<0.001	significan
Number of obs: 28, groups: ID, 10						

Table 5: Results from the GLM investigating the snout temperature of mammals with the interaction of time and pain. There is a significant interaction between time (before and after) and pain status; also, age and species are statistically significant factors.

As can be seen on the Figure 4 below, drawn from raw data, the reason for why there were no significant results for patients with no pain was a lack of data: there were only two mammal patients with no pain, and the after-examination snout temperature was not collected from either of them. Both patients were juvenile red squirrels, and their before-examination snout temperatures were the same: 30,8 Celsius degrees both. Due to the lack of these values, no conclusions can be drawn as to potential differences between pain and non-pain patients, and therefore I will not include these results in the discussion.

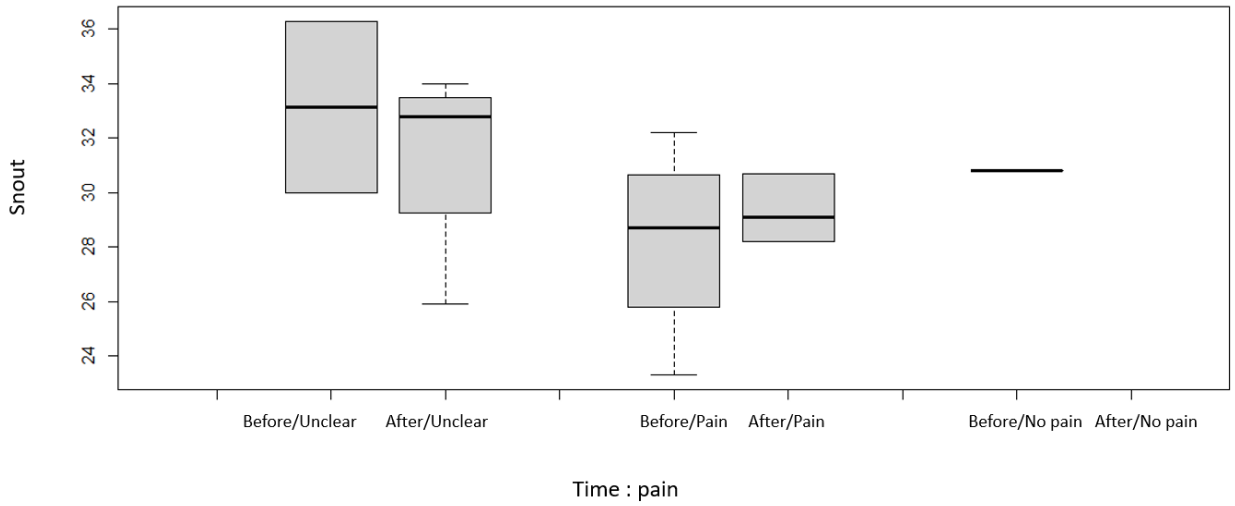

Figure 4. A boxplot of the raw data of the snout temperatures of mammals with the interaction of time and pain. The Y axis is the temperature in Celsius degrees.

4.3. Eye & eye area

The last analyzed temperature was the eye temperature. For birds, the collected temperature was maximum temperature of the eye area. For mammals, the collected temperatures were the maximum temperatures of eyes. These differences of points were decided based on anatomical differences between birds and mammals, e.g., birds having a thinner skull due to which the temperature changes are noticeable from a larger area near the eye – whereas for mammals, the skull is thicker and thus the subtle changes of peripheral temperatures are much more visible from the eye, more specifically the corner of the eye (as noted for example by Stewart et al. 2008).

4.3.1. Eye area temperature of birds

For this, I used Model 3.1. For birds, when tested with the interaction between time and pain, none of the tested terms were significant predictors of the eye area temperature. After removing the nonsignificant interaction, time was found to be a significant factor ($x^2 = 9.937$, $p < 0.001$; see Table 6).

Eye, birds, no interactions			
fixed effects	β ± s.e.	x^2	p-value	
(intercept)	32.631 ± 1.253			
time (after)	-0.614 ± 0.193			
pain status (pain)	0.387 ± 1.338			
pain status (NA)	-0.189 ± 1.091			
age (young)	0.311 ± 1.093			
pain		0.043	0.978	non-sig
time		9.937	0.001	significant
age		0.042	0.835	non-sig
Number of obs: 140, groups: ID, 47; species, 12				

Table 6: Results from the GLM investigating eye temperatures of birds, no interactions.

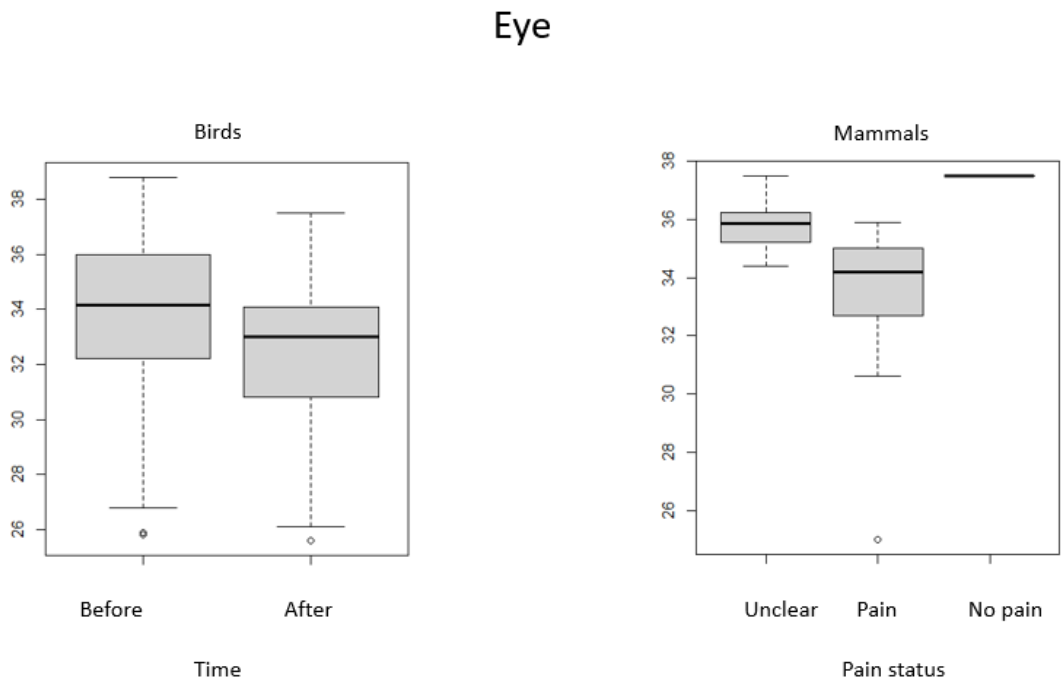

Figure 5. A raw data plot of eye temperatures for both birds and mammals. Left shows values before and after examination for birds as time was statistically significant predictor, right is a plot of pain status for mammals, as this was statistically significant predictor of eye temperature in mammals.

Looking at the left side panel of Figure 5, a raw-data plot of time parameter for bird eye temperatures, it seems that the median of the temperature values of all patients was higher prior to examination and lower after the examination.

4.3.2. Eye temperature of mammals

For this, I used Model 3.2. For mammals too the time-pain interaction was nonsignificant, and the model was changed to not include this interaction. Pain was found to be a significant predictor ($x^2 = 7.248$, $p = 0.026$), age was near significant ($x^2 = 3.504$, $p = 0.061$) time and species were both nonsignificant as seen on Table 7 below.

Eye, mammals, no interactions					
fixed effects	β ± s.e.		x^2	p-value	
(intercept)	36.2 ± 1.885				
time (after)	-0.931 ± 0.924				
pain status (pain)	-2.477 ± 1.346				
pain status (NA)	1.006 ± 2.300				
age (young)	-3.264 ± 2.326				
species (squirrel)	3.558 ± 3.228				
species (hedgehog)	0.641 ± 2.388				
time			0.746	0.387	non-sig
pain			7.248	0.026	significant
age			3.504	0.061	near sig
species			2.926	0.231	non-sig
Number of obs: 28, groups: ID, 10					

Table 7. Eye temperatures of mammals, no interactions.

Looking at the Figure 5 above, the eye temperature median for all patients seemed to be the lowest for pain patients. Similar results can be seen on Table 7, suggesting that pain patients had the lowest temperature value, as compared to other patient groups.

5. Discussion

In this thesis I investigated thermal patterns in patients of the Korkeasaari Wildlife Hospital with pain, without pain, and those whose pain status was unclear. The main question at the heart of my thesis was whether we could use thermal imaging to detect if animals are experiencing pain or not, based on differences in surface temperatures of the head area between pain and non-pain patients. My hypothesis was that the experience of pain would change the surface temperature of certain skin areas of the head, and that this could be detected with a thermal camera. My purpose was to investigate whether there is a change of surface temperature in the designated areas between before and after the examination and whether pain-patients differ in their surface temperature of non-pain patients, and if they do, what is this difference like. My results did not unequivocally support this hypothesis, but some potentially promising indicators for pain were found, which I will next discuss. However, one interesting discovery was that across all patient groups, a drop in the peripheral temperatures was common. It is likely that this is due to a change in the activation of the autonomic nervous system, but the exact mechanism is unknown.

5.1. Birds

There was a statistically significant interaction between pain and time for the crown of the head temperature of birds, meaning that head temperature changed with examination differently in patients depending on their pain status. I found that patients with an unclear- or no-pain-pain status had a statistically significant change in their before- and after-examination temperatures. For both classes of patients, the temperature dropped. This could be due to a different baseline activation of the sympathetic nervous system between the unclear and no-pain patients versus pain patients: it is possible that the temperature had already dropped prior to the examination for pain patients – possibly due to pain – resulting to no further drop during the examination. The crown temperature might be a more reliable indicator of SNS activation for birds than it is for mammals, due to the skull of birds being thinner and thus possibly being able to better show the intricate changes of peripheral temperatures.

For beak temperatures too, the interaction between time and pain was significant. I found that both pain- and non-pain patients had statistically significant changes in their beak temperatures,

whereas unclear patients did not. Both pain- and non-pain patients had a drop in the beak temperature after the examination as compared to the temperature prior the examination.

For eye temperature of birds, there was no interaction between time and pain, but time per se was found to be a significant factor. According to the statistical analysis, temperature was higher prior to the examination and lower after it. This observation however cannot be used to answer the study question about temperature differences between pain- and non-pain patients, but could be interesting for further studies, for example when looking at effects of handling.

Two studies were concluded by Edgar et al. (2011 & 2013) that measured the head area surface temperatures of hens in relation to stressful situations. The first study (Edgar et al. 2011) found a drop in eye temperature following the stressful situation, which is similar to the drop in eye temperature that I found with examination. The second study (Edgar et al. 2013) found that there was a significant drop in the comb temperature and an initial drop in eye temperature followed by a rise over baseline following the stressful situation. The rise was recorded 20 minutes after the handling, and thus cannot be compared with my results that are only based on the immediate moment following the procedure. The changes over a longer period, e.g., 20 minutes or longer, is a relevant topic for further studies.

In the study by Edgar et al. (2013) the main reason for the initial drop in temperature was thought to be stress-induced hyperthermia. It is a phenomenon characterized by an increase in core body temperature and a decrease in the surface temperature. Most likely, this phenomenon is due to vasoconstriction. During vasoconstriction, the blood is redistributed to e.g., muscles and brain, which might result to a decrease in surface temperature. The phenomenon is thought to be caused by emotional stress. (Edgar et al. 2013). This might also be one of the reasons for my similar results, too; however, in my study the core temperature was not recorded, and this information would be necessary to define the phenomena as stress-induced hyperthermia.

The studies by Edgar et al. are drastically different from my main question in that they are not comparing animals in pain with animals without pain. However, I think both these studies and my research show that stressful situations, such as human handling of an animal, affect the surface temperatures of facial areas in birds, and that the imminent change (over the first 1-5 minutes) seems to be a drop in the temperature. Similarity of results between my research and the research

by Edgar et al. also seems to indicate that the temperature changes seem to be similar, no matter the species or if the bird in question is caged or wild.

My most relevant finding was that birds with pain had no significant change of the crown temperature before and after examination, whereas birds with no pain or with an unclear pain status had a drop in this temperature. This might indicate that the baseline sympathetic activation of pain patients was higher, due to which the changes caused by the stressful examination situation are not as visible as they are for other patients. However, this effect was only visible with the crown temperature, but not with beak- or eye temperature.

What is clear from all these results is that in each of these measures – crown, beak, and eye – there was usually some sort of drop in temperature after the examination for some patients. These results did not show unambiguously that these changes would be different between pain- and non-pain patients, but there is a slight indication that the temperature changes, at least as measured from the crown of the head, could be lesser in pain patients as compared to others. This does support my hypothesis that the temperature changes would somehow differ between pain- and other patients, but needs much further investigation before any statements of its relevance can be made.

5.2. Mammals

For mammals, there was no statistically significant interaction between pain and time for the crown temperature, meaning that patterns of temperature change during examination did not differ between pain- and non-pain patients in this measure. After removing this interaction from the model, I found that time and age were significant predictors of crown temperature. I found that the median crown temperature of all patients was higher prior to examination as compared to after. This might indicate that for mammals too, as seems to be the case with birds, crown temperature drops following the stressful examination situation. I also found that the median crown temperature of adults was higher than that of juveniles. Juveniles also had more variation of temperature than adults. The mammal sample of patients included 5 adults, and 8 juveniles; there were both juveniles and adults of hedgehogs and squirrels, and the one rabbit included in the study was an adult. With this variation inside the sample and a relatively small sample size, it is not possible to unequivocally say whether these differences could be indeed due to age, and not for example due to the differences between either species or the individuals, or both.

It is possible to hypothesize that the differences between age groups in mammals could also be due to animals experiencing the situation differently during different phases of age. The study showed that juvenile mammals had more variation across their crown temperature values compared to adults. It is possible that a juvenile animal experiences the world differently to some extent than an adult, like how a child experiences the world differently to an adult in humans, too. This is at least partly due to the different developmental status of the nervous system. I cannot go further in this hypothesis without further studies, but the observed differences between adults and juvenile animals and the connection of this to the development of nervous system should be explored in the further studies.

As I mentioned above, in the context of mammals I will not discuss further the snout temperatures, as I found out that the data was insufficient for reliable analysis.

Regarding the eye temperature, the time-pain interaction was nonsignificant and pain per se was a significant parameter: the eye temperature was lowest for pain patients, as can be seen in the results (Table 6). This is not directly relevant for our study question, but still an interesting suggestion, as it might be related to the higher baseline sympathetic activation of pain patients versus other patients, irrespective of examination.

For example, in a study by Stubsjøen et al. (2009) of using thermal imaging to assess pain in sheep, it was found that *both* in painful procedures and in stressful-but-not-painful sham-procedures the eye temperature decreased. However, a study by Stewart et al. (2010) investigated how castration pain affects the maximum eye temperature of calves. Study consisted of controls that were not castrated but went through a stress- but not pain-inducing sham procedure, calves that were castrated without anesthesia, and controls that were given anesthesia and went through the sham procedure and lastly calves that were castrated with local anesthetic. For both groups that went through castration, the eye temperature had risen almost a degree five minutes after the procedure. (Stewart et al. 2010). The differing results of these prior studies suggest that there is still much to do in the field of using thermal imaging to assess pain in mammals, and I think if something this is also what my results suggest, too.

5.3. Distinguishing stress from pain

One of the questions I brought up in the introduction was that it is both difficult to differentiate between positive and negative states based on the activation of sympathetic nervous system and the peripheral temperatures related to it and detected by the thermal camera, and between different negative states, e.g., stress or fear versus pain. In this research, all the patients were wild animals. For this reason, the mere presence of humans, especially with the addition of being in a new place and in a completely new situation (being examined by a human in the hospital), is most likely very stressful to the animal, no matter which animal is in question, and no matter what their pain status is. Thus, it can be assumed that all or most of the patients in the study were already under a tremendous stress. This situation can be expected to be the same for both pain- and non-pain patients. Due to this, if there is a stress effect on the peripheral temperatures measured, it should be similar across all patients. This reinforces the thought that the temperature differences between pain- and non-pain patients could be indeed caused by pain. As pain is inherently related to stress in its effects on the body (e.g. they both can activate the same hormone-releasing pathways, as explained in the introduction), it is also possible that the pain the patients experience might make them interpret the examination situation more negatively than the patients with no pain interpret it: this is an argument that aligns with the suggestion of Mendl et al. that chronic stress makes animals interpret ambiguous stimuli negatively, whereas wellbeing makes them interpret it more positively (Mendl et al 2009).

How could stress be distinguished from pain by the means of thermal imaging, for example? We are still far from having a univocal answer to this question. Some of my results suggests that the baseline peripheral temperature of animals in pain could be different from that of those not in pain, and this could be due to differences in baseline sympathetic activity. Investigating further the relation of these baseline peripheral temperatures to pain status is one future direction to take. However, how this should be done and by what parameters needs further investigation. It cannot be suggested that there would be a pattern that could be recognizable across all animals experiencing pain, and it is important to not make suggestions too hastily in the first place, as this could result in making suggestions that do the contrary of helping the patients. If we suggest that some type of activation (e.g., a certain type of temperature value or pattern) is always present along with pain, we simultaneously suggest that pain would not be present without this type of activation.

This thought itself might conflict with the definition of pain as suggested by IASP (Raja et al. 2020; see Text boxes 1 & 2 in the introduction), including the additional note: "Pain and nociception are different phenomena. Pain cannot be inferred solely from activity in sensory neurons" (Raja et al. 2020). Thus, we cannot ever be certain of presence or absence of pain based solely on measures done from the outside. What we can possibly detect by thermal imaging is some sort of difference in the activation of the autonomic nervous system, and we might be able to detect whether this activation is of sympathetic or parasympathetic nature – all further suggestions are too early and might pose the risk of a slippery slope argument towards biological essentialism (e.g., the thought that pain would always come with biological markers of some sort).

My proposal for further studies would be to investigate with larger sample sizes, and perhaps with more homogenous groups of animals (e.g., one species at a time and then see how the results compare with the other species) that were not possible to collect with my limited research time. The relationship between the differences in baseline temperatures and the lack of change in temperatures with examination (as suggested by my results with the crown temperature of birds) should also be investigated further. However, I also suggest that the hypothesis that thermal imaging could be used to detect pain is taken with much caution.

5.4. Final conclusions and future suggestions

In this study, I found that rather than showing clear patterns between pain patients as compared to other patients, results varied across measurement points and taxa. One clear pattern that was repeated across my results was that there was a decrease of temperature after the examination as compared to before the examination, both in birds and mammals, and in patients regardless of pain status. This is a result that is supported by prior research, too (see e.g., Edgar et al 2011 & 2013, Stubsjøen et al 2009) and consistent with the idea that handling stress causes a reduction in peripheral temperature. To investigate whether this phenomenon is stress-induced hypothermia, the core temperature of animals would need to be measured – but this was not done in my study.

As only before and after temperatures from within relatively close events were compared to each other, this reduces the significance of many possible error sources: even if there were error sources present, mostly they can be thought to only add random noise, as all recording was done with the same camera settings, and only values that were collected in the same situation but from different

time points were compared. In the future studies where data does not consist only of before and after temperature values, the error sources must be considered more cautiously.

To develop thermal imaging as a technique to detect pain in animals – wild or not – a lot of baseline work still needs to be done: not only with thermal imaging, but also with the questions we choose to ask. The hypothetical difference between the sympathetic activation as reflected by the peripheral temperatures of facial areas, between those animals who are in pain and who are not, was not univocally indicated by my results. I think the questions that should be asked in the future thermal imaging research should be about: what kind of peripheral temperature differences can be detected in the first place, and what physiological processes we can assume to be behind these differences. This should be done without qualitative assumptions, e.g., assuming the activation of the sympathetic nervous system to be associated with either negative or positive feelings. As suggested by Proctor et al. (2015), peripheral temperatures could be similarly affected by positive and negative emotional states. Pain is not simply a physiological process and cannot be reduced to one: this statement alone shows that pain cannot be detected unquestionably with means that are based on measuring the physiological phenomena only.

5.5. Contextualizing my study in the field of pain research and animal welfare

One thing this study has shown is that recognizing pain by scientific tools is difficult. Yet trying to find means to do so is of immense importance. As we gain more tools to make visible the things that often go ignored, such as pain, agony and suffering that animals go through in different contexts, the more we gain tools to show that these things are real, and that these things matter.

Recognizing pain only takes us part way – we also need to find ways of reducing or preventing its occurrence. One obvious approach to avoiding pain is to eliminate the procedures that cause it, but the practicality of this option will depend upon the purpose of the procedure and the availability of feasible alternatives. We can also prevent injury and disease that cause pain or minimize the effects by finding better methods for early diagnosis and treatment (Viñuela-Fernandéz 2005).

The ability of animals to feel pain is still a matter that doesn't go unquestioned, the question of whether animals are capable of feelings is a conversation that has been going on perhaps as long as human life has (Proctor 2012). The capability of animals to feel pain and suffering has been recognized by many notable thinkers and scientists throughout the history, but questioned by at

least as many - e.g., Descartes, who claimed that animals were automata like machines (Proctor 2012). Yet many – such as some ancient thinkers like Hippocrates and Pythagoras, and later Darwin – were known advocates for making animal suffering recognized & minimized.

The problem remains that we simply cannot know, unquestionably, what the other is feeling. This goes both for humans and other animals, and it becomes increasingly difficult if the other is unable to communicate their feelings. According to Helen Proctor (2012), the subjective manner of both perceiving and experiencing emotion has resulted to the sentience of animals being often described as an anthropomorphic assumption, which results to the undervaluing of its credibility as a science (Proctor 2012). Even if we can use neuroscientific tools to match certain brain areas with certain feelings, we are unable to make a straightforward causal links for example because feelings can be felt without expressing them, and, on the other hand, the categories of feelings overlap.

The assumption of being able to measure feelings based on cortical activity can be questioned, too. Antonio & Hanna Damasio (2016) have talked about how even in humans it is evident that "subcortical structures and even the peripheral and enteric nervous system appear to make important contributions to the experience of feelings." (Damasio & Damasio 2016, 3). This suggestion implies that feeling emotions is itself a complex, embodied matter, and measuring cortical activity or any other physiological activity as a tool to recognize affective states is limited and comes with some issues.

From this argument by Damasio & Damasio, I Imply two points. First, we should be wary of making assumptions about feelings or emotions based on any scientific method that uses physiological markers, especially when it comes to animals. This does not mean that developing techniques based on such markers would not be important or necessary to improve the state of animal welfare, but it is important to state that in trials to develop such techniques we should remain cautious and aware of the issues we might face. Second, for this reason it becomes extremely important to develop tools to detect pain that not only consider the brain, but the body as a whole, and as a complex ensemble in which everything is connected.

This thesis consists of a study that only includes wild animals as subjects. Most of the animal welfare studies consist of considering the welfare of farm or companion animals (e.g., Veit et al. 2021). Browning and Veit (2021) argue that often the distinction between captivity and freedom

is overstated in the context of animal welfare, and there is no intrinsic difference between wild and captive animals (Browning and Veit 2021). The data available to us mostly consists of captive animals. Thus, studies such as this where data is gathered of wild animals without producing them additional stress or harm, are important: in this way, we can learn about those animals in the world around us with whom we usually do not have direct interactions. It's of utmost importance that we keep things this way – avoiding direct interactions with wild animals as much as possible – but situations where we are given the opportunity to help wild animals directly, such as is done at the Wildlife Hospital, we also have the opportunity to learn about the new ways we could be able to help them in the future.

Increasing knowledge about pain and its effects on the body gives us better premises to both understand pain as a phenomenon and treating it, especially in animals (Viñuela-Fernández et al. 2007). Based on the current knowledge about physiology and emotion, it can be stated that there is a link between how we feel and what happens in our bodies – and this same statement can and should be extended to animals, too. Thermal imaging is one of the tools we can continue to explore and develop to assess this link, and to investigate its meaning.

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