Classification of Social Interaction in a Porcine Neuropathic Model using Machine Learning

Master's Thesis

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STUDENT REPORT

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Abstract:

Background and aim: Translation of preclinical findings from animal models to treatment of neuropathic pain in humans relies on objective and clinically relevant outcome measures. Although the primary symptom of neuropathic pain is spontaneous pain, the most commonly used outcome measures in porcine models are stimulus-evoked measures that assess hypersensitivity. Detection of pain related behaviours such as aggression could possibly enable quantification of spontaneous pain. Thus, this project aims to develop an objective and clinically relevant outcome measure of spontaneous pain using computer vision and machine learning.
Methods and materials: Pose estimation and tracking methods were utilised for obtaining data which described the movement of three pairs of pigs in a pen. Features related to the pigs' acceleration and distances between the pigs were extracted. Lastly, behavioural classification using K-Means clustering was conducted, followed by post-clustering analysis.
Results: A pose estimation model with an estimated success rate of 70.84% correct node placements was implemented. Three clusters were identified using K-Means clustering and interpreted as representing social interaction, defecation, and the transition state between behaviours.
Conclusion: Pose estimation and tracking was successfully applied. No behavioural differences between pigs with and without neuropathic pain were identified. However, the project is the first step towards an objective and clinically relevant outcome measure for a porcine neuropathic model.

Preface

This master's thesis was completed by Louise Leonhard Andersen and Katrine Mühlenfeldt Høegh Stephansen from group 10414 during the 4th semester of the master's program in Biomedical Engineering and Informatics at Aalborg University. The project was carried out in the period from February 1st to May 31st of 2024 in collaboration with supervisor Suzan Meijs. The authors would like to thank Suzan Meijs for guidance and support throughout the entire project period.

Reading instructions

The project consists of three parts: A problem analysis, a description of methods and results, and a synthesis. In the problem analysis the problem domain is described and the aim of the project is specified. A structured literature search was conducted in order to examine existing literature on the topic of the project's aim. The method involved in conducting the structured literature search is described in Appendix A: *Structured literature search*. The second part of the project report consists of a description of the methods and materials used to address the problem statement. In addition, the results from applying the methods are also presented in the second part of the project. Lastly, the project report includes a synthesis which is comprised of a discussion and conclusion. Moreover, appendices are presented at the end of the report. Included in Appendix B: *PBL Portfolio* is a problem based learning (PBL) portfolio in which reflections upon the project period are provided.

The referencing throughout the project report is performed using the Harvard referencing system for active and passive references. When passive references are applied after a full stop, the reference refers to the entire preceding paragraph. However, if a passive reference is placed before a full stop the reference refers to the preceding sentence only. At the end of the report the bibliography is presented in alphabetical order. Furthermore, the tables and figures used throughout the project are numbered in accordance with the chapter and the order in which they appear. In addition, Biorender.com has been utilised for creating some of the figures used throughout the report.

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Contents

1 | Introduction

Chronic pain affects approximately every fifth adult in Denmark with an incidence rate of 1.8% [Werner et al., 2019]. Worldwide, it has been estimated that about 30% of people suffer from chronic pain [Cohen et al., 2021]. Chronic pain is a problem that affects all aspects of life for the patient, including quality of life in terms of personal and social relations, emotional impact, sleep problems, depression and anxiety, as well as the ability to work. In addition, chronic pain entails consequences affecting society due to loss of productivity and increased societal costs. [Werner et al., 2019; Cohen et al., 2021].

Chronic pain is a general category of pain conditions which last for a substantial period of time. Chronic pain differs from acute pain in terms of the duration of pain. Acute pain serves as a warning signal of tissue damage at the time of damage. Meanwhile, chronic pain extends beyond the normal recovery time for tissue damage. [Werner et al., 2019]. Neuropathic pain is a subcategory of chronic pain which is caused by a lesion or injury of the somatosensory nervous system, resulting in pain originating from the injured nerve fibres [Werner et al., 2019; Fitzcharles et al., 2021]. The incidence of neuropathic pain is expected to rise due to the growing aging population, an increase in cases of diabetes mellitus, and enhanced cancer survival rates. An increase in drug prescriptions and healthcare visits is furthermore associated with managing neuropathic pain. [Colloca et al., 2017]. The assessment of pain and the effect of treatment is highly dependant on the patient's ability to verbally describe the pain experience as well as a clinician's ability to correctly interpret the descriptions [Elsayed et al., 2020; Wagemakers et al., 2019]. In preclinical studies animal models are often used in the study of pain and effect of treatments. Animals obviously do not possess the ability to verbally communicate the pain experience. Thus, outcome measures addressing clinically relevant aspects of the pain experience are crucial for the translatability of preclinical studies using animal models. [Jhumka and Abdus-Saboor, 2022; du Sert and Rice, 2014]. Thus, the initial problem statement has been stated as follows:

How can clinically relevant outcome measures in preclinical studies of neuropathic pain using animal models improve the translation from animals to humans?

Part I

Problem Analysis

2 | The physiology of pain

Pain is defined by the International Association for the Study of Pain (IASP) as *"an unpleasant sensory and emotional experience associated with actual or potential tissue damage"* [IASP, 2020]. Thus, pain acts as an indicator or warning of tissue damage, and the organism is therefore highly dependant on pain perception in order to survive [Werner et al., 2019]. The perception of pain is subjective since the experience of pain is dependant on an emotional as well as a cognitive aspect. The physiological mechanisms underlying pain perception is referred to as nociception while the term pain refers to the subjective experience of pain. [Werner et al., 2019; IASP, 2020]. Nociception is registered and perceived by the nociceptive system which is a subcategory of the somatosensory nervous system. The somatosensory nervous system is the part of the nervous system responsible for the perception of touch, temperature, pressure, and pain. [Colloca et al., 2017].

Pain can be categorised as acute or chronic depending on the duration of the pain. Acute pain acts as a warning of potential damage to the tissue, while chronic pain extends for longer than the ordinary recovery time. Thus, chronic pain is defined as pain that persists for more than three to six months. The acute pain is a result of a normally functioning nociceptive system reacting to a noxious stimulus. However, chronic pain originates from pathological changes in the nociceptive system that results in long-term persistent pain. [Werner et al., 2019]. Chronic pain can be divided into three categories depending on the cause of the pain: nociceptive, nociplastic, and neuropathic pain. Chronic nociceptive pain refers to a normally functioning nociceptive system reacting to noxious stimuli caused by disease or inflammation. On the other hand, nociplastic pain arises from alterations in the interpretation of noxious stimuli, leading to the perception of non-noxious stimuli as painful. Lastly, neuropathic pain is caused by an injury or lesion to the somatosensory nervous system, resulting in pain originating from the injured nerve fibres. [Werner et al., 2019; Fitzcharles et al., 2021]. This project has been directed to focus on neuropathic pain. In order to understand the pathophysiology and underlying mechanisms of neuropathic pain, it is necessary to understand the normally functioning mechanisms of pain perception.

2.1 The nociceptive system

The nociceptive system refers to the physiological system involved in pain perception. The general pain pathway consists of three orders of neurons which are responsible for transmitting the signalling of pain from the peripheral nervous system to the central nervous system. The first order neurons consist of afferent nociceptive neurons in tissue such as skin, muscle, organs, and bone. The activity in the nociceptive neurons are initiated by a specific type of receptors known as nociceptors, which are located on the nerve endings of primary afferent nociceptive fibers. The nociceptors are activated by a noxious stimulus and an action potential is then transmitted from the point of tissue damage to the dorsal horn in the spinal cord. In addition, nociceptors are categorised according to the type of stimuli to which they respond, be it thermal, chemical, or mechanical stimuli. [Werner et al., 2019]. There are three types of sensory nerve fibres: Aβ, Aδ and C fibres. Of these, the Aδ and C fibres are considered the primary afferent nociceptive fibres. The sensory Aβ fibres are responsible for conveying mechanical stimuli such as touch and pressure. Aβ fibres have a thick myelin sheath and a conduction velocity of 35 to 90 metres per second. Meanwhile, the nociceptive Aδ fibres are responsible for conducting thermal and mechanical stimuli such as cold and pressure. Aδ fibres have a myelin sheath and a conduction velocity of 5 to 30 metres per second. Activation of Aδ fibres produces a sharp experience of pain. Lastly, the C fibres are unmyelinated sensory fibres that respond to warmth as a thermal stimulus and other painful stimuli that result in a long-lasting burning sensation. C fibres have a conduction velocity of 0.5 to 2 metres per second. [Colloca et al., 2017].

The second order neurons in the pain pathway consist of ascending nerves which are responsible for transmitting the nociceptive signal from the dorsal horn in the spinal cord to the brainstem and thalamus via the spinothalamic tract. From here, the nociceptive signal is transmitted to relevant deeper areas of the brain, in which the signal is further processed. This transmission to deeper areas of the brain is conducted by third order neurons. The activity in the different areas of the brain, as a result of the nociceptive signal, entails the perception of pain intensity, location, and other evaluating aspects associated with the perception of painful stimuli. [Werner et al., 2019]. In Figure 2.1, a general illustration of the basic elements of the pain pathway is presented.

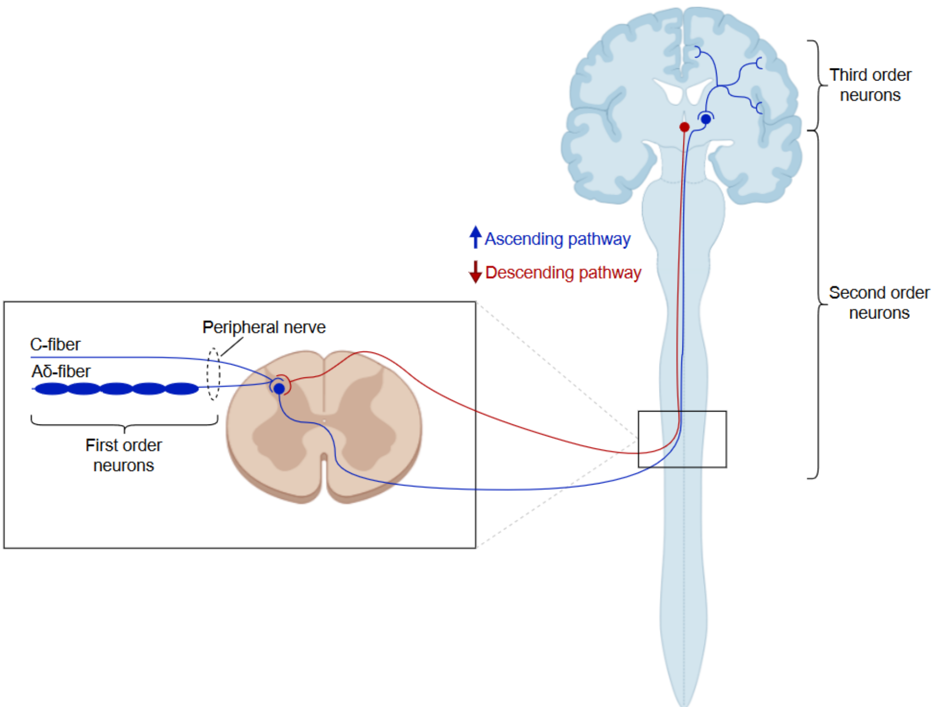

Figure 2.1 *Illustration of the basic elements of the pain pathway, including the first, second, and third order neurons of the ascending fibres. Inspired by Werner et al. [2019] and Colloca et al. [2017]. Created with Biorender.com.*

As illustrated in Figure 2.1, corresponding descending nerve fibres are visible from the brain to the dorsal horn. The purpose of the descending pathways is to modulate the activity in the primary afferent nerves by inhibiting or increasing the activity. [Werner et al., 2019].

3 | Neuropathic pain

Damage to the nervous system can result in impairment of the normal function of the nervous system, possibly entailing analgesia in the denervated area. Analgesia refers to the loss of pain perception in the area in question. However, in some cases pain arises in the denervated area. This type of pain is known as neurogenic or neuropathic pain. [Werner et al., 2019]. Neuropathic pain affects around 7-8% of the north-western European populations [du Sert and Rice, 2014]. In addition, approximately five to ten percent develop neuropathic pain after experiencing nerve damage. However, the prevalence of neuropathic pain is higher for substantial nerve damage such as amputation of extremities or spinal cord injury. In the cases of substantial nerve damage, 50-75% of patients develop neuropathic pain. [Werner et al., 2019]. Neuropathic pain is a general term covering a variety of similar conditions. Common for all these conditions is the development of pain as a result of an injury to the somatosensory nervous system in terms of a lesion or disease. [Colloca et al., 2017; Jensen and Finnerup, 2014].

Neuropathic pain is a diverse group of conditions which can occur as a result of multiple different pathological processes, including trauma, neoplastic, and autoimmune diseases. Examples of neuropathic pain originating from peripheral nerves are diabetic neuropathy and phantom pains as a result of the amputation of an extremity. Causes of neuropathic pain originating from the central nervous system include multiple sclerosis and post stroke pain. [Werner et al., 2019].

3.1 Pathophysiology

The pathophysiology of neuropathic pain involves a cascade of mechanisms and neuroplastic changes that result in sensitisation of the nerve cells. Damage to the somatosensory nervous system results in alterations of the normal nociceptive system allowing for dynamic changes in the nervous system. The dynamic changes are a result of the changes in chemical environment in and around the nerve cells caused by damage to the nerves. The dynamic changes lead to more or less permanent alterations in the excitability of the cells. [Werner et al., 2019]. Gain of excitation and loss of inhibition of the nociceptive signals are evident, resulting in changes of the sensory pathways that possibly entail chronic pain over time [Colloca et al., 2017]. The dynamic changes of the nociceptive system can be divided into peripheral and central sensitisation depending on where the changes occur [Werner et al., 2019]. In Table 3.1, the different types of mechanisms involved in peripheral and central sensitisation is presented.

Peripheral sensitisation	**Central sensitisation**
Ectopic activity	Ectopic activity
Growth of new nerve fibers	Reorganization of synaptic connections in the spinal cord or brain
Awakening of dormant nociceptors	Reduction of descending inhibition on the spinal cord
Increased activity in sprouts	Reduction of segmental inhibition in the spinal cord
Phenotypic changes of peripheral nerves	
Invasion of sympathetic nerve fibers into spinal ganglia	

Table 3.1 *Table presenting the different types of mechanisms involved in peripheral and central sensitisation. [Werner et al., 2019].*

As illustrated in Table 3.1, the dynamic changes can be divided into peripheral and central sensitisation. Peripheral sensitisation involves changes in and around the sensory nerve endings. When damage to nerves occurs, new nerve sprouts develop. Approximately 25 to 50 new nerve sprouts develop for each damaged nerve that has been severed. [Werner et al., 2019]. In addition, alterations of the function and expression of ion channels on the nociceptive fibres result in hyperexcitability [Colloca et al., 2017; Werner et al., 2019]. For example, an accumulation of sodium-potassium channels can appear in the nerve sprouts and damaged nerve endings. This accumulation of sodium-potassium channels results in abnormal nerve activity which can cause sensitisation of the cells. Besides, the new nerve sprouts can innervate other nerve fibres as well as the damaged peripheral nerve ending. Thus, the resulting reinnervation can become disorganised, which, additionally, can result in sensitisation of the cells. Moreover, the changes in the chemical environment around the nerve endings can lead to a release of inflammatory substances, which can also cause sensitisation of the cells. [Werner et al., 2019]. Lastly, the nerves can develop expression of α-adrenergic receptors, which may result in the nerve becoming receptive to catecholamines such as adrenaline and noradrenaline. Thus, additional nerve activity in the damaged fibres can be provoked by activity in nearby sympathetic nerve fibres. In general, peripheral sensitisation leads to the nerve endings becoming extra sensitive due to the additional amount of nerve endings and alterations in ion channels. As a result the nerves display ectopic activity, which makes the nerves more sensitive to sensory inputs such as touch and temperature. [Werner et al., 2019].

Furthermore, central sensitisation involves sensitisation of nerve cells in the central nervous system. The central nerves are exposed to an increased amount of activity due to the spontaneous and increased activity in the damaged nerve fibres. [Werner et al., 2019; Colloca et al., 2017]. Sensitisation of the central cells includes an increase in the amount of activated second-order neurons in the areas responsible for receiving and transmitting the sensory inputs from the peripheral fibres [Colloca et al., 2017]. In addition, the threshold needed for activation of the nerve cells can be decreased, resulting in further nerve activity. Moreover, an increase in the response to painful stimuli in the central nerve cells can occur, and additional nerve cells that were otherwise inactive can be recruited. [Werner et al., 2019]. Lastly, an aspect of central sensitisation is the reduction of inhibitory mechanisms involved in pain modulation. A disinhibition of the pain modulation entails a reduction in the ability to alleviate the pain signals. [Werner et al., 2019; Colloca et al., 2017]. In general, central sensitisation entails hypersensitivity towards painful and non-painful stimuli in terms of hyperalgesia and allodynia. Hyperalgesia refers to the experience of increased pain when exposed to a painful stimulus whereas, allodynia refers to the experience of pain when exposed to a usually non-painful stimulus. [Werner et al., 2019].

Ultimately, the mechanisms involved in neuropathic pain include a cascade of different neuroplastic changes that occur as a result of damage to the somatosensory nervous system. These dynamic changes presumably result in ectopic activity and sensitisation of nerve fibres which cause neuropathic pain. [Werner et al., 2019].

3.2 Diagnosis

In Denmark the diagnosis of neuropathic pain is based on two components: an objective neurological assessment and a subjective anamnesis provided by the patient. The experience of neurological pain is subjective, and the symptoms and extent of the symptoms are therefore individual. However, a likelihood for the pain being neuropathic can be estimated with the use of different questionnaires based on the characteristics of the pain. [Werner et al., 2019]. General symptoms such as hyperalgesia and allodynia are characteristic for neuropathic pain. The symptoms, hyperalgesia and allodynia, affect approximately 15%-50% of patients suffering from neuropathic pain. [Jensen and Finnerup, 2014].

When diagnosing neuropathic pain, it is important to gather as much information as possible about different aspects of the pain. This includes information about when the pain started, whether the pain is spontaneous or gradual, and whether the pain changes when provoked. [Werner et al., 2019]. Furthermore, standardised pain scores aiming to quantify the pain intensity are utilised when diagnosing neuropathic pain. The two most commonly used pain scales are the Visual Analogue Scale (VAS) and Numeric Rating Scale (NRS). Both of these scales are used to quantify pain intensity on a scale from one to ten with VAS including a visual equivalent to the numbers. [Werner et al., 2019]. Lastly, objective neurological assessments is utilised when diagnosing neuropathic pain in addition to the pain scales and evaluation of quality of life (QoL). No standardised objective neurological assessments are available for diagnosing neuropathic pain. However, clinical examinations often include an assessment of increased sensitisation in the affected area. Increased sensitisation can also occur as a result of non-neuropathic pain. Thus additional tests can be used to validate the existence of a lesion or injury to the nervous system in order to confirm neuropathic origin of the pain. Hence, the diagnosis of neuropathic pain is made on the basis of individual anamnesis, clinical examination, and possibly additional relevant tests as illustrated in Figure 3.1. [Mulvey et al., 2014; Werner et al., 2019].

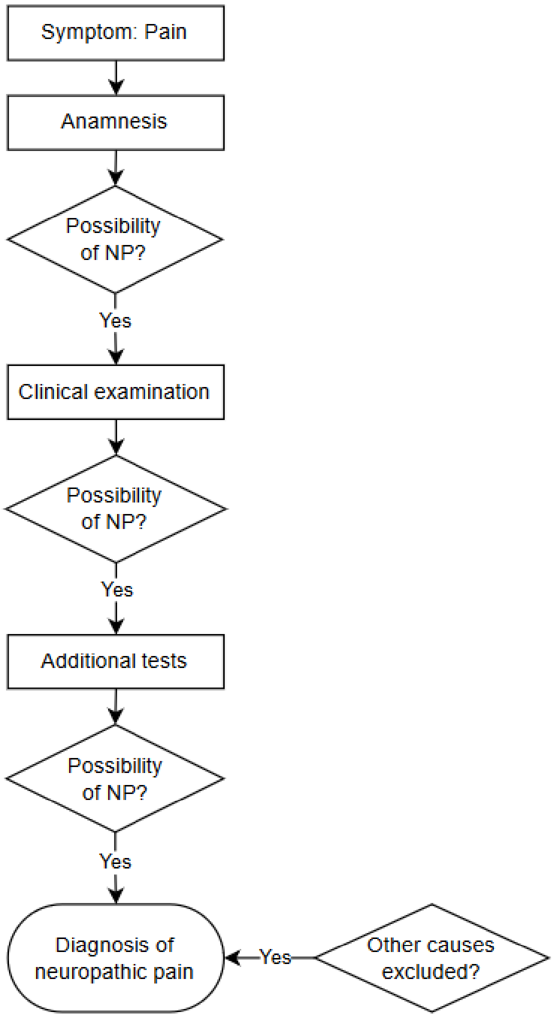

Figure 3.1 *Visualisation of the steps involved in the diagnostic procedure for neuropathic pain. Inspired by Werner et al. [2019]*

Additionally, the QoL of patients with neuropathic pain is affected. This is due to neuropathic pain often being associated with additional problems affecting QoL such as depression, anxiety, and disturbed sleep. Because of the subjectivity of the infliction of neuropathic pain on the patient's QoL, standardised tools for assessing QoL have been utilised. These standardised tools can, for example, be utilised to determine whether or not treatment has an effect on the neuropathic pain in clinical trials. [Colloca et al., 2017]. In a study conducted by Colloca et al. [2017], the use of the SF-36 standardised method for evaluating QoL in patients with neuropathic pain and non-neuropathic pain showed that the QoL in patients with neuropathic pain was significantly lower [Colloca et al., 2017].

In summary, the assessment of neuropathic pain is complex. The diagnosis is highly dependant of the patients' ability to communicate their symptoms and the clinician's ability to correctly interpret them. If there is a lack of successful communication between the patient and the clinician, the assessment and diagnosis of the pain may become inaccurate. As a result the choice of treatment method might be insufficient. [Elsayed et al., 2020; Wagemakers et al., 2019].

3.3 Treatment

The treatment of neuropathic pain is targeted at achieving as much pain relief as possible with the least amount of side effects. It is rarely possible to achieve complete pain relief. Deciding on the course of treatment therefore consists of balancing the side effects of the treatment with maximum pain relief. [Werner et al., 2019]. In addition, no standardised treatment is available due to the heterogeneity in the causes of neuropathic pain. Moreover, the lack of knowledge concerning the origin and pathophysiology of neuropathic pain results in complexity of determining the treatment course. [Werner et al., 2019; Jensen and Finnerup, 2014]. Therefore, numerous different types of treatment options are available when managing neuropathic pain. These treatment options are generally used for treating the symptoms since the cause of chronic neuropathic pain often cannot be treated. [Colloca et al., 2017]. Also, the decisions regarding pain management depend highly on subjective measures of pain perception. According to Zolezzi et al. [2022] the use of insufficient subjective measurement of pain perception has been an obstacle to research in pain management in clinical trials. [Zolezzi et al., 2022]. This results in pain management in clinical practise being non-specific, due to the lack of knowledge regarding the underlying pathophysiology and objective measures of pain perception. [Jensen and Finnerup, 2014].

In Denmark a treatment plan in accordance with the guidelines provided by Sundhedsstyrelsen [2019] should be implemented to determine the course of treatment for patients with neuropathic pain. The treatment plan is individual and should aim at minimising the individual pain perception by 30%-50% using the NRS and improving functional ability. The treatment plan includes a description of the treatment strategy and expected side effects. Also, the treatment plan should include a point in time at which the treatment should be reevaluated or an end date. [Sundhedsstyrelsen, 2019]. Reevaluation of the treatment is performed in order to determine the correct balance between positive effect and side effects. Only the patient is capable of evaluating whether or not a given treatment has the desired effect in terms of improvement in pain perception and whether it counterbalances the side effects. The role of the pain specialist is then to provide the patient with the opportunity to try different medical preparations under current evaluation. [Sundhedsstyrelsen, 2018].

The treatment methods of neuropathic pain can be divided into three categories: pharmacological, non-pharmacological, and surgical interventions. [Sundhedsstyrelsen, 2019; Werner et al., 2019]. The primary method used for treatment of neuropathic pain consist of pharmacological treatment. However, non-pharmacological treatment should also be incorporated in the pharmacological treatment, since these methods can contribute to improvement of physical and psychological function. [Sundhedsstyrelsen, 2019]. The medical preparations used for pharmacological treatment are often not specific in their mode of action due to the lack of knowledge concerning the underlying pathophysiology of neuropathic pain. [Jensen and Finnerup, 2014]. Non-pharmacological treatment deals with the aspects of pain management that do not involve medication. This includes training, rehabilitation, improved sleeping patterns, counselling, etc. The use of non-pharmacological treatment does not necessarily result in pain relief, but it might contribute to improved pain management and better QoL. [Sundhedsstyrelsen, 2019]. Lastly, surgical options exist for treatment of neuropathic pain. The surgical methods include the severing of certain nerves in order to remove the pain, but the risk of inducing new pain is great with this type of treatment. Thus, surgical treatment of neuropathic pain is very rarely used in clinical practise. [Werner et al., 2019]. In Table 3.2, the primary pharmacological and non-pharmacological treatment options are presented.

Pharmacological	**Non-pharmacological**
Antikonvulsiva • Pregabalin • Gabapentin *Serotinin and Noradrenaline* Reuptake Inhibitor (SNRI) • Duloxetin *Tricyclic antidepressants (TCA)* • Amitriptylin	• Exercise • Relaxation • Interdisciplinary rehabilitation • Good sleep habits • Social counselling • Distribution of activities throughout the day

Table 3.2 *Table illustrating the primary pharmacological and non-pharmacological treatment options for managing neuropathic pain. Inspired by Sundhedsstyrelsen [2019].*

4 | Models of neuropathic pain

Preclinical modelling of pain is an important aspect of translational success of findings in preclinical trials to implementation of new treatment options in clinical practise [Sadler et al., 2022]. When modelling neuropathic pain, producing irreversible damage is required in order to induce neuropathic pain in test subjects. As a result, it is not possible to utilise human test subjects when examining the underlying mechanisms of neuropathic pain and potential of the appertaining pharmacotherapy in preclinical trials. Thus, properly validated animal models are needed. The mechanisms involved in the induced neuropathic pain should ideally produce sensory deficits comparable to symptoms observed in humans, allowing the evaluation of neuropathic pain mechanisms and potential pharmacotherapies. [Jaggi et al., 2011]. The mechanisms involved in human chronic pain are complex, but animal models can be utilised in preclinical trials to provide insight into the complex biological mechanisms causing chronic pain. The design of preclinical trials is therefore of great importance to pain research when aspiring to provide translatability of results from experiments using animal models on humans in clinical practise. [Sadler et al., 2022].

4.1 Animal models of neuropathic pain

As neuropathic pain can be caused by a combination of different factors, neuropathic pain does not have a single etiology. Consequently, different types of animal models have been created and evaluated in order to further understand the different mechanisms of neuropathic pain and the potential of related pharmacotherapy. [Jaggi et al., 2011]. The different types of animal models differ in terms of the choice of model organism, the method of inducing neuropathic pain referred to as injury model, laboratory environment, and outcome measure. [Sadler et al., 2022].

4.1.1 Model organism

The choice of model organism affects the translatability from models to humans. A valid pain model should reflect the pathophysiology and psychological aspects of the disease in order to translate results from the model to humans [Meijs et al., 2021]. According to an analysis of literature published in the journal *Pain* conducted by Sadler et al. [2022], the use of rats and mice were the most common while larger mammals like dogs and pigs were rarely used as model organisms in preclinical research of pain [Sadler et al., 2022]. Multiple pain models using rodents like rats or mice have therefore been applied and researched. These models have contributed to the understanding of the underlying mechanisms of pain processing. However, limitations regarding the use of different species in pain models are evident in terms of translatability between the model organisms and humans due to differences in physiology. In pharmaceutical trials the promising results often fail to translate from rodents to humans. When compared to rodents the physiology of pigs is more similar to humans. This includes physiological resemblance between humans and pigs in terms of structure and function of the nervous system as well as skin and skin innervation. In addition, the response to pharmaceuticals in pigs is more similar to that of humans. [Castel et al., 2016; Meijs et al., 2021]. This has been demonstrated in studies where observations regarding the presence of an analgesic effect from treatment with the drug aprepitant failed to translate from rodents to humans while the observations in pigs could be replicated in a clinical trial using humans as test subjects [Castel et al., 2016]. Consequently, in the remainder of the project report the focus will be on porcine neuropathic models.

4.1.2 Injury model

Multiple different injury models have been used to induce neuropathic pain in animals during preclinical trials [Jaggi et al., 2011]. In rodents the sciatic nerve crush model and peripheral nerve injury (PNI) models are commonly used. PNI models can include ligation of selected nerves or spared nerve injury (SNI) in which one or more nerve branches are severed. [Castel et al., 2016]. However, a mixed neuritis model is the only neuropathic pain model which has been described in pigs. The mixed neuritis model involves inducing neuritis by combining immune activation and physical injury, resulting in neuropathic pain. [Hellman et al., 2024]. In Castel et al. [2016], a porcine neuropathic pain model is developed by surgically inducing peripheral neuritis trauma (PNT) in pigs. Neuropathic pain was induced by performing loose ligation of the sciatic nerve in the PNT-injured pigs. [Castel et al., 2016]. Additionally, the development of a common peroneal nerve injury (CPNI) model for a porcine neuropathic pain model is described in Hellman et al. [2024]. The CPNI model is an adaptation of the sciatic nerve injury model which has been validated in rodents. Specifically, the CPNI involves ligation of the common peroneal nerve, which is a branch of the sciatic nerve. [Hellman et al., 2024].

4.1.3 Outcome measures

A direct measure of pain does not exist due to the subjective nature of pain perception. The assessment of pain in humans is therefore often based on subjective outcomes reported by the patient. [Meijs et al., 2021]. In animals there is a need for measures that are not based on verbal communication. For this purpose, testing stimulus-evoked nociception and nocifensive behaviour as a response to sensory stimuli is often utilised as an outcome measure in animal models. [Meijs et al., 2021; du Sert and Rice, 2014]. However, the assessment of uninfluenced spontaneous pain-related behaviour is rarely used as an outcome measure of pain in animal models. This is contradictory with the fact that spontaneous pain is a symptom which most patients with neuropathic pain experience while sensory gain is a much less frequent symptom. [du Sert and Rice, 2014]. Moreover, animal models of pain that rely on stimulus-evoked measures have previously failed to translate successfully to human clinical settings [Deuis et al., 2017]. Approximately 15%-50% of patients with neuropathic pain experience hyperalgesia and allodynia while all patients with neuropathic pain experience spontaneous pain to some extent [Werner et al., 2019]. Thus, a mismatch is arguably evident regarding the translation of outcome measures in animal models to the symptoms observed in humans. [du Sert and Rice, 2014].

Analysing the display of a variety of complex pain-related behaviours can be a method for quantifying non-evoked pain in animals. Pain-related behaviour can, among other things, include touch avoidance, social isolation, or aggressive demeanour. However, the display of behaviour might differ depending on the individual as well as multiple factors such as species, age, gender, history of trauma, and environment. [Jhumka and Abdus-Saboor, 2022]. Research has been conducted to investigate different types of methods for utilising ethologically relevant behaviours as an outcome measure. This includes assessment of pain using the facial expressions of the animals as a method of detecting non-evoked pain-related behaviour. In addition, changes in burrowing behaviour in rodents, voluntary activity such as wheel running, and activities such as licking, lifting or shaking the affected paw have been analysed to address spontaneous pain-related behaviour. [du Sert and Rice, 2014]. However, measuring spontaneous pain-related behaviour is complex since replication of the studies can be difficult. This is due to the need for a better understanding of the factors affecting the animal behaviours and how to control these factors in preclinical trials. These factors for example include changes in the physical environment in which the animals are placed. Furthermore, inter-animal variations in spontaneous pain related behaviour are evident, which can make the interpretation of averaged group-wise findings difficult. [du Sert and Rice, 2014].

The use of home cage monitoring has also been researched for assessment of pain in rodents. Advantages of home cage monitoring includes the reduction of stress in the animals as a result of the analysis being conducted under standard housing conditions. However, video-based home cage monitoring is restricted by the need for manual analysis of behaviour. [Tappe-Theodor et al., 2019]. The methods of behavioural analysis are often very time-consuming and involve a high degree of ethological expert knowledge regarding the target species. Additionally, the manual decoding of behaviours is not free of bias due to inter-observer variability. [Jhumka and Abdus-Saboor, 2022; Siegford et al., 2023]. To allow for some of the problems associated with manual behavioural assessment, studies focusing on the development of computer vision methods for behaviour recognition and classification have been conducted in the fields of ethology and neuroscience. The development of methods for pose estimation and behaviour classification has contributed to advances in quantifying animal behaviour. Thus, the use of computer vision methods in pain research could potentially contribute to the development of outcome measures addressing non-evoked pain-related behaviour, improving the translatability of pain research. [Jhumka and Abdus-Saboor, 2022].

5 | Porcine behaviour analysis

In pain research, not much literature is available specifically on the topic of automated porcine behaviour analysis. However, animal behavioural analysis has been a topic of growing interest in estimating animal welfare in the animal production industries [Siegford et al., 2023]. In the pig production industries, aggressive and harmful social behaviour reduces animal welfare and entails economic consequences for the pig production [Liu et al., 2020]. Negative social behaviour in pig production can occur due to factors such as confined environment, diet, and changes in the group composition which results in instability of the group hierarchy [Viazzi et al., 2014].

For this reason detection of aggressive and harmful behaviour has been a topic of interest in order to minimise or prevent negative social behaviour. [Liu et al., 2020]. With the intention of detecting negative social behaviour, video-recordings using cameras have often been used. Still, manual decoding of multiple hours of animal behaviour videos is time-consuming. To eliminate the need for manual decoding of the videos, research has been conducted on the use of computer vision in terms of automated behaviour analysis (ABA) strategies. [Siegford et al., 2023]. According to Siegford et al. [2023], the motivations for conducting research in ABA are numerous and diverse. ABA strategies could potentially be used to scientifically gain a deeper understanding of the animals and provide insights into human physiology. Additionally, Siegford et al. [2023] suggests that ABA could be utilised in detection of pain and emotion as well as for gaining an understanding of the mechanisms in the brain. [Siegford et al., 2023]. Thus, the utilisation of ABA strategies in the context of a porcine neuropathic pain model should be investigated further.

5.1 Automated behaviour analysis

Analysis of animal behaviour can be automatised using computer algorithms that are capable of recognising different types of animal behaviour based on data such as video or sensor data. The purpose of ABA strategies is therefore to identify patterns in the data that are characteristic of specific types of animal behaviour, and utilising these patterns to recognise the behaviour when presented with new data. [Siegford et al., 2023]. Recently, the development of methods for pose estimation and behaviour classification has contributed to advances in quantifying animal behaviour in the fields of ethology and neuroscience. Traditionally, approaches for the analysis of video data have involved time-consuming manual annotations and expert knowledge regarding the target species and ethology. The use of supervised as well as unsupervised machine learning methods have been utilised for behavioural classification. [Luxem et al., 2023]. In the context of automated pig behaviour analysis, research on video-based computer vision methods of ABA has been conducted due to video surveillance having been implemented in many farms [Yang and Xiao, 2020].

5.1.1 Related work

Based on the results from the structured literature search described in Appendix A: *Structured literature search*, automation of porcine behaviour analysis has been a popular topic for research in pig production, but not in the field of neuropathic pain modelling. However, research concerning the use of ABA strategies in pig production may potentially be utilised as outcome measures in neuropathic pain research. From the included articles in the structured literature search it is evident that most studies use deep learning as a method of implementing ABA. Specifically, different types of convolutional neural networks (CNN) has been utilised. In addition, other machine learning approaches such as clustering and linear discriminant analysis (LDA) have been used to automatically analyse animal behaviour. In Table A.3 in Appendix A.1: *Related work*, the distribution of the utilised methods in the resulting articles from the literature search are presented.

From the distribution of utilised methods described in Table A.3 in Appendix A.1: *Related work*, it is evident that the majority of the resulting studies focus on CNN-based methods. Specifically, the methods Visual Geometry Group-16 layers (VGG-16), Residual Network (ResNet), and You Only Look Once (YOLO) have been used in multiple studies. However, the aims of the different studies are not necessarily directly comparable even though all the related literature focuses on ABA. The methods implemented in the behaviour analysis vary since behavioural analysis is a complex topic involving ethological knowledge regarding the behavioural patterns of the specific animal [Siegford et al., 2023]. Thus, some studies focus on video-based ABA in terms of classification of aggressive vs. non-aggressive episodes while other studies aim to classify the porcine behaviour in multiple different categories. For example, the study described in Fraser et al. [2023] focused on the detection of aggressive and non-aggressive episodes while the study in Chen et al. [2017] aimed to classify according to the amount of aggressive behaviour in the categories medium and high aggressiveness. Also, the characteristics associated with specific types of behaviour are not clearly defined, and behavioural analysis therefore often involves ethological expert knowledge. The resulting ABA implementation in different studies is therefore highly dependant of the choices regarding classification categories, and the labelling of the data. [Siegford et al., 2023]

Multiple of the resulting studies focusing on CNN's indicate a potential in the utilisation of deep learning-based strategy for video-based ABA. For example, the results from Fraser et al. [2023] show effectiveness of a differential-based deep learning approach for detection of aggressive behaviour in pigs. The results from the study showed that detection of aggressive subtypes such as head-biting was the easiest ones to classify with a sensitivity of 95% while non-aggressive subtypes like mounting were the hardest to classify with a sensitivity of 63%. In general, it is concluded that the study showed that using a CNN along with an image differential approach could result in a meaningful method of ABA. [Fraser et al., 2023]. As opposed to the majority of the results from the structured literature search, few articles investigate the possibility of applying unsupervised learning for analysing porcine behaviour. In Chen et al. [2017], hierarchical clustering was used to classify medium and high aggression among group-housed pigs based on an acceleration feature which was extracted from video sequences. The results from the classification show an accuracy of 95.82% with a sensitivity of 90.57% and specificity of 96.95% for recognising medium aggression as well as an accuracy of 97.04% with a sensitivity of 92.54% and specificity of 97.38% for recognising high aggression [Chen et al., 2017].

6 | Problem definition

Throughout the problem analysis it has been established that neuropathic pain is a complex diagnosis involving elaborate pathophysiology and varying symptoms. The process of diagnosis and treatment of neuropathic pain is based on successful communication between the patient and the clinician. In addition, the treatment options often entail side effects which have to be outweighed by the positive outcomes of the treatment.

Furthermore, it has been established that the use of animal models is a significant aspect of pain research. The use of pigs as model organisms has a potential when compared to rodents due to the similar physiology of pigs to humans and promising results in earlier studies. In the context of pain research using porcine models, a need for objective outcome measures reflecting the spontaneous pain-related behaviour has been described. Such an outcome measure that reflects the spontaneous pain-related behaviour without impacting the model organism could potentially provide clinically relevant measurements of the effect of medication for neuropathic pain treatment of the animal. Aggressive traits could potentially be an indicator of pain. Thus, analysis of the amount of aggressive social interaction could possibly be utilised as outcome measure representing pain-related behaviour in a porcine neuropathic model. Thus, this problem delimitation leads to the following problem statement:

How can an objective and clinically relevant outcome measure be developed using computer vision and machine learning to classify social interaction in a porcine neuropathic model?

Part II

Methods and Results

7 | Methods and materials

The following chapter contains descriptions of the methods and materials used to address the problem statement throughout the project period. Initially, a general overview of the solution strategy is presented along with a description of the data source. Later, the individual methods involved in the strategy will be elaborated upon.

In order to address the problem statement different methods have been applied. The methods have been divided into three categories aiming to address the relevant aspects of the problem solution. The aims of the three categories are pose estimation and tracking of the pigs, preprocessing of the pose estimation and tracking data, and classification of social interaction, respectively. In Figure 7.1, an overview of the three categories are presented along with the methods applied. Pose estimation and tracking of the pigs were performed using Social LEAP Estimates Animal Poses (SLEAP), which is an open-source deep learning-based framework for pose estimation of multiple animals [Pereira et al., 2022]. The use of SLEAP for pose estimation and tracking enabled the extraction of relevant features for examining social interactions. The features were then used to examine the possibility of classifying behaviours, including social interaction, into meaningful categories using a clustering analysis. Specifically, the unsupervised clustering approach, K-Means, has been chosen for grouping data based on similarities. Lastly, a post-clustering analysis was performed in order to examine and interpret the characteristics of the resulting clusters. The post-clustering analysis constitutes the basis for examining the behavioural patterns, including social interaction, represented in groups of pigs with and without neuropathic pain.

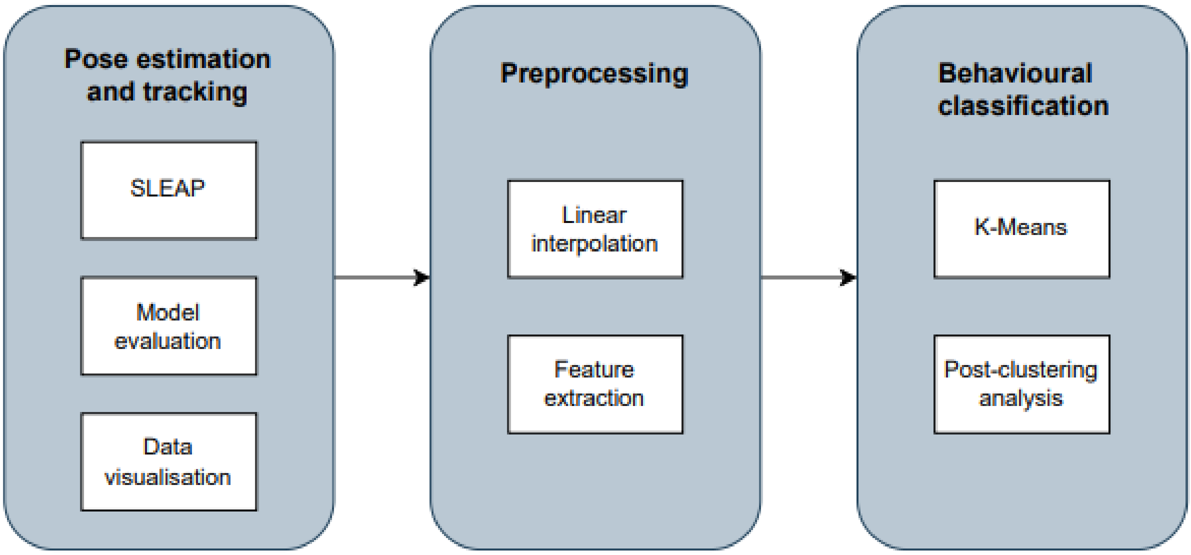

Figure 7.1 *Illustration of the general structure of the solution strategy, consisting of three components: Pose estimation and tracking, preprocessing, and behavioural classification.*

7.1 Source of data

The data used in this project consisted of videos of pigs that were included in a study of neuropathic pain at Aalborg University. All procedures involved in the study were approved by the Danish Veterinary and Food Administration under the Ministry of Environment and Food in Denmark (protocol number: 2020-15-0201-00514). The pigs were housed in pairs of two in a home pen with a cycle of 12 hours of light and 12 hours of darkness. If this time period the pigs were recorded during six hours of daytime and twelve hours of nighttime. The pigs housed together were siblings. They were fed twice a day and had unlimited access to water. The pigs were involved in training sessions once a day and were provided with different types of toys according to the trial protocol.

As illustrated in Figure 7.2, the trial procedure was initiated by the pigs undergoing an habituation period of minimum two weeks in order to get used to new surroundings and procedures. Afterwards, one week of baseline data was collected. The pigs were then randomly separated into an intervention group and a control group. During data analysis in this project, blinding of the researchers was applied. Thus, the researchers were not aware of the distribution of pigs in the control -and intervention group. After conducting the data analysis, the researchers were provided with the knowledge of what pigs belonged to the control and what pigs belonged the intervention group.

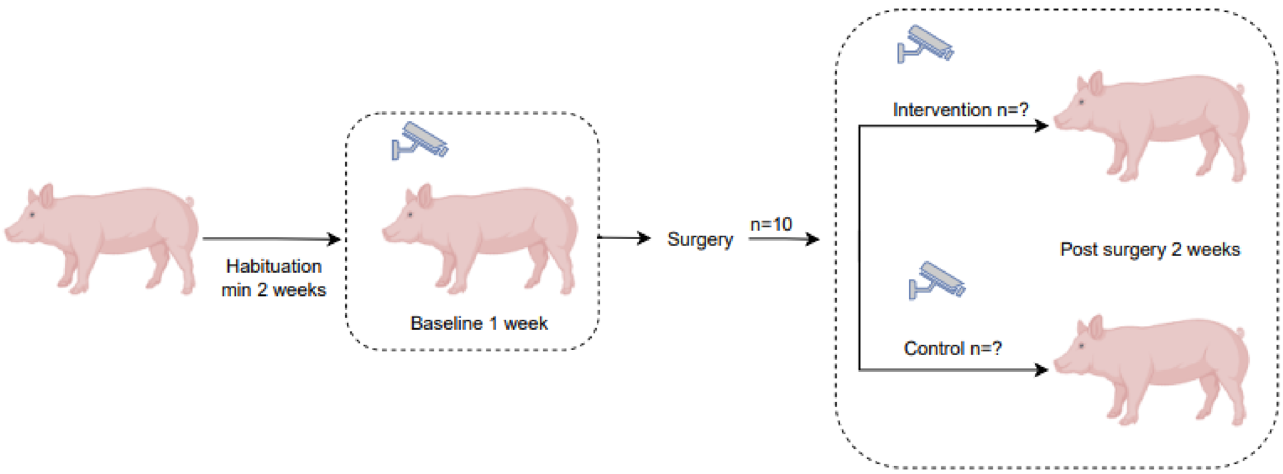

Figure 7.2 *Illustration of the trial procedure, consisting of an habituation period, baseline period, and post surgery period. Created with Biorender.com.*

During the trial, the intervention group was induced with neuropathic pain using the SNI model while the control group were not induced with neuropathic pain. The pigs in both groups went into surgery. However, the radial nerve was severed only in the pigs assigned to the intervention group as illustrated in Figure 7.3.

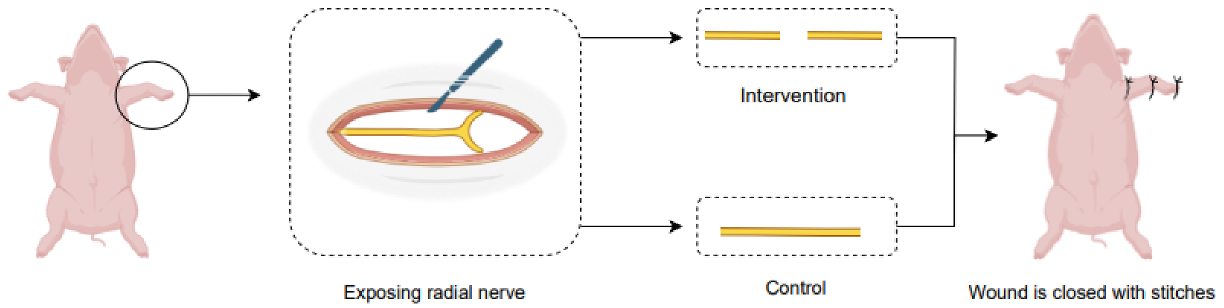

Figure 7.3 *Visualisation of the surgery procedure in which all pigs underwent surgery but the radial nerve was only severed in the SNI intervention group. Created with Biorender.com.*

The total amount of available data provided for the project consisted of videos from eight pairs of pigs in a home pen during six hours of daylight. Of these, it was chosen to exclude two pairs of pigs, specifically the 9-10 and 7-8 groups. The exclusion was made due to one of the groups expressing so aggressive behaviour that separation of the pigs was necessary. Meanwhile, the other pair consisted of two pigs belonging to an intervention and a control group, respectively. Due to challenges associated with distinguishing between the individual pigs during tracking, the pair consisting of mixed groups were excluded.

All available video material consisted of five minute videos. In addition, videos from a varying number of days for each of the groups were available. In Figure 7.4, a visualisation of the total amount of available data is presented.

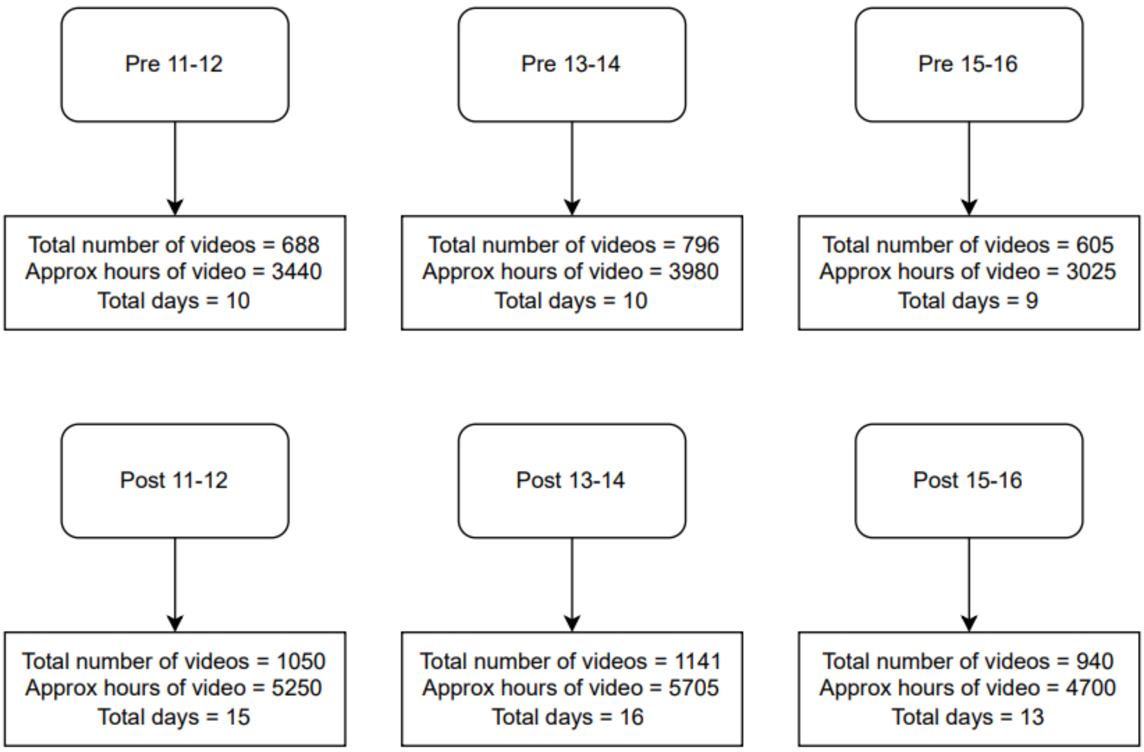

Figure 7.4 *Illustration of the total amount of available data, including the total number of videos, amount of time, and days.*

7.2 Pose estimation and tracking

In order to identify and classify social interaction, tracking and pose estimation of the pigs has been performed using the deep learning based framework SLEAP. SLEAP is an open source graphical user interface (GUI)-based software which can be used to predict animal poses and track the movement of the animals across video sequences [Pereira et al., 2022].

Before deciding on the use of SLEAP for pose estimation and tracking of the pigs in the videos, the use of the proprietary software EthoVision by Noldus was tested. EthoVision is built upon a contrast-based method of detecting and tracking animals in video sequences [Luxem et al., 2023]. The original intention was to utilise EthoVision for identification and extraction of video sequences containing social interaction, enabling extraction of relevant features for classifying different types of social interaction. However, the videos that constitute the data source in this project contain a somewhat challenging environment for contrast-based object detection and tracking methods. Thus, the use of EthoVision proved not to achieve good results due to the lack of sufficient contrast between the pigs and the background (e.g., the wall and bedding material). In addition, the background in the videos changes throughout the collection of videos because the bedding material is movable. In Appendix C: *EthoVision* a description is provided of the test conducted for examining the tracking performance using EthoVision. Additionally, examples of images are provided, displaying the issues that occurred using the contrast-based detection methods implemented in EthoVision.

On the basis of the poor results obtained using contrast-based methods, the choice was made to change the methodological strategy in the project. As described in [Luxem et al., 2023], methods for pose estimation and behavioural classification are currently revolutionising behaviour quantification in the fields of ethology and neuroscience. Thus, a choice was made to investigate the potential in utilising deep-learning based methods for pose estimation and tracking instead of the contrast-based methods implemented in EthoVision. For deep learning-based methods a large amount of annotated data is needed during the model training [Siegford et al., 2023]. SLEAP is an open source GUI-based framework that enables pose estimation and tracking of multiple animals with a minimal amount of manual annotations [Pereira et al., 2022]. Since the data provided for this project does not contain annotations of any kind and the process of manual annotation is very time-consuming, the use of SLEAP has been chosen for pose estimation in this project due to its sample efficiency.

7.2.1 SLEAP

SLEAP was used to generate predictions of pose estimations throughout all video sequences. The pipeline for using SLEAP is illustrated in Figure 7.5. Moreover, the individual steps in the SLEAP pipeline will be elaborated upon in the following sections.

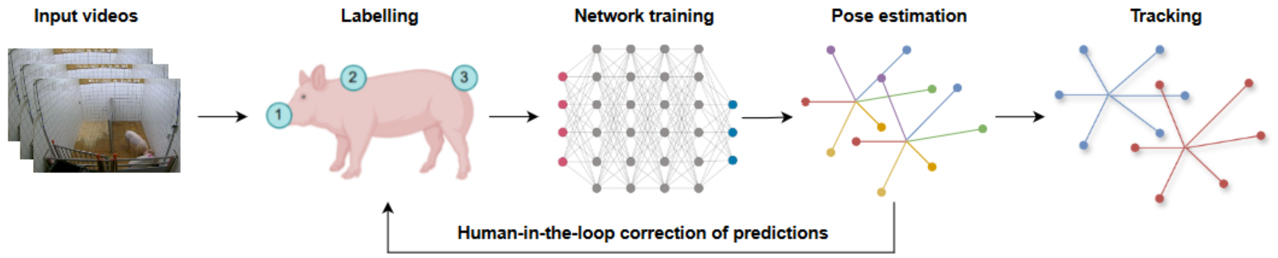

Figure 7.5 *Illustration of the SLEAP pipeline, consisting of five steps in which steps two to four are repeated until model convergence is achieved. Inspired by SLEAP [2024].*

Initial labelling

The first step in the SLEAP pipeline consists of manual annotation of a subset of representative frames from each video. The labelling procedure consists of manual placement of skeletons on all animals present within a frame. The skeletons are composed of three nodes which are placed on the snout, shoulder, and tail of each pig, respectively. The three nodes are combined via edges between snout and shoulder as well as shoulder and tail, creating a skeleton. The node placement on a pig is illustrated in Figure 7.6. The placement of nodes has been chosen due to the easily identifiable points of the pig, and because consistency is of importance when manually placing the nodes.

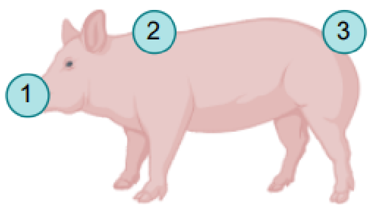

Figure 7.6 *Illustration of the skeleton used for annotation in SLEAP. The annotation includes three nodes placed on the snout, shoulder, and tail, respectively. Created with Biorender.com.*

The initial labelling should be performed on frames that are representative of the entire videos in order to train a robust model for predicting labels on new frames. A stride sampling method was used to select 20 frames with fixed intervals in each of the six videos for the initial labelling. The stride sampling method was chosen due to a large amount of variation within the datasets being evident between the days as a result of the changes in camera angles. Thus, it is argued that a representative data set should at least include frames from all days, incorporating all possible camera angles. In addition, it is important to include any atypical scenarios that might appear in the training data in order to improve the robustness of the model. In this project atypical scenarios could include frames in which people enter the pen or the pigs being unusually positioned.

Network training

After the manual labelling a deep learning model is trained on the manually labelled frames. In SLEAP, it is possible to choose between two different types of models: A top-down model and a bottom-up model. These two models differ in terms of method for modelling the relationship between the instances and their body parts. In the top-down approach, the detection of instances and their body parts is divided into two parts. At first, the instances are detected by locating the center point of the instances. Next, the body parts of the individual instances are detected on the basis of the location of the center point. In the bottom-up approach, all body parts in a frame are located first after which they are grouped into instances based on the connectivity between the body parts. The choice of whether to use the top-down or bottom-up approach depends on the data. [Pereira et al., 2022]. According to Pereira et al. [2022], the bottom-up approach might perform the best on datasets with many animals in which the animals constitute a large fraction of the frames. Conversely, the top-down approach might perform the best in datasets with fewer animals. [Pereira et al., 2022]. In this project, the top-down approach has been chosen due to the dataset containing frames with two pigs that do not occupy large fractions of the frames.

In SLEAP, UNet is the primary network architecture [Pereira et al., 2022]. The UNet network architecture is a symmetrical U-shaped architecture which was originally developed for semantic segmentation of images [Ronneberger et al., 2015]. A UNet consists of an encoder part and a decoder part as illustrated in Figure 7.7. The purpose of the encoder is to downsample the input image thereby extracting the most important characteristics of the input image. Meanwhile, the purpose of the decoder is to upsample the image to its original size including the newly acquired information. Thus, the UNet is used to generate a new image that includes the important characteristics of the original image. The nature of the important characteristics depends on the training data since the model is trained to detect the specific characteristics in the training data. [Ronneberger et al., 2015; Pereira et al., 2022]. In order to recover the details from the original image during upsampling, skip connections combine the early downsampling blocks in the encoder with the corresponding upsampling blocks in the decoder as illustrated in Figure 7.7. The skip connections concatenate the feature maps from the encoder blocks with the feature maps from the corresponding decoder blocks in order to include details from the original image in the new generated image. [Pereira et al., 2022].

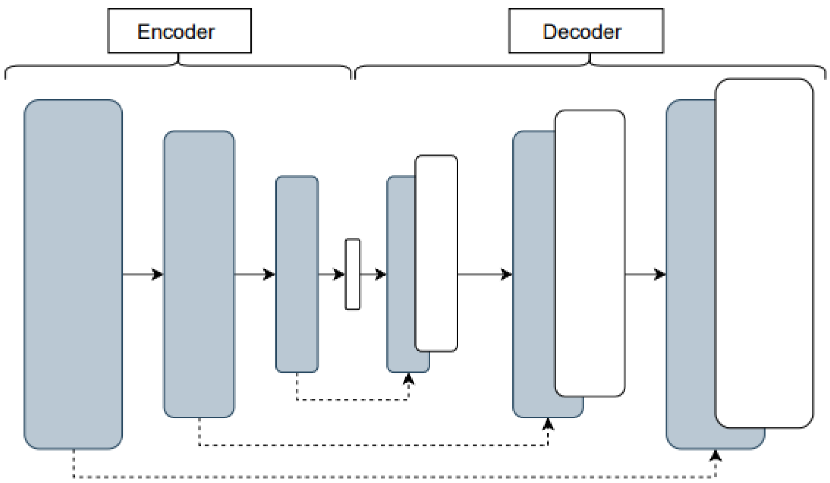

Figure 7.7 *Illustration of the general UNet architecture. The dashed lines represent skip connections. Inspired by Brownlee [2021].*

The individual blocks in the encoder and decoder of a UNet consist of convolutional layers which include a predefined number of filters used for kernel convolution during network training. The filters include weights and biases which are updated during the model training through an optimisation process known as backpropagation [Munro, 2011]. The aim of updating the weights and biases through backpropagation is to minimise a loss function that represents the difference between the predicted output from the model and a ground truth. [Ronneberger et al., 2015; Pereira et al., 2022]. Specifically, the convolutional blocks in the UNet used in the SLEAP model consist of one or more two-dimensional convolutional layers in which a kernel size of 3x3 is used. In addition, a stride of one is used in the encoder while a stride of two is used in the decoder. Moreover, the Rectified Linear Activation Unit (ReLU) activation function is used [Pereira et al., 2022]. The purpose of the ReLu activation function is to map the output of the convolutional layers to a desired output range. The ReLU activation function is a nonlinear function which is defined as presented in equation 7.1 and visualised in figure 7.8. As a result, the use of the ReLU activation function entails unchanged positive values while negative values are set at zero. [Arat, 2019].

$$f(x_i) = \begin{cases} x_i & \text{if } x_i \leq 0 \\ 0 & \text{if } x_i < 0 \end{cases} \tag{7.1}$$

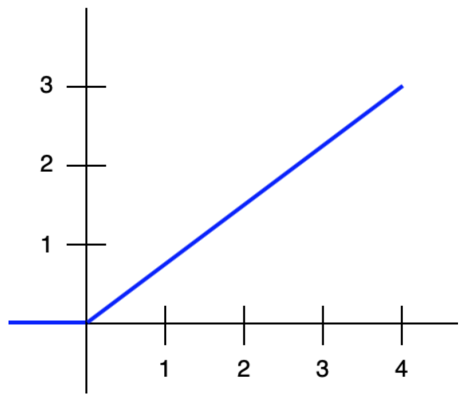

Figure 7.8 *Illustration of the ReLU activation function.*

Furthermore, the encoder blocks in the UNet architecture of SLEAP include max pooling layers with a kernel size of 3x3 and a stride of two [Pereira et al., 2022]. The purpose of utilising max pooling layers is to reduce the dimensions of the feature maps by performing a max pooling operation. In the max pooling operation the maximum element of regions in the feature map is selected. Thus, only the most prominent features are selected and included in the remainder of the network training. [Thomsen, 2020].

Finally, transfer learning is the backbone of SLEAP as pretrained weights can be used in the encoder blocks of the network architecture. The UNet has therefore previously been trained on multiple datasets for detecting the characteristics of the input images which are important for determining the node placement. The datasets used for the previous training consist of 7,636 labelled frames with 15,441 animal instances in total from seven different datasets. The subject animals in these existing datasets were flies, mice, bees, and gerbils. When using SLEAP on a new dataset, transfer learning is therefore available by initialising the weights using the pretrained weights and afterwards tailoring these to the new dataset during training, based on the manually labelled frames from the new dataset. [Pereira et al., 2022].

In summary, the network training in the SLEAP pipeline involves training a UNet for predicting the animal poses represented by skeletons with three nodes. The default architecture of the UNet has been employed, and key aspects of this architecture have been described to elucidate the underlying mechanisms of SLEAP. Lastly, transfer learning is used to initialise the model weights, while the inclusion of manually labelled frames in the training dataset allows for tailoring the model to the specific dataset [Pereira et al., 2022].

Pose estimation

The third step in the SLEAP pipeline is referred to as pose estimation and involves predicting labels on new frames using the previously trained model. The predicted labels should then be manually corrected in order to improve model performance by increasing the size and representativeness of the training set. The corrections should be performed on predictions which are not correct, thus expanding the training set to include frames which the model did not predict correctly. The manual correction of the predictions after inference is referred to as the human-in-the-loop part of the training procedure. The manual corrections are less time-consuming than the initial labelling due to the skeletons already having been placed more or less correctly within the frames. After performing the corrections the model should be trained again on the basis of the expanded training set. The steps involving network training and pose estimation, including human-in-the-loop correction of the labels, should be repeated until the model converges. In this project model convergence has been evaluated using the object keypoint similarity (OKS), precision, and recall metrics based on a validation set as well as visual examination of the model predictions. Thus, the training and inference steps have been repeated until the precision and recall metrics did not improve any further, and the majority of predictions seemed to be correctly placed visually.

Tracking

Eventually, when a satisfactory model performance has been achieved, and the best trained model has been identified based on the evaluation metrics, pose estimation should be conducted for all frames in all of the videos. The final pose estimation differs from the previous pose estimation steps in terms of the number of frames on which predictions should be performed and the inclusion of a tracking method. It is possible to include a tracking method on the final pose estimation due to predictions being performed on adjacent frames as opposed to the previous predictions on randomly selected frames. The purpose of the tracking method is to connect the individual instances across the frames in two tracks, enabling analysis of the movement of the individual instances [Pereira et al., 2022]. In this project the method used for tracking the instances across the frames is the method referred to as intersection of union

(IoU). IoU is a simple tracking method that is used to determine which track the individual instances belong to based on the overlap between the bounding boxes surrounding an instance across frames [Subramanyam, 2021]. In Figure 7.9, an illustration is presented, visualising the IOU used to track the movement of a pig across adjacent frames.

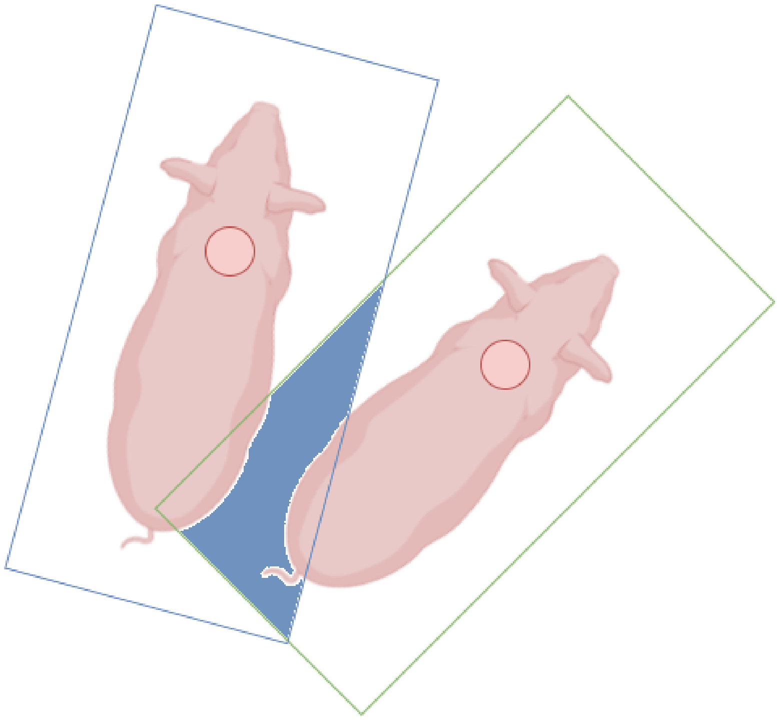

Figure 7.9 *Illustration of the IoU between bounding boxes surrounding the pigs in two adjacent frames used to track the pigs across time. Created with Biorender.com.*

After completing the final inference and tracking, the pose estimation and tracking data is exported as .h5 file, enabling analysis of the movement of the pigs in further analysis.

7.2.2 Evaluation of pose estimation and tracking

For evaluating the quality of the pose estimation and tracking of the final model, two tests have been conducted in this project. The first test involves estimating the error rate in the prediction of nodes during pose estimation. The amount of incorrectly identified nodes in the final pose estimation is estimated using 20 randomly selected frames from each of the six videos. The estimate has been made by visually evaluating the placement of the nodes in the 120 randomly selected frames. The placement of the nodes were then categorised as either correct or incorrect. The categories have been defined on the basis of whether or not an observer would be able to place the nodes correctly. Thus, if the snout, shoulder or tail is visible for an observer within the frame, the nodes can be placed correctly. Likewise, if any of the body parts are not visible within the frame, it is still possible to place some of the nodes correctly.

When estimating the error rate of the pose estimation using randomly selected frames it is not possible to evaluate the tracking of the individual pigs. This is due to the assessment of the tracking being dependant on adjacent frames. During the trial procedure the pigs were not marked using any identifiable markers such as a colour-coding system. As a result, the two pigs in the same pens were more or less indistinguishable for an outside observer during the days in question. Thus, re-identification of the individual pigs across the days has not been found possible. Differentiation between the individual pigs in each pen across the days has therefore not been possible during tracking. However, it is possible for an observer to distinguish between the individual pigs within adjacent frames from the same day because the previous placement of the pig can be taken into account. On the basis of an observer's ability to visually evaluate the tracking performance during continuous video sequences, the performance of the tracking algorithm has been evaluated. During the tracking evaluation, the tracking of two pigs on a single day has been manually examined. The manual examination provides an insight into the types of errors that occur during tracking.

7.3 Preprocessing

Extraction of relevant information is necessary in order to analyse the social interaction among the pigs on the basis of the pose estimation and tracking data from SLEAP. The raw pose estimation and tracking data consist of a large number of location coordinates in two separate tracks for each of the two pigs in every video. These coordinates are stored within a locations matrix for each video in which the columns represent the number of frames, the number of nodes, and the x- and y-coordinates for each node, respectively. In order to extract the relevant information for analysing social interaction using unsupervised machine learning methodologies, preprocessing of the raw pose estimation data has been necessary. The purpose of the preprocessing has been to handle missing data and extract only the relevant information from the raw pose data in terms of features.

7.3.1 Missing data

Missing data is evident within the coordinates of the locations matrix when one or more nodes have not been predicted in one or more frames. The lack of nodes can occur in cases where certain parts of the pigs or the entire pig disappears within the frames. Also, a lack of nodes can occur if the deep learning model for pose estimation fails to predict one or more nodes within the frames. In order to compensate for the missing data within the coordinates, linear interpolation has been applied with the purpose of estimating the values of the missing coordinates based on the known coordinates. Linear interpolation is used to approximate the missing coordinates, using a linear function between the known coordinates, assuming a linear connection between the coordinates [Kreyszig et al., 2011].

7.3.2 Feature extraction

For enabling analysis of social interaction among the two pigs using unsupervised machine learning methods, extraction of relevant features from the raw pose data has been necessary. The aim of this project has been to examine and analyse social interactions between two pigs. Social interaction includes aggression which can potentially be utilised as an outcome measure in a porcine neuropathic model. In existing literature the detection and classification of aggression in pigs have been investigated [Chen et al., 2017]. Thus, the extracted features should express relevant information regarding the aggression expressed by the pigs. In Chen et al. [2017], the acceleration of the pigs has been utilised as a feature for addressing aggressive social interaction. In addition, Chen et al. [2023] describes the distance between different nodes and angles between the animals' position as relevant features for examining social interaction between two animals. Consequently, the acceleration of the snout and shoulder nodes as well as distances between selected nodes of the two pigs have been determined as relevant to the analysis of social interaction in this project. Multiple distances have been considered relevant since the pigs can be positioned differently in relation to each other during interaction. The distances which have been considered relevant for the analysis of social interaction are illustrated in Figure 7.10.

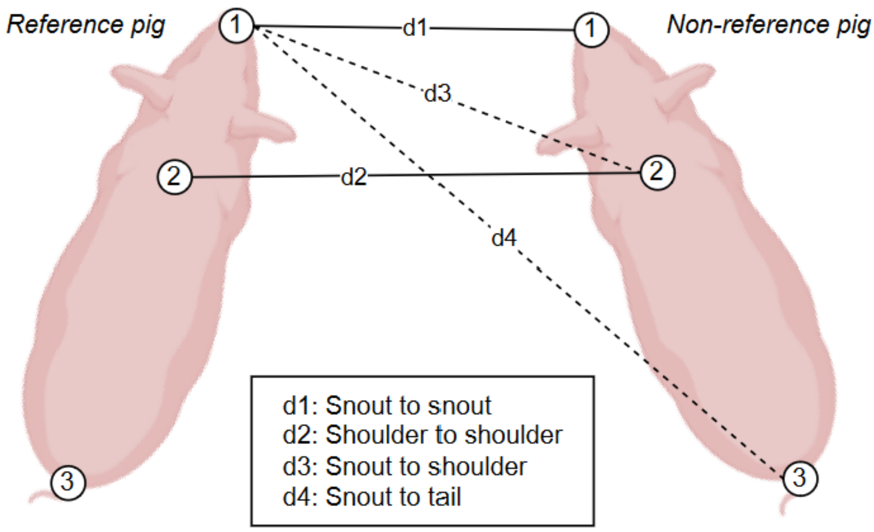

Figure 7.10 *Illustration of the included distance features. The circles represent the three nodes, and the lines represent the distances calculated based on the node coordinates. The dashed lines represent distances that are dependant on the choice of reference pig. Created with Biorender.com.*

The distances have been calculated as the euclidean distance between the respective node coordinates. Of these, the snout to snout and shoulder to shoulder distances do not depend on the choice of a reference pig since these distances will always be the same. However, the distances from snout to shoulder and snout to tail depend on the choice of reference pig. For this reason the distances have all been calculated from a reference pig to a non-reference pig. Thus, all distances are calculated twice on each frame using both pigs as reference one at a time.

Furthermore, the acceleration of the snout and shoulder nodes on the individual pigs have been calculated. Firstly, in order to calculate the acceleration, the velocity of the nodes on the individual pigs has been calculated using a Savitzky–Golay filter. By using a Savitzky–Golay filter, the variation in the coordinates has been smoothed, enabling the calculation of the first derivative in each point which corresponds to the velocity of the node. Afterwards, the acceleration of each point from the previous frame to the current frame has been calculated by dividing the time difference between frames with the change in velocity between frames. The change in time has been derived from the known frame rate of 6.38 frames per second, resulting in a change in time of 0.157 seconds between adjacent frames. In Figure 7.11, an example of location coordinates describing the movement of the shoulder node on one of the pigs during one day is visualised.

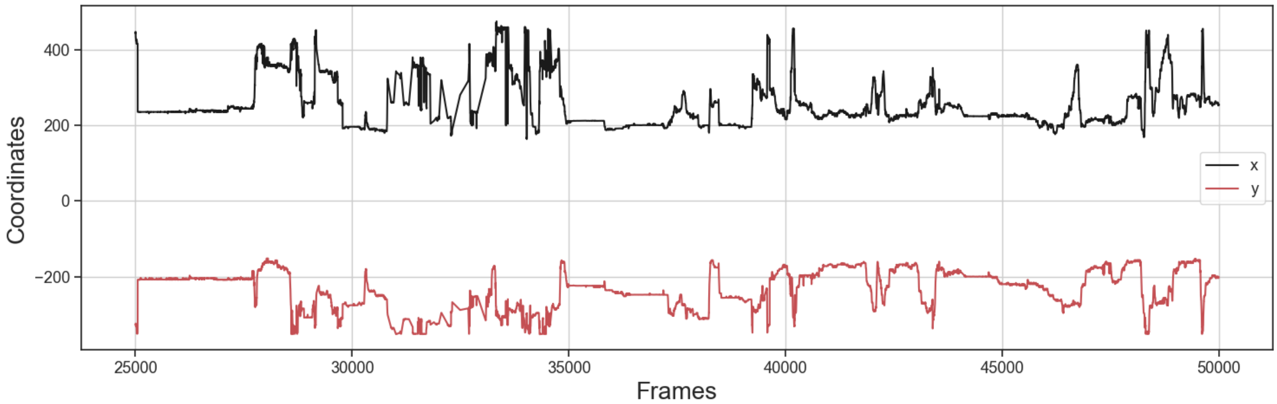

Figure 7.11 *Illustration of coordinates representing the movement of one pig during one day. The y-coordinates are represented as negative in order to separate the x- and y-coordinates within the plot.*

On the basis of the location coordinates, as presented in the example provided in Figure 7.11, the velocity and acceleration have been calculated and visualised. In Figure 7.12 the acceleration of all nodes corresponding to the movement of the pig presented in the example provided in Figure 7.11 is visualised.

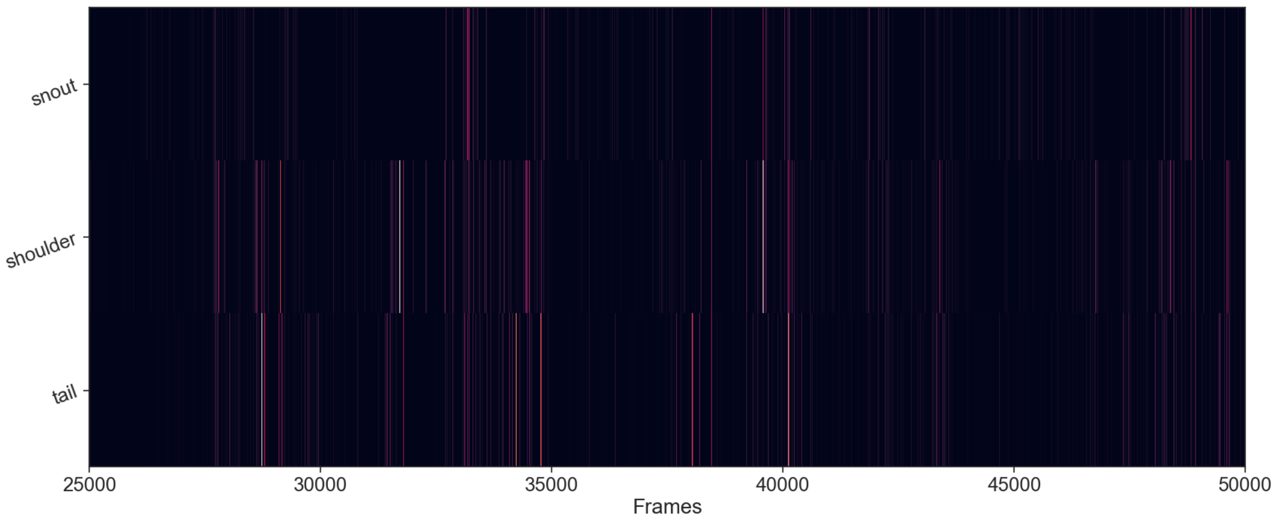

Figure 7.12 *Illustration of acceleration of all three nodes for one pig during one day.*

From Figure 7.12 it is evident that the acceleration of the different nodes on one pig varies. Different types of behaviour arguably results in different accelerations of the individual nodes on one pig. Thus, it is argued that multiple accelerations should be incorporated as features in the remainder of the analysis.

Finally, the extracted features have been combined in a table as presented in Table 7.1. In this table the columns represent the individual features while the rows represent the individual pigs in each of the videos. As it has not been possible to distinguish between the individual pigs in the respective videos, it has been chosen not to distinguish between them during the data analysis. Thus, the data setup involves extracting the features regarding distance and acceleration from a reference pig in relation to a non-reference pig. Every frame in the video has therefore been represented twice in the collected data; once with each of the two pigs as reference pigs.

Video	Frame	Instance	acc1	acc2	acc3	d1	d2	d3	d4
1	1	0	0.00	0.00	0.00	4.28	19.5	44.79	64.91
1	2	0	-0.66	-1.68	0.26	8.67	45.243	21.90	68.01
1	1	1	-0.65	-1.40	0.21	44.56	34.45	54.87	74.56
1	2	1	-0.64	-0.48	0.17	12.89	54.90	65.34	84.29

Table 7.1 *Table presenting the extracted features consisting of accelerations for two nodes, snout and shoulder, as well as the selected distances. The data presented in the table is fictive.*

Lastly, the extracted features have been standardised by moving the mean of the features to 0 and scaling the features to one unit variance. Scaling the features ensures that all features contribute equally to the distance calculations during the k-Means clustering.

7.4 Behavioural classification

In order to analyse behavioural patterns based on the extracted features, the use of unsupervised machine learning methods was investigated. An advantage of unsupervised learning is the fact that these methods do not require labelled data [Luxem et al., 2023]. The process of manually labelling the data by identifying different types of behaviours in video sequences is time-consuming and risks being biased if the observers do not possess sufficient ethological knowledge [Siegford et al., 2023]. Also, the use of unsupervised learning for behavioural analysis enables the identification of patterns within data for describing behaviours that are not clearly defined. However, the interpretability of the output of unsupervised machine learning methods can be challenging. [Luxem et al., 2023]. As a result the use of unsupervised learning was found suitable for the behavioural classification in this project.

The aim of this project was to examine the possibility of using unsupervised machine learning methods for classifying aggressive social interaction among pigs. For this purpose K-Means clustering was used to define clusters within the dataset consisting of the extracted features regarding distance measures and acceleration of nodes. K-Means is one of the most commonly used clustering algorithms [Menaker et al., 2022]. The purpose of clustering algorithm for behavioural classification was to divide the dataset into a number of clusters based on the movement patterns within the dataset. Afterwards the clusters were examined by means of a post-clustering analysis in order to interpret the characteristics of the resulting clusters. In order to be able to interpret the behavioural patterns within the clusters, an assumption was made based on the studies described in Chen et al. [2023] and Chen et al. [2017] regarding aggression as being characterised by high acceleration of nodes combined with short distances between the two pigs.

7.4.1 K-Means clustering

K-Means clustering is a type of unsupervised machine learning method used to divide data into smaller groups or clusters. Clustering algorithms aim to define the clusters on the basis of increasing similarity of the data points within the clusters and decreasing similarity of data points in different clusters. [Rodriguez et al., 2019]. In K-means clustering the means of a predefined number of clusters are used to determine the similarity of points within the clusters. Given random initial means, the K-Means clustering algorithm is designed to assign each data point to the cluster with the smallest euclidean distance. The means of the clusters are then updated by calculating the means of the data points included in the individual clusters. The process of assigning cluster labels to data points according to the smallest euclidean distance and updating the cluster means is then repeated until a convergence criteria is met. [Jin and Han, 2011]. In this project the convergence criteria of a maximum of 300 iterations or changes smaller than 0.0001 in the updated means have been applied. In figure 7.13 the iterative steps of updating the cluster means according to the smallest euclidean distance is illustrated.

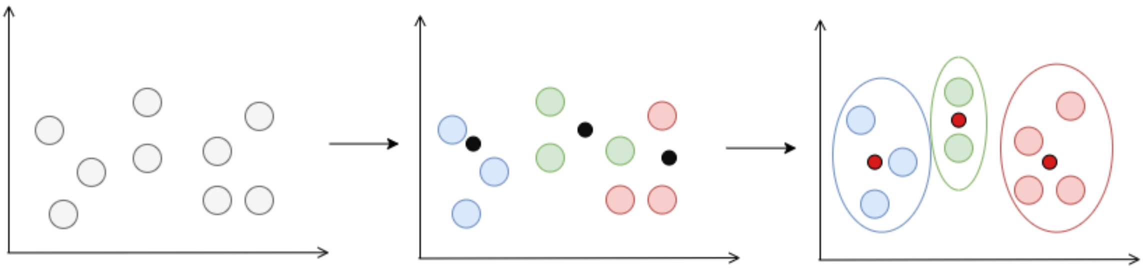

Figure 7.13 *Illustration of the iterative steps involved in the K-Means clustering algorithm.*

In order to determine the most ideal number of clusters within the dataset, the elbow method was utilised. The elbow methods includes running the K-Means clustering algorithm with an increasing number of clusters and calculating the within cluster sum of squares (WCSS) for all models. The WCSS at different number of clusters are then visualised in a plot, enabling visual analysis of the location of the inflection point in the graph. The location of the inflection point indicates the most optimal number of clusters as the the WCSS does not decrease substantially when more clusters are included in the K-Means clustering. [Verma, 2023].

Principal component analysis

The resulting clusters from the K-Means clustering algorithm consist of high-dimensional data, incorporating all six features. In order to visualise the high-dimensional data in fewer dimensions, a principal component analysis (PCA) was used to reduce the dimensionality by computing uncorrelated linear combinations of the original features known as principal components (PC). The use of dimensionality reduction using PCA minimises the loss of information while preserving the variability of the original dataset and increasing the interpretability of large datasets [Jolliffe and Cadima, 2016]. In order to determine the number of PCs needed for visualising the majority of the variance within the data, a scree plot has been constructed. In a scree plot the cumulative explained variance by the PCs are illustrated. The purpose of implementing a PCA to visualise the clusters in lower dimensions is to visually examine the separation of the clusters.

7.4.2 Post-clustering analysis

After using the K-Means clustering algorithm to define a number of clusters within the dataset, a post-clustering analysis was performed in order to examine and interpret the characteristics of the clusters. The post-clustering analysis included conducting descriptive statistics for each feature in the resulting clusters. The purpose of the descriptive statistics is to describe the characteristics of the data within the individual clusters. Thus, the descriptive statistics allows examination of the differences in the data distribution in the individual clusters. In addition, the importance of each feature in the clusters has been examined by means of a feature importance plot, visualising the explained variance. The feature importance plot was used to interpret the most important characteristics of the cluster separation. On the basis of the characteristics of the clusters and the importance of the individual features in each cluster, interpretation of the behavioural patterns within the clusters was performed. Lastly, the distribution of the data in the different clusters before and after the intervention was examined in order to analyse potential differences between the groups with and without neuropathic pain.

8 Results

The following chapter contains results obtained from the utilised methods in the project period. The results include a detailed description of the utilised data, an evaluation of the pose estimation and tracking performance, visualisation of the raw pose estimation and tracking data as well as results from the clustering and post-clustering analysis.

Smaller samples of the video material were selected for the data analysis in order to minimise the factors which could possibly affect the outcome of the analysis. An hour's worth of videos was determined to be a suitable time interval to represent the pigs' undisturbed daily activities. Thus, the intention was to perform the analysis on videos of moments when the pigs were alone and undisturbed, thereby reflecting the non-evoked behaviour of the animals. In Figure 8.1, an illustration of the selected data is presented. Moreover, the information about the groups being intervention or control is provided.

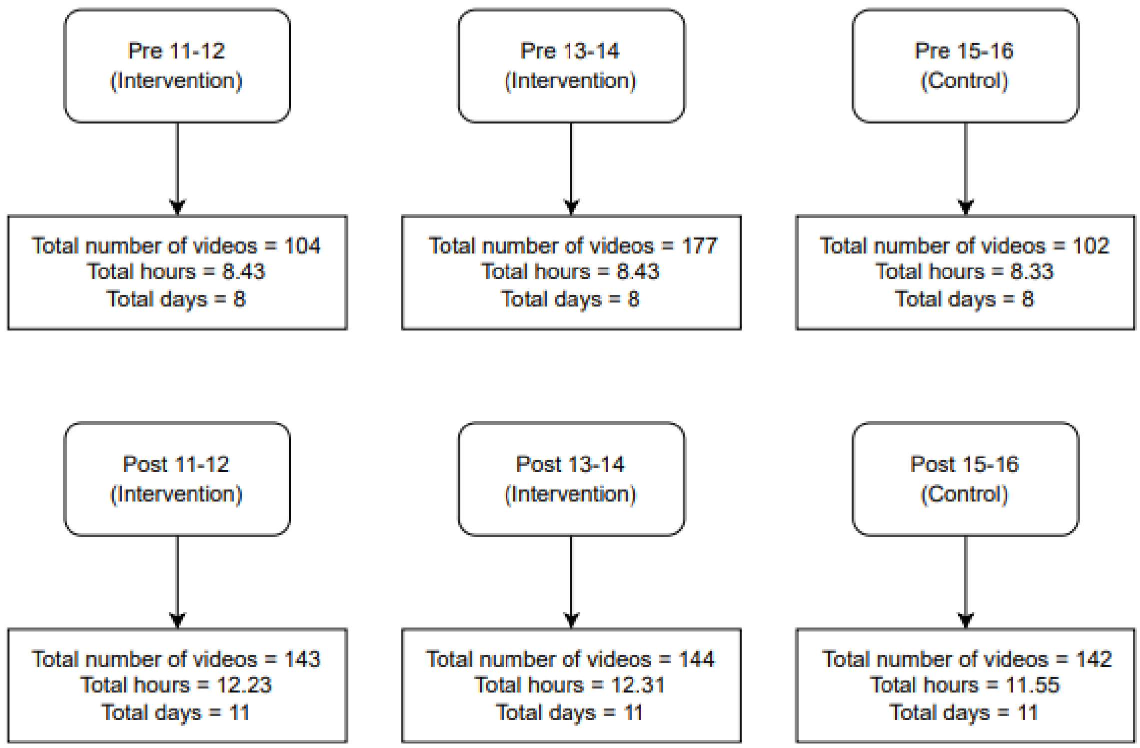

Figure 8.1 *Illustration of the amount of available data after performing the data selection.*

The included time intervals were chosen based on a manual examination of the activity of the pigs during the days. The time interval from 11:00 am to 12:00 pm each day was chosen because the pigs seemed to be relatively alone and undisturbed at this time of the day. However, it was occasionally necessary to instead collect video material from the time interval between 10:00 am and 11:00 am the same day due to the pigs being disturbed by for example training sessions for longer periods of time during the original time interval from 11:00 am to 12:00

pm. Furthermore, missing data was evident since data from all 14 days of the post surgery periods was not available. On some days the recording stopped earlier than intended and on other days the recordings were completely missing. This arguably happened as a result of the camera sometimes overwriting existing recordings and the staff sometimes forgetting to turn on the camera after an SD-card change. In order to achieve similar data sets from each of the three pens, a choice was made to limit the number of days for each pen in accordance with the least available number of days for the groups containing data from the baseline and intervention period, respectively. As a result data from eleven different days were extracted from the post-intervention groups while data from eight days were selected from the baseline groups as illustrated in Figure 8.1. For all six groups the five minute videos from the selected time interval were merged into one video. Thus, the remaining data analysis was performed using six videos.

8.1 Pose estimation and tracking

Pose estimation and tracking of the pigs have been performed by means of SLEAP. The model performance has been continuously evaluated on validation data during the human-in-the-loop training procedure in order to determine when to stop the training procedure. In addition, an estimate of the number of incorrect identifications of nodes as well as an evaluation of the tracking performance has been provided.

8.1.1 Model evaluation

During network training the model performance was continuously evaluated to determine convergence of the model. Specifically, the OKS, precision, and recall metrics were evaluated after each training session. If the model performance increased, the training set was expanded by means of human-in-the-loop correction of predictions. Network training and human-in-the-loop corrections were performed by means of five iterations in total. In Table 8.1 the evaluation metrics for each iteration are presented.

Iteration	Model	OKS	Precision	Recall
5	Centered instance, n = 427	0.07678	0.96347	0.96789
	Centroid, n = 427	0.56213	0.96795	1.00000
4	**Centered instance, n = 385**	**0.13394**	**0.97326**	**0.98913**
	Centroid, n = 385	**0.50140**	**0.99145**	**1.00000**
3	Centered instance, n = 318	0.09364	0.97006	0.96429
	Centroid, n = 318	0.54070	0.99074	1.00000
2	Centered instance, n = 230	0.06272	0.97143	0.95327
	Centroid, n = 230	0.43564	1.00000	1.00000
1	Centered instance, n = 120	0.09899	0.96667	0.96667
	Centroid, n = 120	0.50392	0.97619	1.00000

Table 8.1 *Table showing the evaluation metrics used to determine convergence of the pose estimation models. The variable n refers to the number of frames in the training set and the bold text indicates the iteration in which convergence was achieved.*

From Table 8.1, it is evident that model convergence was achieved during the fourth iteration. Thus, the pose estimation model which had been trained on a training set consisting of 385 manually labelled frames was utilised to predict skeletons on all frames in all six videos. Furthermore, the precision-recall curves for the final centered instance model (n = 385) at different levels of OKS-scores is presented in Figure 8.2.

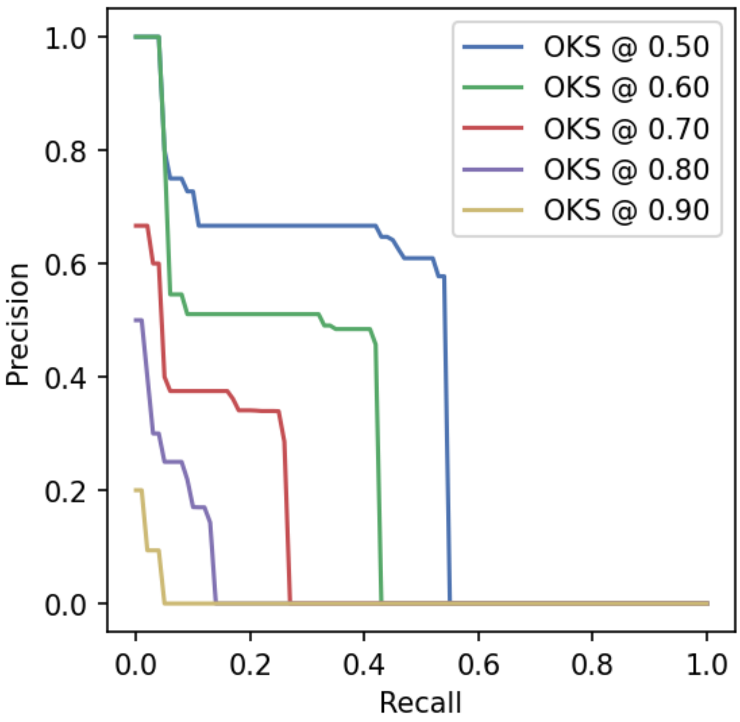

Figure 8.2 *Visualisation of the precision-recall curves for the final centered instance model (n = 385) at varying levels of OKS.*

From Figure 8.2 it is evident that the precision and recall values increase along with a decrease in the OKS-score. The values of precision and recall in the fourth iteration during the network training, as presented in Table 8.1, are relatively high. This is consistent with the OKS-score being relatively low with a value of 0.13.

8.1.2 Estimating the error rate in pose estimation

The number of incorrectly identified nodes in the final pose estimation has been estimated using 20 randomly selected frames from each of the six videos. The estimate was made by visually evaluating the placement of the nodes in the selected frames and assigning the frames to the "correct" or "incorrect" categories according to the evaluation of node placements. An example of a frame containing all three correctly placed nodes are illustrated in Figure 8.3 while an example of some correctly placed nodes are illustrated in Figure 8.4. Both of these examples are categorised as correct nodes placement because an observer would place the nodes similarly.

Figure 8.3 Example of a frame containing all three correctly placed nodes on both pigs.

Figure 8.4 Example of a frame containing correctly placed nodes on all visible parts of the pigs.

Furthermore, Figure 8.5 illustrates an example of incorrect node placement which occurs when nodes are missing from the body parts of the pigs that are visible to an observer. Incorrect placement of nodes can also occur when the nodes are not placed on the pigs as illustrated in Figure 8.6.

Figure 8.5 Example of a frame containing missing nodes on one of the pigs.

Figure 8.6 Example of a frame containing incorrect node placement at points other than on the pigs.

During estimation of the error rate in the pose estimation, the characteristics of the observed node placements were documented as a means of evaluating the type of mistakes that occurred. In Table 8.2, the observed placement of the nodes is presented. In Appendix D: *Examples of node placements*, examples of frames from the different categories are presented.

Description	Category	Percentage	Total
1) Correct skeleton with missing nodes that were not visible	Correct	26.67%	70.84%
2) All three nodes correctly detected	Correct	38.33%	
3) One pig correctly detected while the other pig was hidden behind the gate	Correct	4.17%	
4) One pig hidden behind the other pig	Correct	1.67%	
5) Exchange of nodes in the skeleton	Incorrect	6.67%	29.16%
6) Only one pig detected while the other one was visible in the image	Incorrect	5.83%	
7) The only predicted node was a shoulder node which was not placed correctly but still on the pig	Incorrect	1.67%	
8) A slight misplacement of a single node but it was still on the pig	Incorrect	1.67%	
9) Both skeletons placed on one pig	Incorrect	4.17%	
10) One skeleton spanned across both pigs	Incorrect	5%	
11) Detection of nodes at points other than on the pigs	Incorrect	0.83%	
12) Nodes incorrectly placed while its ideal location is visible	Incorrect	3.32%	

Table 8.2 *Table containing the descriptions of the observed node placement as well as the corresponding categories, describing whether or not the type of node placement was considered correct or incorrect.*

In addition, the number of times the different node placements were observed in the randomly selected frames is provided in Table 8.3, enabling examination of the distribution of the errors across videos.

Desc.	Video 1	Video 2	Video 3	Video 4	Video 5	Video 6	Total	%
1)	6	3	7	5	6	5	32	26.67%
2)	6	11	8	7	8	6	46	38.33%
3)	1	0	0	2	0	2	5	4.17%
4)	0	1	0	0	1	0	2	1.67%
5)	1	1	2	2	0	2	8	6.67%
6)	1	1	1	1	1	2	7	5.83%
7)	1	0	1	0	0	0	2	1.67%
8)	2	0	0	0	0	0	2	1.67%
9)	1	0	1	1	1	1	5	4.17%
10)	0	2	0	0	2	2	6	5%
11)	0	1	0	0	0	0	1	0.83%
12)	1	0	0	2	1	0	4	3.32%

Table 8.3 *Table containing the number of observations of the different types of node placements in each video. In the descriptions colour-coding of the correct and incorrect categories is applied. Thus, green indicates correct node placement while red indicates incorrect node placement.*

From Table 8.2, it is evident that 70.84% of the observed frames contained satisfactory pose estimation in terms of correctly placed nodes. Thus, the estimated error rate is 29.16% based on 120 randomly selected frames. Additionally, it is apparent from Table 8.3 that the distribution of the occurrence of the different types of node placements is comparable in all six videos.

8.1.3 Tracking evaluation

An evaluation of the tracking performance was conducted by manually examining the tracking of two pigs across a single day. The manual examination provided an insight into the types of errors that occurred during tracking. In Table 8.4, descriptions of the different types of errors that were observed during the tracking evaluation are presented along with the frequency of occurrence during the one hour video.

Description	Category	Frequency
Switching of the skeletons occur when the pigs get close to each other or in front of each other	Incorrect	5
One or both pigs are behind the gate, after which the tracks of the pigs are swapped during reidentification.	Incorrect	5

Table 8.4 *Table presenting the types of observed scenarios which could entail switching of the tracks.*

From Table 8.4, it is evident that the tracks on the individual pigs tended to switch in situations where one or more of the pigs disappeared fully or partially behind the gate. Additionally, the tracks switched when the pigs were moving closely to each other, particularly in cases where one of the pigs moved in front of the other from the perspective of the camera. In Figures 8.7 and 8.8, an example of a track switch occurring as a result of one of the pigs partially disappearing behind the gate is presented.

Figure 8.7 *Original track*

Figure 8.8 *Switched track*

Similarly, In Figures 8.9 and 8.10 an example of a track switch occurring as a result of the two pigs being located near each other is presented.

Figure 8.9 *Original track*

Figure 8.10 *Switched track*

The timestamps of the track switching occurrences were noted, enabling examination of the amount of time in which the tracks were switched. The time spent in the individual tracks along with the timestamps of the beginning and end of the time interval are presented in table 8.5.

Track	Time
Start (timestamp)	10:55:13
Original	04:22
Switched	00:03
Original	25:30
Switched	03:28
Original	12:25
Switched	04:18
Original	01:35
Switched	00:23
Original	00:32
Switched	00:44
Original	11:49
Stop (timestampe)	12:00:22

Table 8.5 *Table presenting the time spent in the individual tracks along with the timestamps of the beginning and end of the time interval.*

In Figure 8.11 a corresponding pie chart is presented illustrating the total amount of time in which the original tracks and the switched tracks were observed within the hour. The original track was considered as the observed identification of the individual pigs in the beginning of the specific day while the switched track was considered the opposite identification.

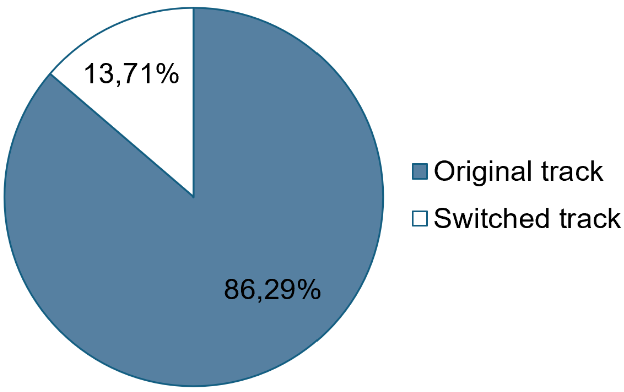

Figure 8.11 *Pie chart illustrating the time distribution across the original and the switched track.*

From the pie chart visualised in Figure 8.11 it is evident that the original identification of the individual pigs was observed in 86.29% of the time while the switched identification was observed in 13.71% of the time. Thus, it is evident that switches sometimes occur during the days and it was therefore not possible to distinguish between individual pigs based on the tracks. As a result, distinguishing between the individual pigs has not been employed throughout the rest of the data analysis.

8.2 Data visualisation

The tracking data consists of coordinates for each of the three nodes on the two pigs. These coordinates are stored within a locations matrix for each video in which the columns represent the number of frames, the number of nodes, and the coordinates for each node, respectively. In order to visualise the movement of the pigs, an example of a heatmap of the pigs' movement within one hour is visualised in Figure 8.12 along with an image of the pen in Figure 8.13.

Figure 8.12 *Example of an image of a pen.*

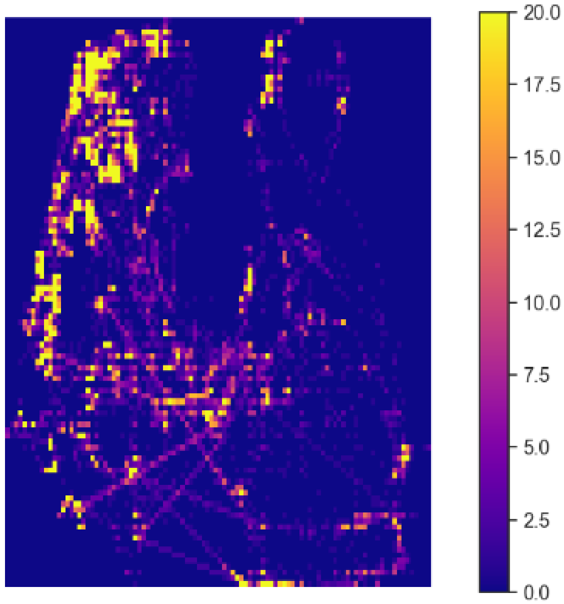

Figure 8.13 *Example of a heatmap illustrating the movement of the pigs within the pen during one hour.*

From the video material it was evident that the pigs often were located in the side of the pen with the bedding material in which they slept. This is supported by the heatmap of oberservations made by the computer vision model as seen in Figure 8.13.

Also, the amount of social interaction has been examined using the distance between the two pigs. When the pigs are closer to each other, the likelihood of their interacting is larger than when they are far apart. In Figure 8.14 an example of a histogram illustrating the number of observations at certain distances for group post 11-12 is presented. The histograms for the remaining groups are presented in appendix E: *Observations of distances.*

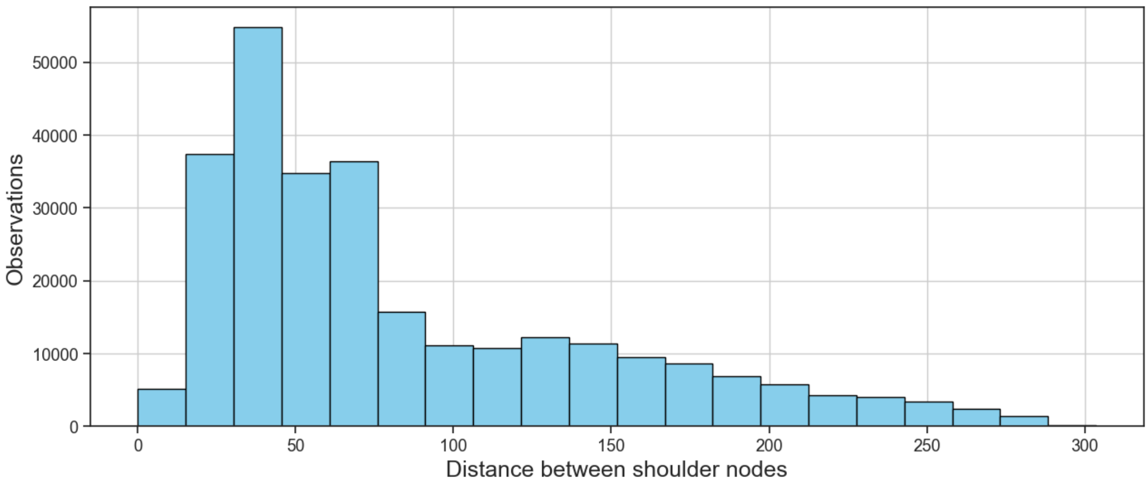

Figure 8.14 *Histogram visualising the amount of observations at certain distances between the two pigs.*

From Figure 8.14 and the histograms for the remaining groups it is evident that the histogram is skewed to the left. Thus, the pigs have often been located near each other, increasing the possibility of social interaction.

Furthermore, an example of a visualisation of the acceleration of the snout node across frames for group post 11-12 is illustrated in figure 8.15, providing a visual indication of the pigs' activity and movement across time. In Appendix F: *Acceleration across frames* visualisations of the snout acceleration across frames for the remaining groups are provided.

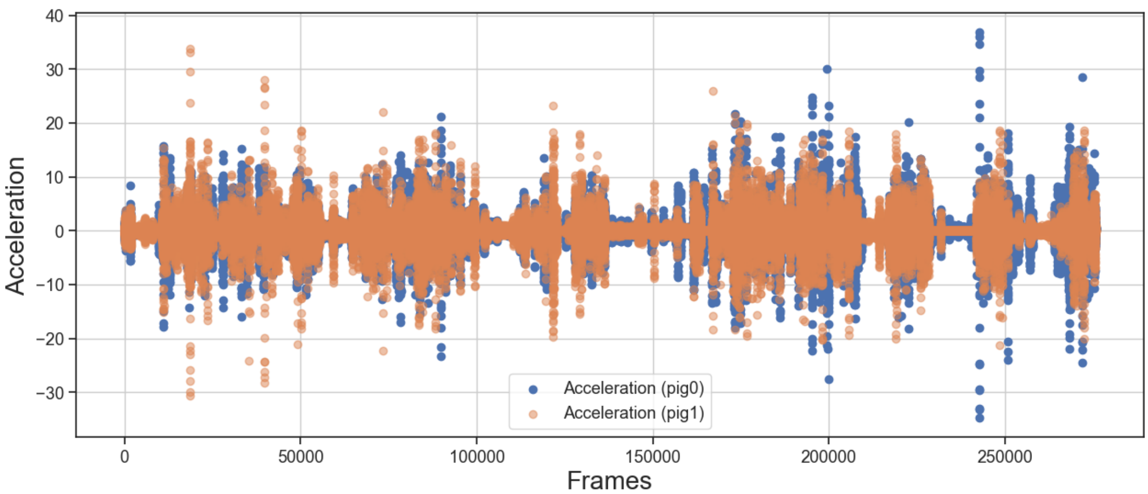

Figure 8.15 *Illustration of the acceleration of the snout node across time in terms of frames.*

From Figure 8.15 it is apparent that the acceleration of the snout nodes on the pigs appears to be somewhat synchronised, suggesting similar acceleration patterns at specific time intervals.

Lastly, the snout acceleration and distance between the shoulder nodes on the pigs in group post 11-12 have been visualised in Figure 8.16. The acceleration across distance diagrams for the remaining pens are presented in Appendix G: *Acceleration across distance*.

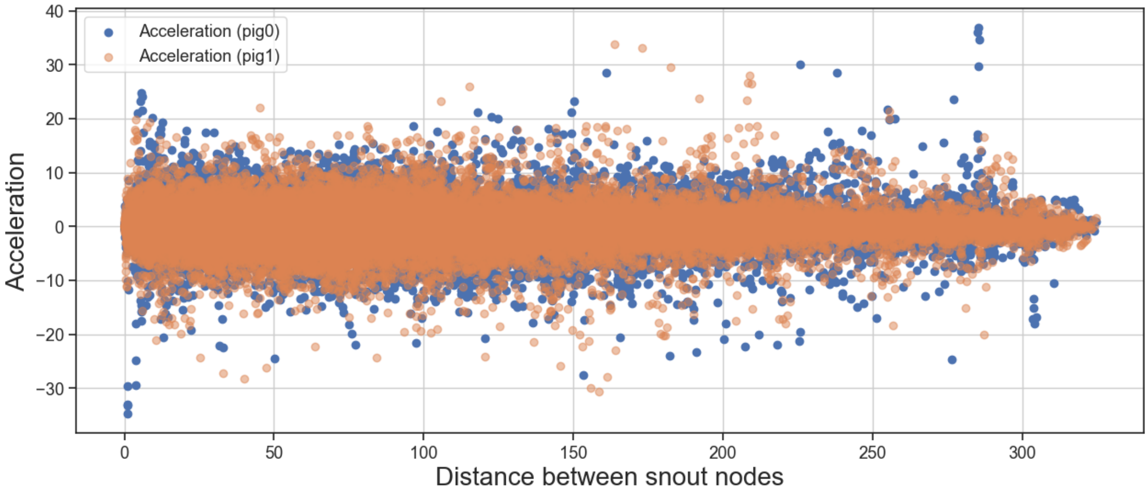

Figure 8.16 *Illustration of the acceleration of the snout nodes in relation to the distance between the shoulder nodes on the individual pigs.*

It is evident from Figure 8.16 that the greatest variation of the acceleration appears to occur at the smaller distances between the pigs. Meanwhile, outliers are evident at all distances.

8.3 Clustering analysis

In order to analyse the potential movement and behavioural patterns in the data using K-Means clustering, the ideal number of clusters was estimated using the elbow method. In Figure 8.17 the resulting graph from applying the elbow method is presented.

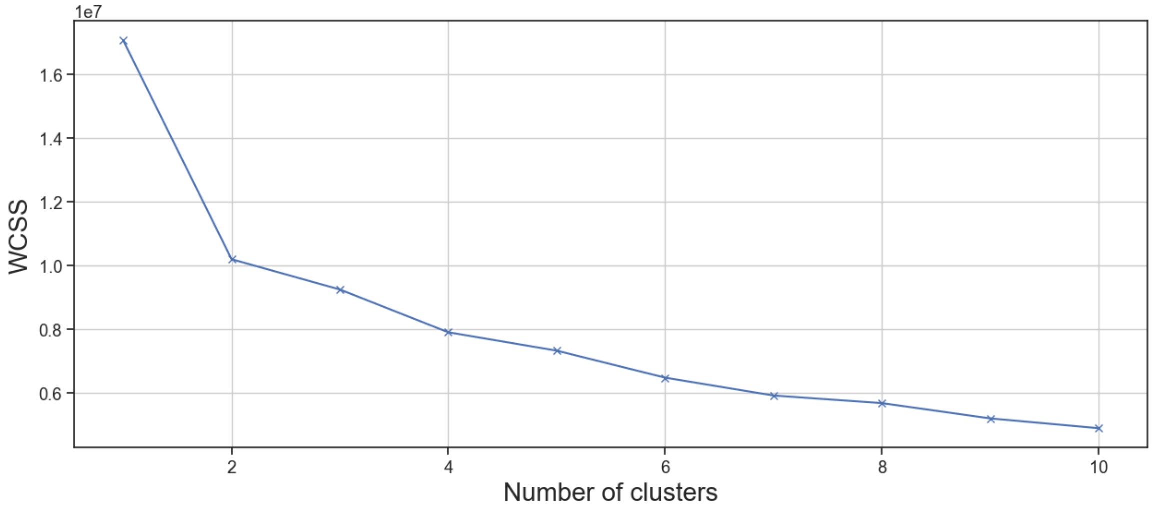

Figure 8.17 *Visualisation of the resulting graph from applying the elbow method to the dataset, displaying the WCSS at varying numbers of clusters in the K-Means clustering.*

It is evident from Figure 8.17 that two to three is the optimal number of clusters for the K-Means clustering on this dataset. A choice was made to conduct the analysis using three clusters. This was due to the movement data being continuous in terms of the transitions from different types of behaviours being fluent. Thus, it can be argued that the data might not contain clearly defined natural clusters. As a result, the choice of defining three clusters were made in order to incorporate the transition between at least two behavioural clusters.

The knowledge regarding the optimal number of clusters was then used to define three clusters in the dataset using K-Means clustering. On the basis of the six extracted features, K-Means clustering was used to define three clusters based on the best possible separation between the data points included in the different clusters. In Figure 8.18 an example is shown, illustrating the resulting clusters when displayed in relation to acceleration of the snout node and the distance between the snout nodes of each pig.

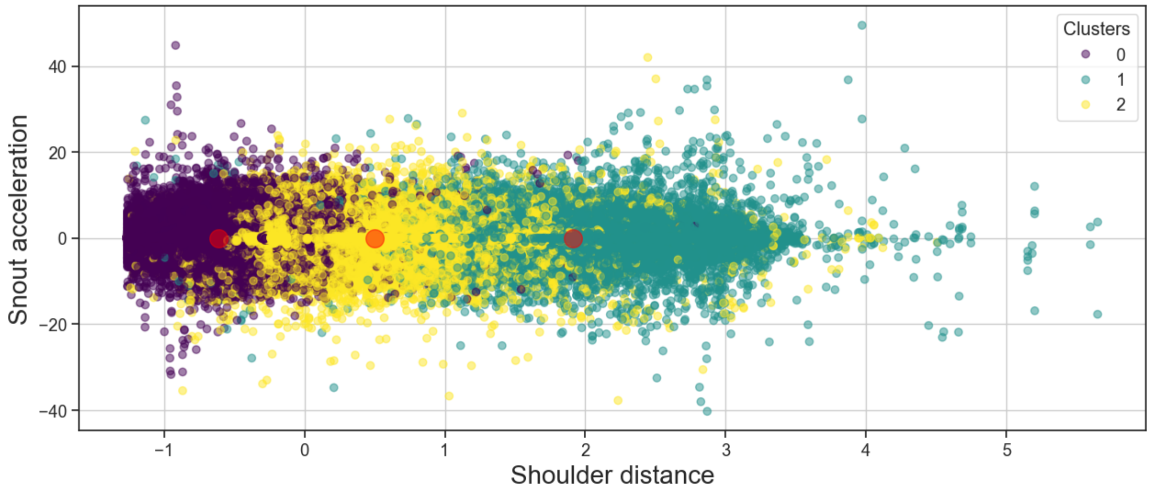

Figure 8.18 *Example of a cluster plot when displayed in relation to snout acceleration and the distance between the snouts of the two pigs. The colouring of the data points represent the appertaining cluster while the red dots represent the cluster means.*

From Figure 8.18 it is evident that clearly separated clusters are not visible when visualising the clusters using the two dimensions: Snout acceleration and shoulder distance.

8.3.1 Principal component analysis

PCA analysis has been used to reduce the dimensionality of the feature space by computing uncorrelated linear combinations of the original features. Using PCA to visualise the results from the clustering analysis enables visual examination of the separation between clusters in two dimensions. Before performing PCA, the number of principal components was determined using the cumulative explained variance by the PCs. In Figure 8.19 the cumulative explained variance by the PCs are visualised in a scree plot.

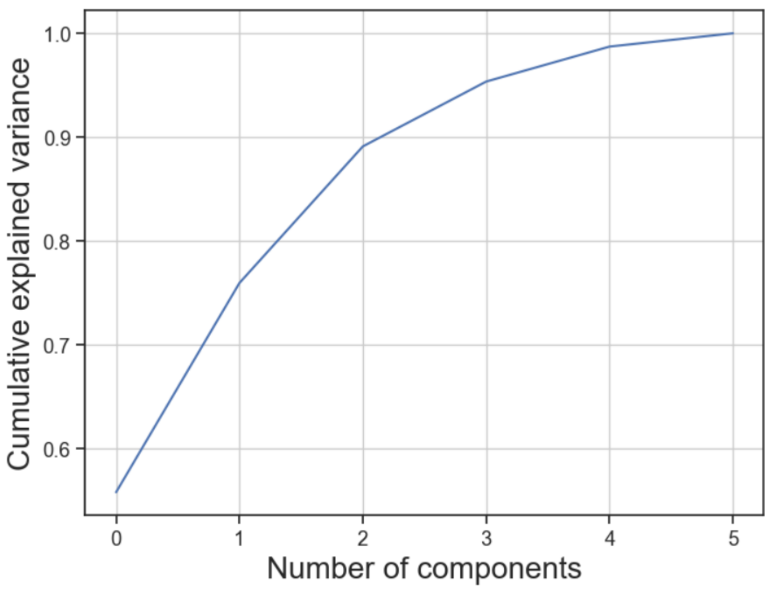

Figure 8.19 *Scree plot illustrating the cumulative explained variance from the PCA.*

From Figure 8.19 it is evident that approximately 95% of the variance in the dataset can be explained by the first four PCs while approximately 75% can be explained with the first two PCs. On the basis of the two first PCs, PCA plots have been constructed to show the distribution of the data with respect to the first two PCs. Thus, about 75% of the variance within the dataset is to be represented in the PCA plots. Two PCA plots have been constructed and visualised in figure 8.20 and 8.21 representing the clusters and the groups, respectively. In both PCA plots, the means of the three clusters or the six different groups are visualised. By including the means of the clusters or groups in the plots, it was possible to visually examine the distribution of the data.

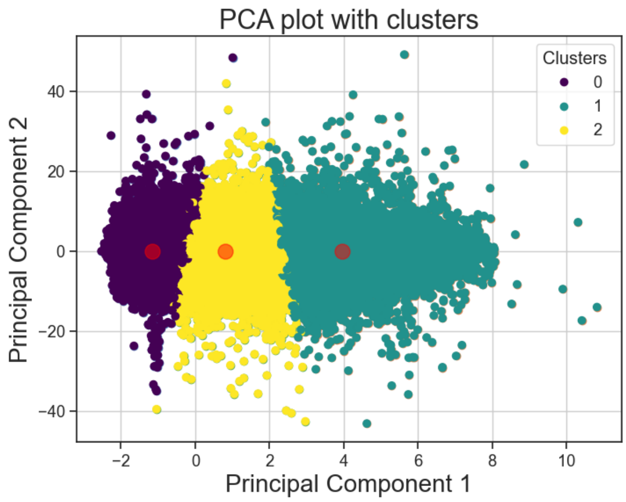

Figure 8.20 *Visualisation of a PCA plot, displaying the distribution of the data in the clusters in relation to the two first PCs.*

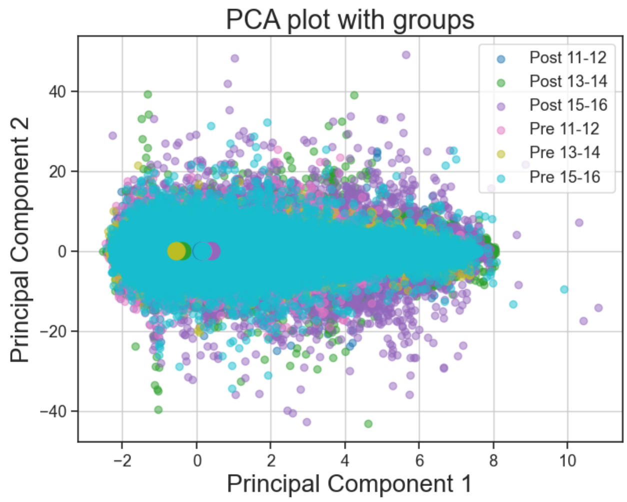

Figure 8.21 *Visualisation of a PCA plot, displaying the distribution of the data in the different groups in relation to the two first PCs.*

From Figure 8.20 it is evident that the data points in the three clusters are not clearly separated within the PCA feature space. This suggests that the movement and behavioural patterns which the clusters potentially represent are not naturally separated. Additionally, it is evident from Figure 8.21 that no visible difference in the movement patterns between the six groups exist on the basis of the two first PCs which represent around 75% of the variance within the dataset.

8.4 Post-clustering analysis

In order to gain insight into the results from utilising the K-Means clustering algorithm on the dataset, a post-clustering analysis was performed. The post-clustering analysis consisted of a visualisation of the feature importance and calculating descriptive statistics of the data within the individual clusters. The importance of the individual features in describing the clusters has been examined by visualising the variance explained by the features. In Figure 8.22 an illustration of the feature importance is presented.

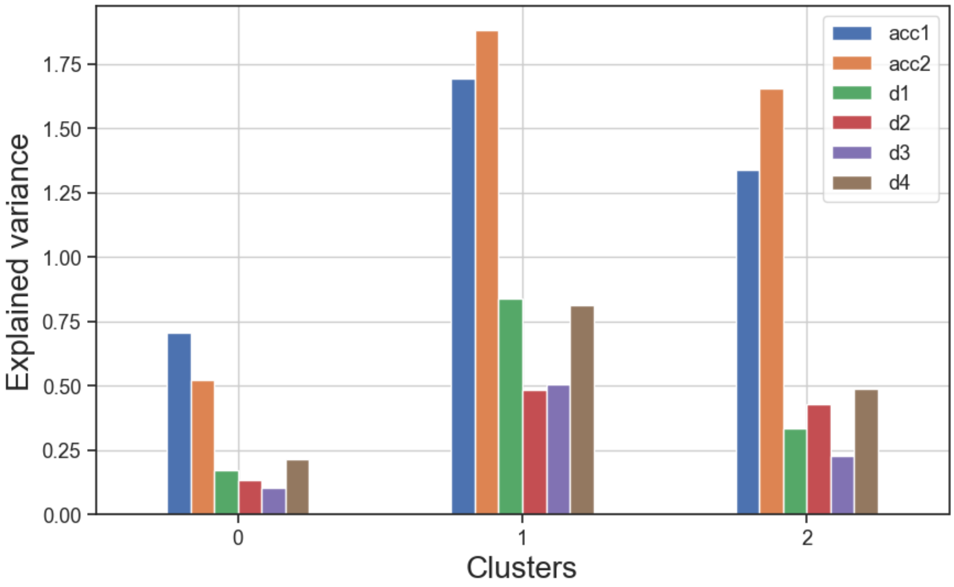

Figure 8.22 *Plot illustrating the feature importance in the clusters in terms of the variance explained by the features.*

From Figure 8.22 it is apparent that the features regarding snout and shoulder acceleration appear to be the two features that explain the highest degree of variance within the clusters. Thus, the two acceleration features appear to be of importance since these features have the greatest influence on the model predictions.

Furthermore, descriptive statistics have been calculated for all included features in each of the three resulting clusters. The descriptive statistics is performed on the scaled data and includes the means of the features in each cluster, the minimum and maximum values, the standard deviation, and the 25th, 50th, and 75th percentile. In Table 8.6 an example of descriptive statistics for the acc1 feature concerning the acceleration of the snout node is presented. In Appendix H.1: *Descriptive statistics*, the descriptive statistics for the remaining features are provided.

Cluster	Mean	Std	Min	25%	50%	75%	Max
0	0.011	0.839	-31.740	-0.076	-0.000	0.079	44.814
1	0.005	1.302	-40.317	-0.112	-0.000	0.112	49.476
2	-0.027	1.157	-37.788	-0.098	-0.000	0.080	41.997

Table 8.6 *Table containing the descriptive statistics for the acc1 feature, representing acceleration of the snout node.*

In addition, the means of each feature along with the standard deviations in the clusters have been combined in Table 8.7 to examine the characteristics of the data within the clusters. The means of each cluster constitute a six-dimensional coordinate in the feature space.

	acc1		acc2		d1		d2		d3		d4	
	Mean	Std	Mean	Std	Mean	Std	Mean	Std	Mean	Std	Mean	Std
C 0	0.01	0.83	0.01	0.72	-0.57	0.41	-0.62	0.36	-0.62	0.32	-0.52	0.47
C 1	0.01	1.30	0.00	1.37	1.96	0.92	1.92	0.70	1.92	0.71	1.92	0.90
C 2	-0.03	1.16	-0.02	1.29	0.39	0.58	0.50	0.65	0.41	0.48	0.30	0.70

Table 8.7 *Table containing the mean values and corresponding standard deviations of features in the three clusters. In the first column C indicates the respective clusters.*

It is apparent from the coordinates of the cluster means presented in Table 8.7, that cluster 0 is characterised by the smallest distances and highest accelerations when compared to the two other clusters. Meanwhile, cluster 1 is characterised by the largest distances and medium accelerations. Lastly, cluster 2 is characterised by the lowest accelerations and medium distances.

Furthermore, the proportion of observations in the individual clusters for each of the six groups were calculated in order to examine the distribution of the data from each group in the individual clusters. On Figure 8.23, the proportions of observations within the groups are visualised in a bar plot. The specific percentages of the calculated proportions are available in Table H.6 in Appendix H.2: *Proportions within clusters.*

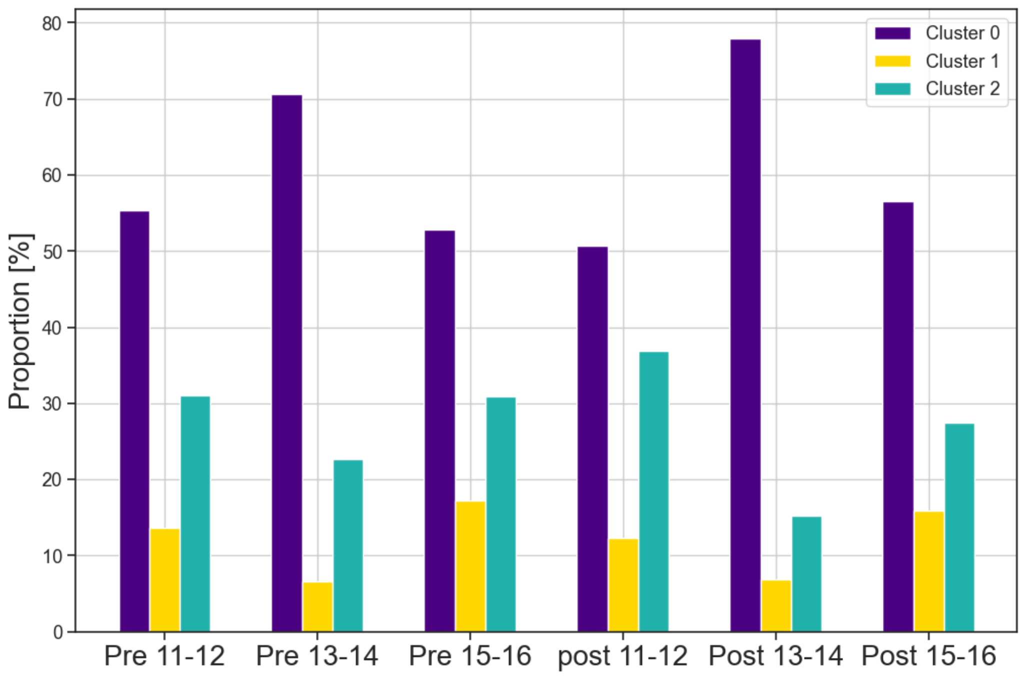

Figure 8.23 *Bar plot illustrating the proportion of observations within the three clusters in each group.*

Based on the amount of observations from each group within each cluster as visualised in Figure 8.23, the changes in the number of observations from baseline to intervention period have been calculated. The resulting changes are provided in Table 8.8. These results were used to determine whether or not a difference between the number of observations in each cluster was evident before and after intervention and whether these changes were similar across intervention and control groups.

Pairs of pigs	Cluster	Change [%]
11-12 (Intervention)	0	-4,69%
	1	-1,21%
	2	5,89%
13-14 (Intervention)	0	7,25%
	1	-0.03%
	2	-7,51%
15-16 (Control)	0	4,79%
	1	-1,36%
	2	-3,42%

Table 8.8 *Table presenting the change in proportion of observations within the three clusters for each pair of pigs before and after the intervention. Information about whether the groups were control or intervention groups is also provided.*

From Table 8.8, it is evident that the proportion of observations in cluster 0 increases in two out of three pairs from baseline to the intervention period while one pair decreases. Additionally, the proportion of observations in cluster 1 decreases in all three pairs. Lastly, the proportion of observations in cluster 2 decreases in two out of three pairs while one increases.

Part III

Synthesis

9 Discussion

The aim of the project was to examine the possibility of utilising computer vision and machine learning methodologies for classifying social interaction among pigs in a neuropathic model. Specifically, the project focused on detection of aggressive social interaction with the purpose of using aggression as an outcome measure for addressing non-evoked pain related behaviour. The use of outcome measures focusing on non-evoked pain related behaviour for addressing spontaneous pain have the potential to improve the translatability of findings from preclinical trials using porcine neuropathic models to humans in a clinical setting. This is due to spontaneous pain being the primary symptom of neuropathic pain in humans.

9.1 Results

The results obtained throughout the project were based on videos of three different pairs of pigs. The video material was divided into six groups in total based on whether the videos were collected in the baseline period or the intervention period for each pair. Pose estimation and tracking data was obtained by means of SLEAP. K-Means clustering was used to classify behavioural patterns based on features regarding acceleration of the pigs and distances between the pigs extracted from the pose estimation and tracking data. When analysing the changes in proportions of observations in each cluster for the three pairs of pigs from the baseline period to the intervention period, no correlation seems to be evident across intervention and control groups. Similarly, no correlation is apparent between baseline and post-surgery periods. Thus, the data analysis conducted during this project arguably does not indicate a correlation between experiencing neuropathic pain and the expression of different types of behaviour.

9.1.1 Pose estimation and tracking

The quality and validity of the result of the behavioural analysis are dependant on the quality of the input data. According to Low et al. [2022], the performance of classification methods can be significantly affected since the quality of the extracted spatio-temporal features relies on accurate spatial depiction. Thus, the quality of the pose estimation and tracking data obtained by means of SLEAP should be addressed. The evaluation of the pose estimation in terms of the estimation of the error rate of node placements suggests that 70.84% of 120 randomly selected frames contained correctly placed nodes. Assuming that the 120 frames are representative of all the frames in the videos, the visual examination of the performance indicates that the placement of nodes are considered correct in the majority of the frames. Furthermore, the tracking performance was evaluated by manually examining the tracking of two individual pigs across one hour. From this evaluation, it is evident that switches between the individual tracks on the pigs do occur in cases where the pigs are moving closely to each other, disappear behind each other or disappear behind the gate. Similarly, it has not been possible to distinguish between the individual pigs across the different days. It can be discussed how the occurrence of the switches between tracks has potentially affected the data throughout the rest of the analysis. On one hand, the switches result in a miscalculated acceleration between the adjacent frames since the switch is interpreted as movement. On the other hand, the miscalculated accelerations constitute a small proportion of the accelerations used in the data analysis. For addressing the issues regarding the switching between tracks, methods for improving the likelihood of correct reidentification could have been implemented during the trial. Such measures include the use of colour-coding of the pigs to ensure correct identification. Additionally, filming the pen with a top-down view would minimise the risk of the pigs disappearing within the frames. However, placing the camera with a top-down view has not been possible during the trial due to the construction layout of the pens.

Furthermore, the visualisation of the features extracted from the pose estimation and tracking data regarding acceleration of nodes and distances between the pigs suggest that the pigs interact with each other during the videos. This is due to the pigs often being located near each other while the accelerations of the two pigs seem to be somewhat synchronised when visualised across frames. Thus, it is argued that there is a potential in analysing social interaction based on the extracted features from the pose estimation and tracking data. It can be discussed whether the perspective of the camera has influenced the extracted features regarding distance and acceleration, impacting the results obtained in the clustering analysis. Variations of the distance measure arguably occur depending on the pigs' location in the pen. However, it has not been possible to assess the extent of this influence on the extracted features in the current analysis. If further analysis were to be conducted, the impact of the camera's perspective on the extracted features should be examined more closely.

9.1.2 Clustering analysis

For conducting the clustering analysis, the optimal number of clusters has been determined using the elbow-method. However, determining the appropriate number of clusters for this analysis can be challenging due to the continuous nature of the data. The transitions of the pigs moving from one type of behaviour to another result in fluent transitions between the different types of behaviour. Fluent transitions between classes in data can be challenging for unsupervised classification using the K-Means algorithm. In the K-Means clustering algorithm, it is assumed that all data points belongs to a specific cluster [Kharkar, 2023; Rodriguez et al., 2019]. Thus, when the transitions between the clusters are fluent, the data point may be ambiguously assigned to individual clusters, reducing the accuracy of the clustering. Additionally, the fluent transitions between clusters may result in the model converging to a solution which does not accurately represent the natural grouping within the data. This is a result of the K-Means clustering being sensitive to the initial placement of the cluster centroids [Rodriguez et al., 2019]. Furthermore, the expression of different behaviours is complex and possibly not clearly distinguishable [Larsen et al., 2023]. It is therefore recognised that multiple types of behaviours with fluent transitions exist in the dataset due to the pigs moving freely around in the pen as they engage in their daily activities. Examples of observed behaviours include social interaction, sleeping, eating, playing, and defecating. The knowledge about the existence of multiple types of different behaviours within the data should be recognised when considering the optimal number of clusters. However, the data does not necessarily reflect all the behaviours in terms of the selected features. The elbow plot indicates an inflection point at approximately two clusters. Although the elbow plot may not display a definitive inflection point, considering the complexity and continuous nature of the behaviours displayed in the data. When considering a model with two clusters, it is therefore recognised that more than two types of behaviours exist and the continuous nature of the behavioural data could entail the need for a cluster containing the transition between behaviours. Another approach could be to apply other unsupervised classification methods that may be better suited for data with overlapping clusters. For example, the fuzzy K-Means algorithm is an extension of the K-Means method that allows for classification with overlapping clusters [Cleuziou, 2009].

Furthermore, the resulting clusters arguably represent the behaviours which occur most often and contain distinguishable movement patterns. However, the clusters may not only represent one type of behaviour due to the complexity of behavioural patterns. Thus, a possibility is evident of the less frequently expressed behaviour types disappearing within the clusters. For interpreting the behavioural patterns in the clusters, the mean values of the clusters within the original feature space have been examined. Based on the mean values for each feature, the highest acceleration and lowest distances are indicative of the data in cluster 0. An assumption of aggressive social interaction being characterised by high acceleration and low distance was made on the basis of the studies described in Chen et al. [2023] and Chen et al. [2017]. It can therefore be argued that the behavioural patterns related to aggressive social interaction might be located in cluster 0. However, the proportion of observations belonging to cluster 0 was higher than the other two clusters, indicating that most of the time the pigs expressed this respective behaviour. Thus, cluster 0 does not solely indicate aggression, as the pigs would have been separated if they displayed such a high level of aggression. Moreover, it can be difficult to distinguish between aggression and other similar behaviours like playing for an observer as well as a computer vision model. This is due to both types of behaviours occurring sporadically and being short-lasting [Larsen et al., 2023]. Thus, it can be argued that cluster 0 potentially represents social interaction. Additionally, the proportion of observations are the smallest in cluster 1 which is characterised by the largest distances and the medium acceleration. As a result cluster 1 could arguably represent defecating behaviour, which could possibly be characterised by larger distances between the pigs and varying amount of acceleration. The characteristics of the defecating behaviour are coherent with the findings from the data analysis due to the pigs often not defecating at the same time, resulting in larger distances between them. In addition, the proportion of observations in cluster 1 for each group being the smallest, in comparison to the other two clusters, is consistent with defecating behaviour being a less frequent type of behaviour. Lastly, it can be argued that cluster 2 represents the transitions between different types of behaviour due to cluster 2 being located between the other two clusters in the features space.

9.2 Methodological considerations

The structured literature search revealed that no other studies focusing on automatic recognition of aggression among pigs as an outcome measure for non-evoked pain had previously been conducted. However, the possibilities of utilising automated behaviour analysis for recognising aggression have been extensively researched in the field of pig production. Thus, experiences and methodological considerations from this research can arguably be applied to the study of aggression in pigs as a measure of non-evoked pain in pain research. As a result, the computer vision and machine learning methods used throughout this project have been inspired by research of automated behaviour analysis in the field of pig production as well as studies of automated behaviour analysis in other species.

9.2.1 Behavioural classification

In this project, unsupervised machine learning has been chosen as a strategy for examining the possibility of classifying behavioural patterns. The structured literature search revealed that the majority of studies focusing on automated behaviour analysis in pigs have employed supervised deep learning based methods. The deep learning based methods have the ability to accurately detect specific predefined behaviours [Siegford et al., 2023; Luxem et al., 2023]. However, the deep learning based methods require a substantial amount of labelled training data which can be difficult to obtain due to manual labelling being time-consuming and potentially biased. Also, the process of labelling specific types of behaviours requires extensive knowledge regarding the ethology of the target species. [Siegford et al., 2023]. Similarly, other supervised learning methods also require the knowledge of ground truth labels. As opposed to the supervised learning methods, unsupervised machine learning methods do not require labelling or knowledge regarding the expressed behaviour as ground truth. However, unsupervised methods require extraction of relevant features for conducting a meaningful analysis. Thus, the researchers should be able to determine what features could possibly be relevant for the specific analysis. On the contrary, deep learning methods allow predictions based on complex patterns that may not be apparent to researchers. According to Luxem et al. [2023], unsupervised machine learning methods are suitable if the purpose of the analysis is examining the spatio-temporal structure of a behaving animal. It allows for a more exploratory approach, uncovering hidden structures within the data without predefined labels. Similarly, Chen et al. [2023] argues that their implementation of an unsupervised machine learning method enables unbiased identification of behavioural patterns. However, interpretability of the output of unsupervised techniques can be challenging. Thus, the unsupervised learning methods can be challenging to validate when compared to the relatively straightforward evaluation process in supervised learning.

It can be discussed whether the choice of the K-Means clustering algorithm is the most optimal machine learning method, considering the results and experiences from this data analysis. The spatio-temporal data of the pigs' movement in the pens does not contain clearly defined clusters representing different types of behaviours due to the continuous nature of the data. As a result, it can be argued that the K-Means clustering algorithm may not perform optimally on spatio-temporal data. Instead, it can be argued that features that do not represent spatio-temporal information within the data should be extracted for the K-Means clustering algorithm to perform optimally. In Menaker et al. [2022], an example of the use of K-Means clustering for behavioural analysis in dogs is described. For this analysis, no spatio-temporal features are used and the nature of the data is therefore not continuous. On the contrary, the methods described in Chen et al. [2023] are designed specifically to spatio-temporal behavioural data. For the behavioural classification in this pipeline, hierarchical clustering is applied, based on the argument that behavioural data has a hierarchical nature [Chen et al., 2023]. As a result, it can be argued that comparison of different unsupervised machine learning methods should be a focus of further work. Specifically, the potential of hierarchical clustering may be valuable for the data used in this project.

Furthermore, in the study described in Chen et al. [2017], hierarchical clustering has been used to determine a threshold for classifying medium and high aggression between pigs. For this analysis, key frame sequences were extracted to minimise the amount of computation required in the analysis. The key frame sequences consisted of subsets of at least five seconds in which the pigs were located near each other and specific movement patterns occurred. Hereby, the amount of video material included in the feature extraction process was limited to the sequences of interest. [Chen et al., 2017]. Similarly, the study described in Viazzi et al. [2014] reduces the amount of video sequences included in the classification analysis by extracting episodes of social interaction. As mentioned earlier, the intention of utilising EthoVision during this project was to extract the sequences in the videos containing social interaction, enabling the classification of different types of social interaction. It can be argued that limiting the amount of video material to only include relevant sequences where aggression or social interaction occurs could potentially refine the evaluation of behavioural patterns within the resulting clusters. Consequently, the resulting clusters from the classification analysis would more likely represent different types of social behaviours rather than other more frequent behaviours. Thus, the aggression and other types of social interactions might not disappear within the clusters due to the large amount of behavioural data. Furthermore, it can be discussed whether an implementation of a threshold in terms of distance between the pigs could have been applied during the analysis of social interaction using the pose estimation data. On one hand, a threshold in terms of distance between the pigs could potentially enable the analysis to focus solely on social interaction due to the assumption of the pigs being located close to each other when interacting. On the other hand, the challenges posed by the perspective of the camera result in variations in the distance measurements depending on the pigs' location within the pen.

In Chen et al. [2017], the classification of medium and high aggression is performed by defining thresholds for the acceleration involved in the different types of aggression episodes. The thresholds were defined using hierarchical clustering. [Chen et al., 2017]. It can be discussed whether the approach of determining one or more thresholds based on the amount of acceleration could be advantageous in the data analysis conducted throughout this project. Though would the use of a threshold in the analysis possibly enable distinguishing between aggressive social interaction and non-aggressive or positive social interaction. On the other hand, the analysis in this project includes movement data from multiple different behaviours and the different types of social interaction might therefore not be displayed in different clusters. However, it can be argued that one of the clusters potentially reflects social interaction. Thus, further analysis of the data within that particular cluster, e.g. using thresholds, might provide valuable insight into the different types of social interaction and possibly enable classification.

Consequently, validation of the utilised methods and interpretation of behavioural patterns in the clusters are needed in order to assess whether the behaviours are in fact correctly classified. In the studies described in Chen et al. [2017] and [Viazzi et al., 2014], manually labelled data is used to evaluate the model performance. However, according to Siegford et al. [2023] the process of manually labelling animal behaviours should be performed by observers with expert ethological knowledge in order to minimise bias. As a result, it can be argued that validation of the behavioural interpretation of the resulting clusters from the data analysis conducted in this project could be of relevance if further analysis were to be performed.

9.3 Scientific relevance

In this project it is described how the use of clinically relevant outcome measures in preclinical studies using animal models are a prerequisite of achieving translational success of findings from preclinical trials to humans in a clinical setting [Deuis et al., 2017]. It can be discussed whether aggression can be utilised as an outcome measure representing non-evoked pain. In Jhumka and Abdus-Saboor [2022] it is described that aggressive traits can be one of many different types of complex behaviours which can be demonstrated by animals as a means to express pain. Conversely, the complexity of aggression in pigs affects the possibility of automatic classification of social interaction. Pigs are gregarious animals that establish a hierarchy within a group [Landsberg and Denenberg, 2024]. The hierarchy affects the behaviour of the individual animals, and potentially compromises the possibility of associating aggression with the experience of pain. According to Landsberg and Denenberg [2024], aggressive behaviour among pigs who have already established a hierarchy does not necessarily involve physical fights but can instead involve warnings in terms of threatening postures. Thus, aggressive behaviour can be expressed in ways which challenge the assumption of high acceleration and short distances being characteristic of aggression.

The implementation of new outcome measures in pain research requires establishing clinical relevance as well as thorough validation of the utilised methodologies. During this project, it has been established that there is a growing interest in behavioural analysis for pain quantification in rodents [Tappe-Theodor et al., 2019]. This is due its potential in translatability from preclinical studies to the clinic by addressing the spontaneous pain aspect of chronic pain [Tappe-Theodor et al., 2019; Deuis et al., 2017]. In rodents, the use of non-stimulus evoked outcome measures has been researched and methods such as home-cage monitoring are increasingly becoming available [Tappe-Theodor et al., 2019]. However, to the knowledge of the authors, a similar interest in the field of porcine neuropathic models has not been evident. This project provides a first step in the direction towards an objective, unbiased and clinically relevant outcome measure in a porcine neuropathic model. Nevertheless, validation of new methods for pain quantification are challenging due to the complex nature of the pain experience and difficulties in defining the specific behavioural measures related to non-evoked pain [Deuis et al., 2017]. Accordingly, animal behaviour is complex and no single behavioural assay is capable of describing all aspects of the pain experience [Deuis et al., 2017]. As a result, the necessary validation of behavioural outcome measures of pain is challenging, and implementing new measures is a long and complex process.

10 Conclusion

The aim of the project was to develop an objective and clinically relevant outcome measure in a porcine neuropathic model using computer vision and machine learning for classification of social interaction. Pose estimation and tracking methods were used to obtain pose and tracking data, representing the movements of the pigs throughout the pen. Based on an evaluation of the pose estimation performance, it can be concluded that the node placements were correct in 73.34% of the 120 randomly selected frames. Meanwhile, the evaluation of the tracking performance revealed that distinguishing between individual pigs within a pen was not possible as a result of occasional track switches during the reidentification process. However, it can be concluded that the tracking algorithm performed adequately for the extraction of distance and acceleration features, provided that individual pigs were not differentiated. Furthermore, the interpretation of the three resulting clusters from the K-Means clustering analysis indicate that social interaction, including aggression, might be represented in cluster 0. Meanwhile, cluster 1 and cluster 2 arguably represent defecating behaviour and the transition state between behaviours, respectively. Based on the proportion of observations within the respective clusters before and after the intervention, no correlation between the behavioural patterns from this analysis is evident. As a result, it can be concluded that the behavioural classification performed during this project does not indicate a difference in behaviour expressed by pigs with and without neuropathic pain. Lastly, the scientific relevance of this project constitute the first step towards an objective and clinically relevant outcome measure for quantifying spontaneous pain in a porcine neuropathic model.

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A Structured literature search

To identify and evaluate the existing literature in the field of automated porcine behaviour analysis using computer vision, a structured literature search was conducted. In order to perform the structured literature search, a PICO-scheme was constructed as illustrated in figure A.1. The PICO-scheme contained relevant keywords structured in coloumns and combined in the search with the use of the boolean operators OR and AND. The search using the PICO-scheme has been performed in two databases: PubMed and Web of Science. These databases were chosen in order to identify existing literature in the field of medicine with the use of PubMed, while also expanding the search to include other relevant scientific fields by utilising the Web of Science database. Furthermore, the resulting number of hits from each database are presented in the PICO-scheme. Of these, ten duplicates were identified, and thus the structured literature search resulted in 78 hits in total.

	AND			
	Patient	Intervention [Abstract]	Comparison	Outcome [Abstract]
OR	Pain Welfare	"Machine learning" “Object detection” "Image proccessing" "Deep learning" "Machine vision" "Computer vision"	Porcine Swine Pig Piglet Sow Gilt Boar	“Behavior recognition" "Behavior analysis" "Social interaction" "Social behavior" Interaction Behavior Aggression
Web of science: 74	894.557	570.243	545.825	5.891.135
PubMed: 14	1.218.393	180.027	370.916	1.829.545

Table A.1 *PICO framework used to perform the structured literature search.*

In addition, the inclusion and exclusion criteria presented in table A.2 were specified in order to aid the process of sorting the literature identified with the use of the PICO-scheme. The purpose of the inclusion and exclusion criteria was to specify requirements concerning the availability, format, and contents of the resulting literature in order to determine their relevance.

Inclusion criteria	Exclusion criteria
• Danish or English • Full text available • Porcine studies • Must focus on interaction with other animals or toys	• Human studies • Studies on other animals than pigs • Focus on farrowing

Table A.2 *Inclusion and exclusion criteria for the structured literature search.*

Conducting the structured literature in PubMed and Web of Science entailed 78 articles in total. These articles were then examined in two sessions. In the first session, the articles were included or excluded on the basis of title and abstract. In the second session, the full text of the remaining included articles were examined. The resulting amount of included and excluded articles are illustrated in figure A.1.

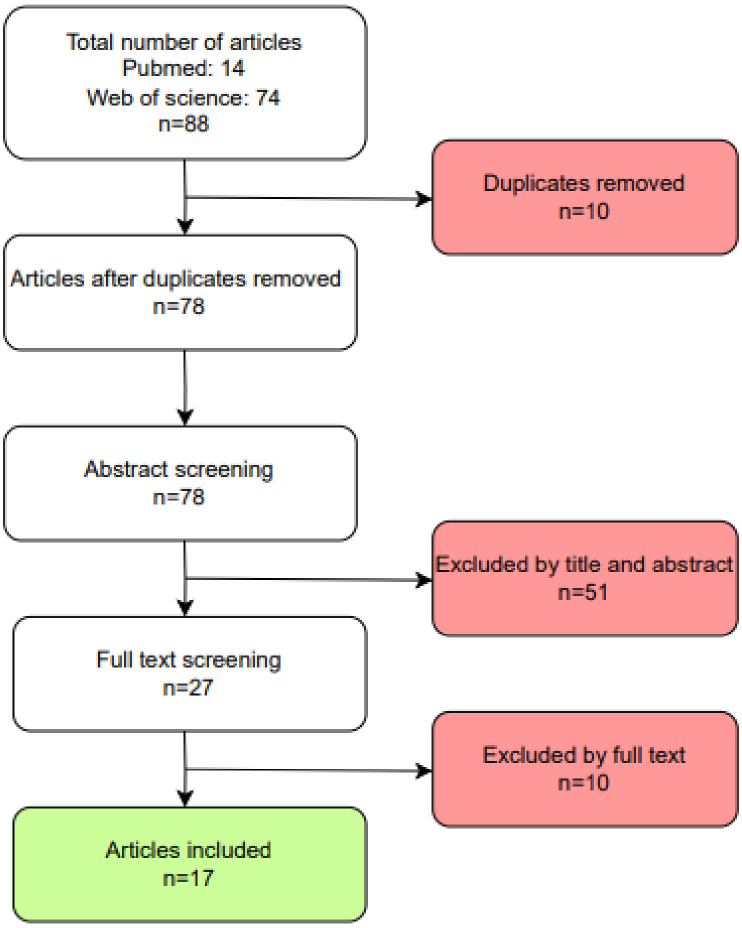

Figure A.1 *Illustration of the number of included and excluded articles from the structured literature search. Excluded articles are highlighted within a red box, while the ultimately included articles are represented within a green box.*

A.1 Related work

An overview of studies described in 13 of the articles included from the structured literature search has been constructed, consisting of keywords or sentences describing the methods, aim and peformance of the utilised methods. In table A.3, the overview of the related work is presented. The citations corresponding to the individual articles are available in the "Method" column of the table along with a short description of the utilised method.

Method	**Aim**	**Performance**
CNN-LSTM network with ResNet34 implemented. [Low et al., 2022]	Classification of pig playing and non-playing behaviors.	Classification accuracy of 92%
YOLO [Tran1 and Thanh, 2023]	Classification of different behaviours such as postures and moving behaviours.	90% accuracy
YOLO [Odo et al., 2023]	Detection of ear biting	Average precision values of 98%, 97.5% and 85.6%, 80.9% on two different data sets
VGG-16 with Resnet-50 implemented [Liu et al., 2020]	Classification of tail-biting behavioural patterns.	Accuracy of 89.23%
CNN-LSTM and CNN-CNN [Hakansson and Jensen, 2022]	Classification of a sequence as either containing or not containing a biting event.	LST model: Sensitivity of 89% CNN model: Sensitivity of 37%

Table A.3 continued from previous page

Method	Aim	Performance
InceptionV3 [Chen et al., 2020a]	Classification of pig enrichment engagement (EE) or non-EE behaviour	EE with blue ball: An accuracy of 96.5%, a sensitivity of 96.3% and specificity of 96.6% EE with golden ball: An accuracy of 96.8%, a sensitivity of 96.2% and specificity of 96.8% EE with wooden beam: An accuracy of 97.6%, a sensitivity of 97.8% and specificity of 97.6%
VGG-16 [Chen et al., 2020b]	Classification of aggressive and non-aggressive episodes	Accuracy of 97.2%
VGG-16 [Fraser et al., 2023]	Detection of aggressive behaviors in pigs using behaviour subtypes.	Accuracy of 79%, sensitivity of 77%, and specificity of 81%
A temporal shift module (TSM) is inserted into four 2D convolutional neural network models: ResNet50, ResNeXt50, DenseNet201, and ConvNext-t [Ji et al., 2023]	Detection of aggressive behaviors in pigs using behaviour subtypes.	ResNeXt50-T (TSM inserted into ResNeXt50) showed best results, including an accuracy of 95.69%, sensitivity of 95.25%, and specificity 96.07%.

Table A.3 continued from previous page

Method	Aim	Performance
Clustering [Durand et al., 2023]	Estimation of welfare status in sows by identification of three clusters: scapegoat, gentle and aggressive	Accuracy rate of 72%
Clustering [Chen et al., 2017]	Detection of aggressive behabiours among pigs by designing recognition rules of medium and high aggression.	Medium aggression: Accuracy of 95.82%, sensitivity of 90.57%, and specicity of 96.95% High aggression: Accuracy of 97.04% , sensitivity of 92.54%, and specificity of 97.38%.
An approach referred to as the TSML-model, which combines mutual learning with two stream neural [Hao et al., 2023]	Recognition of pig behaviours including: fighting, drinking, eating, investigating, lying, and walking.	Accuracy of 96.52%
LDA [Viazzi et al., 2014]	Classification of aggressive interactions.	Accuracy of 89.0%, sensitivity of 88.7%, and specificity of 89.3%

Table A.3 *Table containing an overview of the methods, aims, and performances of 13 of the articles included from the structured literature search. The overview consist of keywords or sentences describing the methods, aim and performance of the studies described in the articles. Additionally, the citation of the articles are provided.*

B PBL Portfolio

The following appendix contains a problem based learning (PBL) portfolio in which the experiences gained from using PBL methodologies throughout the project are described and reflected upon. PBL has been the foundation of the entire project period and the application of appertaining methodologies has entailed valuable experiences and learning processes.

B.1 Time management

During the project period, time management has been prioritised for ensuring completion of all the important aspects of the project within the given time frame. For managing the entire project period and the overall deadlines, a Gantt-Scheme was prepared during the initialisation of the project. The initial draft for the Gantt-scheme contained important overall deadlines. Afterwards, the Gantt-scheme was regularly updated during the project period, as the goals and deadlines of the project became more clearly defined or changes in the strategy occurred. Additionally, meetings and events involving other people than the group members were noted in the Gantt-scheme. On figure B.1, an extract of the Gantt-scheme is presented.

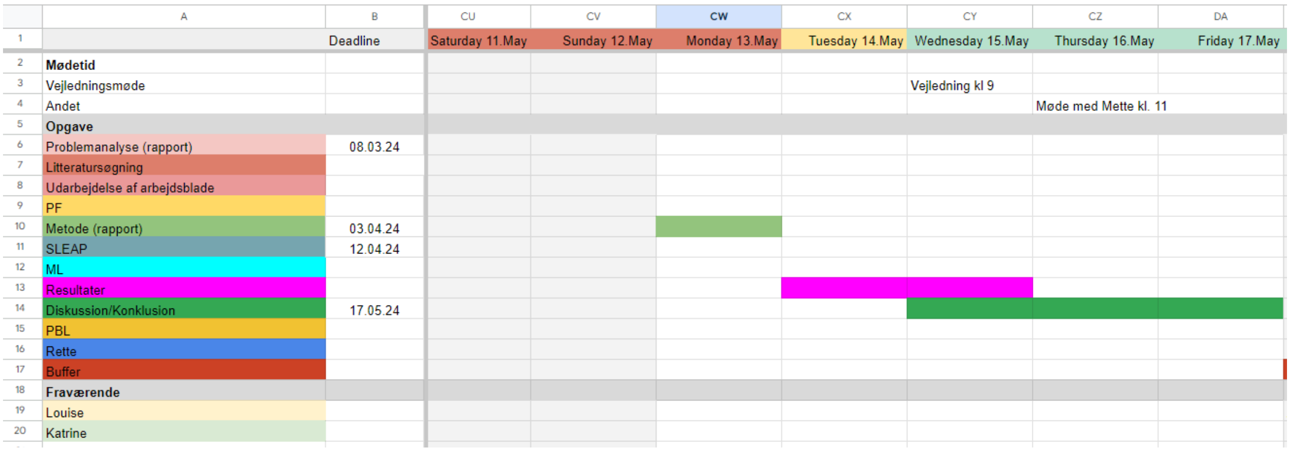

Figure B.1 *Extract of the Gantt-scheme used to manage the time and deadlines throughout the project period.*

The purpose of the Gantt-scheme was to maintain an overview of the entire project period and essential deadlines for ensuring project completion within the time frame. Furthermore, detailed planning of the weeks were conducted in a document, which was updated at least once a week. This document was referred to as the log. The log was based on the overall plans in the Gantt-scheme but contained more detailed descriptions of which tasks should be completed on specific days. In addition time and place of scheduled meetings were noted in the log. At the end of each day, it was evaluated whether or not the tasks were completed and whether more time should be set aside for some tasks.

During the project period, several large alterations of the strategy for addressing the problem statement were performed, including the change of computer vision method for obtaining the pose and tracking data. These alterations entailed challenges regarding the time management due to the need for continuous reevaluating deadlines and goals. Additionally, the group members own expectations for the outcome of the project had to be aligned during reevaluation of the Gantt-scheme and new deadlines. The reevaluation of goals and deadlines were sometimes challenging due to the exploratory nature of the project. New knowledge and potentially relevant methodologies continuously became available and decisions had to be made regarding the course of strategy and goals of the project. In turn, compromises had to be made in order to reach the desired goals within the given time frame. In this context, it was necessary to select a deadline for including new methods and incorporating new knowledge.

B.2 Learning process and experiences

The process of completing the master's thesis has entailed reflections upon the learning process and experiences gained throughout the entire project period. The group members have had to continuously align expectations in regards to the outcome of the project. The changes in the course of strategy throughout the project has entailed a need for defining compromises in terms of the group members' expectations due to the limited time period. It has been a learning process for the group members to rest in the prospect of possibly not achieving the desired outcome. However, the aim of always utilising the correct methodologies based on well-justified scientific reasoning has been the foundation of the decision-making process during the project. Furthermore, the group members sought to gain new knowledge throughout the entire project period. For example, contact were established with multiple external sources, providing expert knowledge regarding ethology and the use of specific software appliances. As a result, the group members actively sought to continuously improve their knowledge on topics relevant for different aspects of the project.

C | EthoVision

The following appendix contains a description of the simple test performed to examine the tracking performance using contrast based tracking with the use of the proprietary software EthoVision by Noldus.

The tracking performance using EthoVision was addressed by visually examining the tracking of the pigs during two randomly selected five minute videos. The tracking performance was assessed by visually examining whether the two pigs were more or less correctly identified by the software. Correct tracking was classified by the center mass being located on the individual pigs when they were visible in the frames. On table C.1, timestamps of incorrect and incorrect tracking during the five minute video.

Tracking	Video 1	Video 2
Incorrect	00:42-00:52 00:59-01:24 01:57-02:22 03:05-03:57 04:10-04:19 Total time = 121 sec = 40.33%	04:50-04:56 Total time = 6 sec = 2%
Correct	00:00-00:42 00:52-00:59 01:24-01:57 02:22-03:05 03:57-04:10 04:19-05:00 Total time = 179 sec = 59.67%	00:00-04:50 04:56-05:00 Total time = 294 sec = 98%

Table C.1 *Table presenting the results of the test of contrast-based methods using EthoVision.*

From table C.1, it is evident that the tracking performance was correct during 59.67% of the first five minute video and incorrect during 40.33% of the time. Additionally, the tracking performance were correct during 98% of the second five minute video and incorrect during 2% of the second video. Thus, the contrast-based tracking performed better during the second video. However, the pigs were sleeping throughout the second video and therefore only moving very little. In the first video, the pigs were awake, moving and interacting with each other. As a result, the first video was more representative of the scenarios of interest in this project.

On figure C.1, an example of an image in which the tracking has been considered correct is illustrated.

Figure C.1 *Example of an image in which the contrast-based tracking using EthoVision is considered correct. The red points indicate the center of mass while the green and yellow colouring represent the tracking of the individual pigs. The orange colouring represents noise.*

The contrast-based tracking displays issues when the two pigs were located near each other or the gate. On figures C.2, C.3, C.4 and C.5, examples of images in which the tracking were incorrect as a result of the pigs being near each other or the gate.

Figure C.2 *Example of an incorrect tracking in which both pigs are detected as one.*

Figure C.3 *Example of an incorrect tracking in which one of the pigs are correctly detected while the other pig is incorrectly detected as the bedding material.*

Figure C.4 Example of an incorrect tracking in which one of the pigs were correctly detected while the other pig is incorrectly detected due to it disappearing behind the gate.

Figure C.5 Example of an incorrect tracking of both pigs on the same pig due to the pig disappearing behind the gate.

Furthermore, the change in camera angle across different days proved to be challenging when using contrast-based tracking. Before performing the tracking using EthoVision, an empty arena should be defined. As the varying camera angles result in differences between the defined arena and the backgrounds, the contrast-based tracking performs poorly across days. On figure C.6 and C.7 examples of incorrect tracking as a result of varying camera angles and backgrounds is illustrated.

Figure C.6 Example of an incorrect tracking with a lot of noise as a result of the varying camera angles.

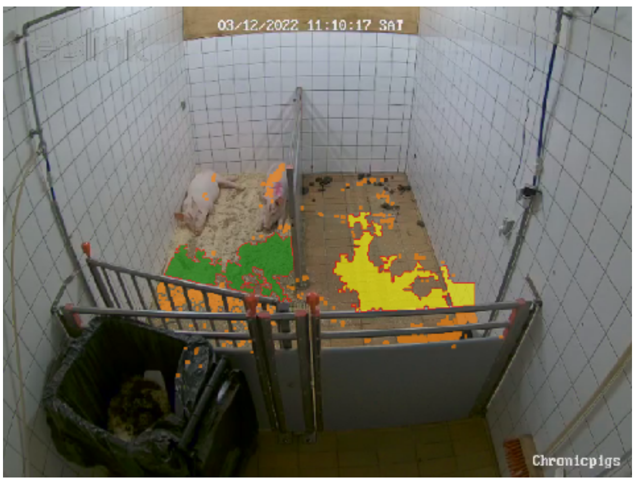

Figure C.7 Example of an incorrect tracking with a lot of noise as a result of the varying camera angles.

D | Examples of node placements

The following appendix contains examples of node placements in the different categories described in the estimation of error rate in pose estimation.

In the following table examples of frames displaying scenarios from each of the 12 categories to which different types of node placements have been assigned. Accordingly, the differentiation between correct and incorrect node placement in the different categories are specified using colour-coding of the descriptions in the table. Red indicates incorrect node placements while green indicates correct node placements.

Description	Example
1) Correct skeleton with missing nodes that were not visible	
2) All three nodes correctly detected	

3) One pig correctly detected while the other pig was hidden behind the gate	
4) One pig hidden behind the other pig	
5) Exchange of nodes in the skeleton	

6) Only one pig detected while the other one was visible in the image	
7) The only predicted node was a shoulder node which was not placed correctly but still on the pig	
8) A slight misplacement of a single node but it was still on the pig	

9) Both skeletons placed on one pig	
10) One skeleton spanned across both pigs	
11) Detection of nodes at points other than on the pigs	

12) Nodes incorrectly placed while its ideal location is visible.	

E | Observations of distances

In the following appendix, histograms illustrating the amount of observations at different distances between the pigs in each of the groups are presented.

On figures 7.4 to E.5 the histograms for the remaining groups are presented, providing a visual representation of the distribution of data in terms of distances between the pigs within each group.

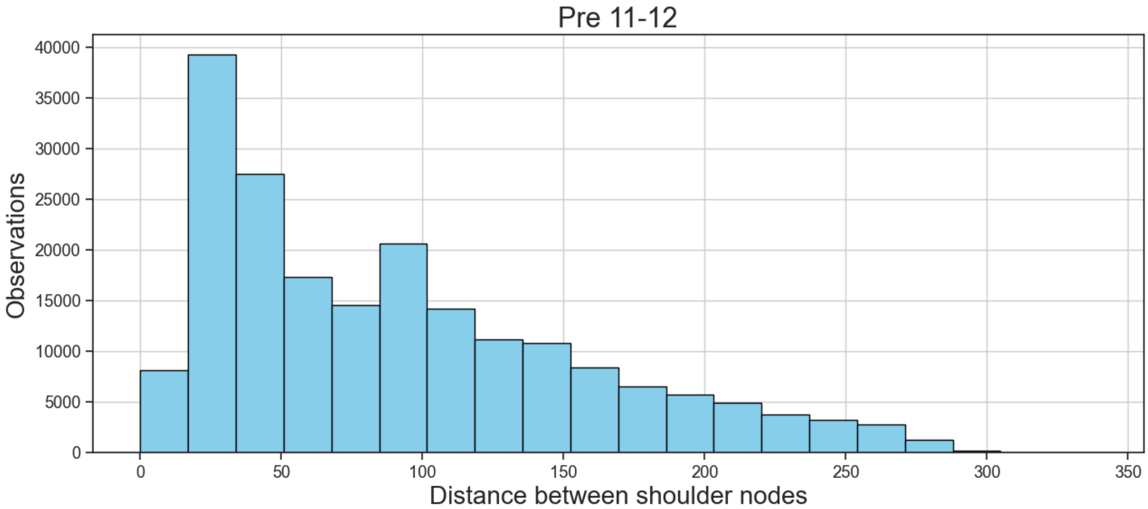

Figure E.1 *The bar plot illustrates the distribution of proportions for group Pre 11-12*

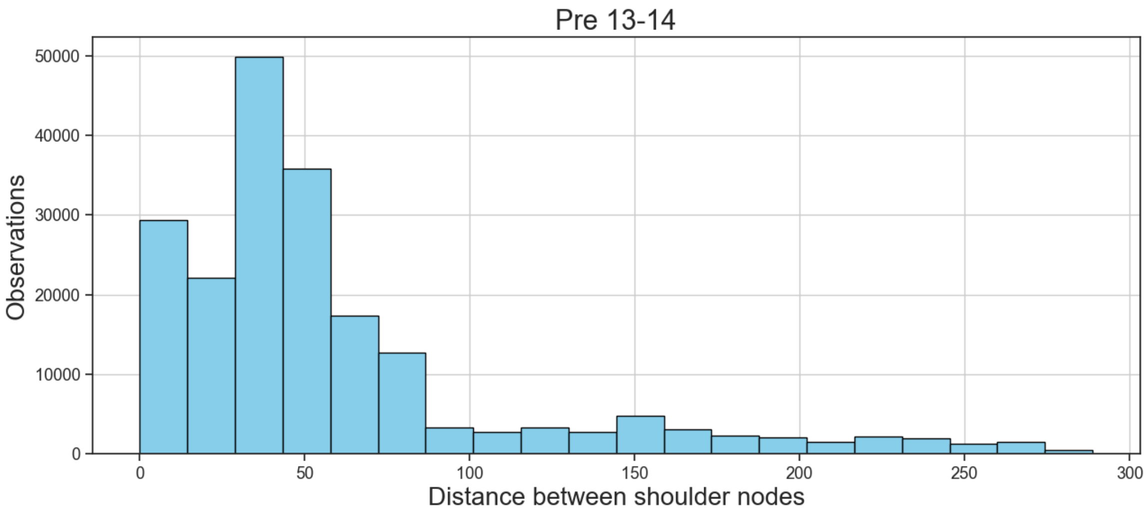

Figure E.2 *Bar plot illustrating the distribution of observations across distances for group Pre 13-14.*

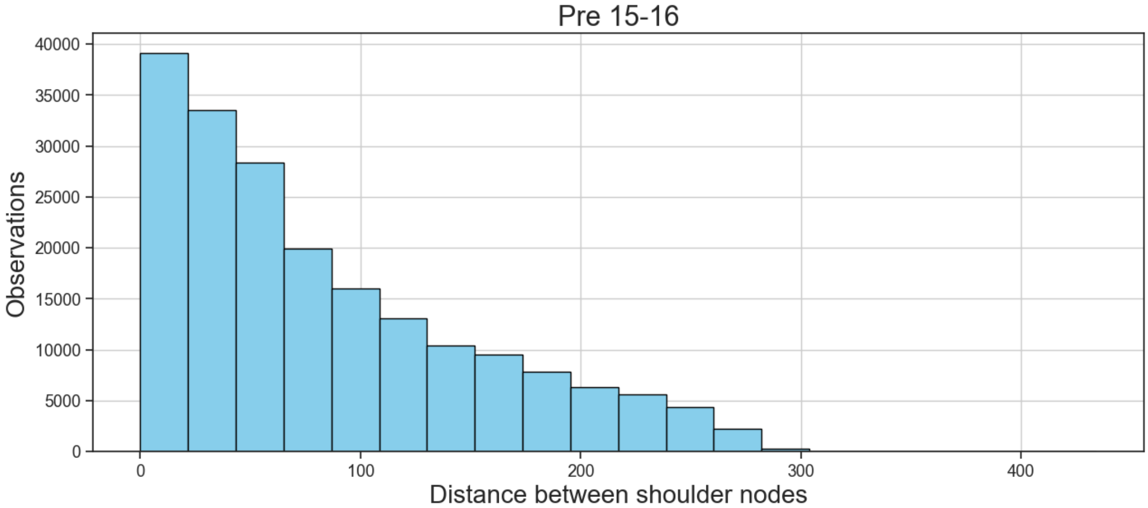

Figure E.3 *Bar plot illustrating the distribution of observations across distances for group Pre 15-16.*

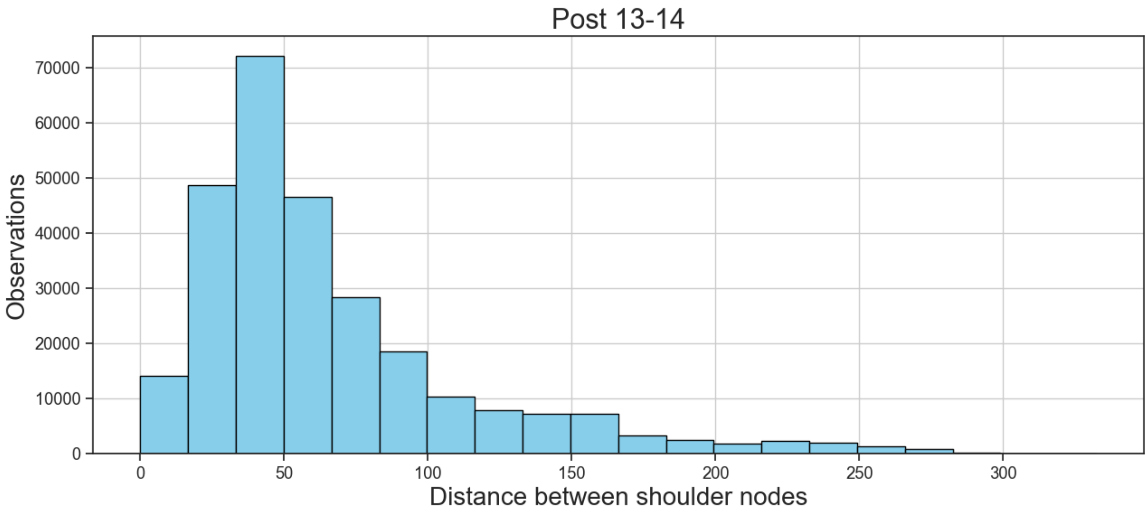

Figure E.4 *Bar plot illustrating the distribution of observations across distances for group Post 13-14.*

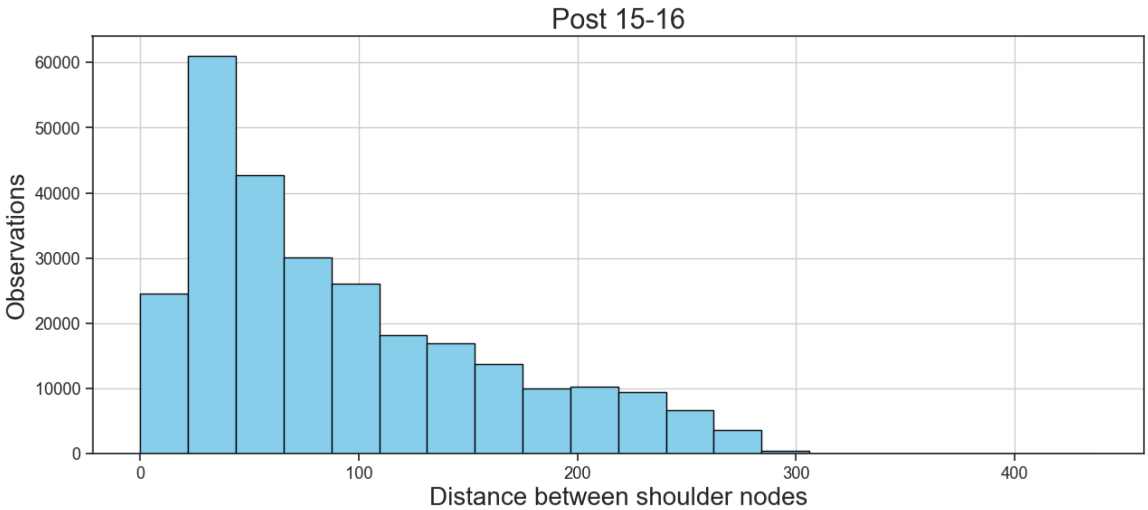

Figure E.5 *Bar plot illustrating the distribution of observations across distances for group Post 15-16.*

F | Acceleration across frames

The following appendix contains scatter plots showing the acceleration data for the two pigs in each pen across frames.

On figures F.1 to F.5 scatter plots of snout acceleration across frames for the remaining groups are presented. On all plots, the x-axis represents the number of frames, while the y-axis indicates the acceleration of the snout nodes on the individual pigs.

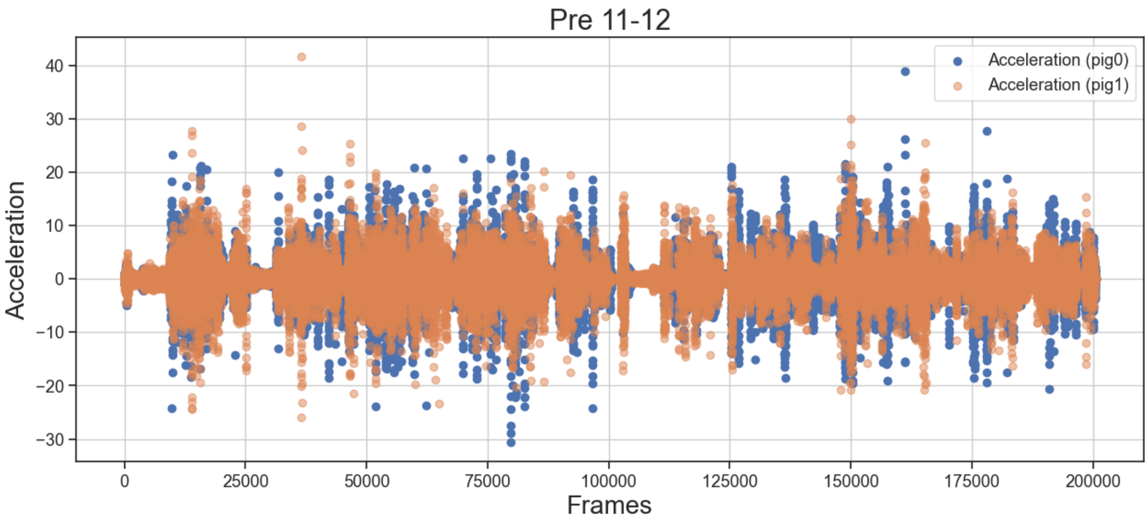

Figure F.1 *Scatter plot illustrating the snout acceleration data for the two pigs in group pre 11-12 across frames.*

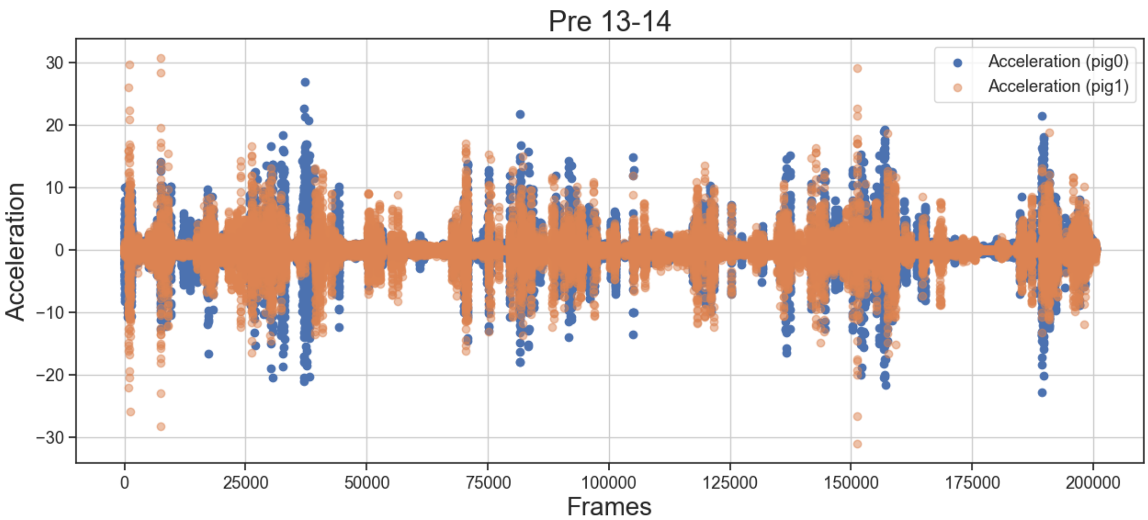

Figure F.2 *Scatter plot illustrating the snout acceleration data for the two pigs in group pre 13-14 across frames.*

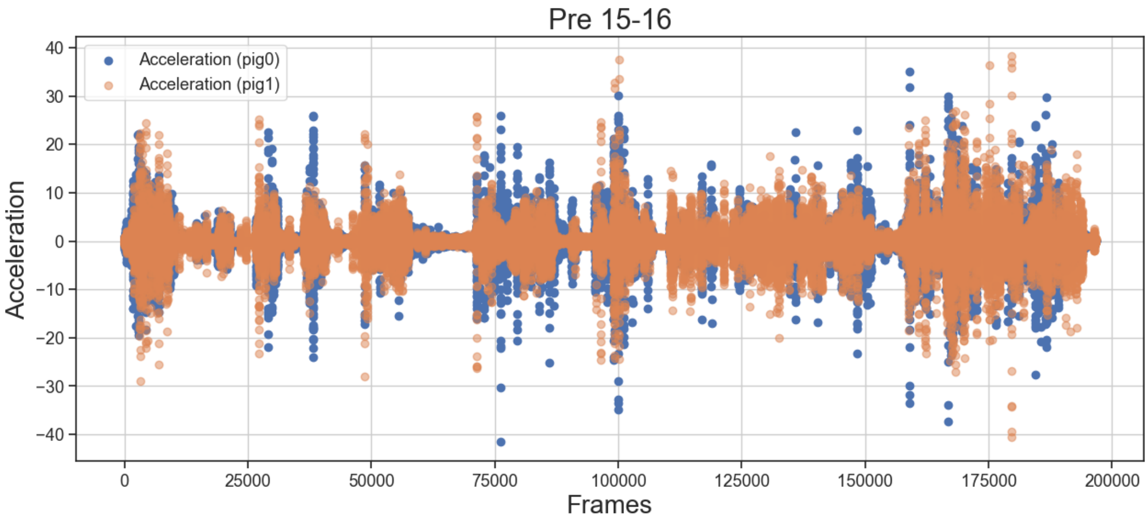

Figure F.3 *Scatter plot illustrating the snout acceleration data for the two pigs in group pre 15-16 across frames.*

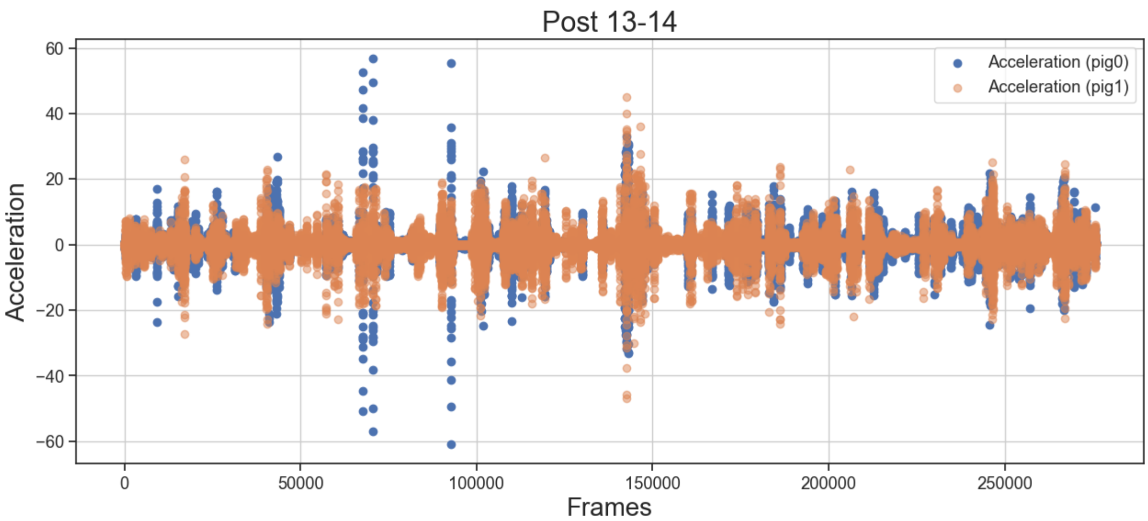

Figure F.4 *Scatter plot illustrating the snout acceleration data for the two pigs in group post 13-14 across frames.*

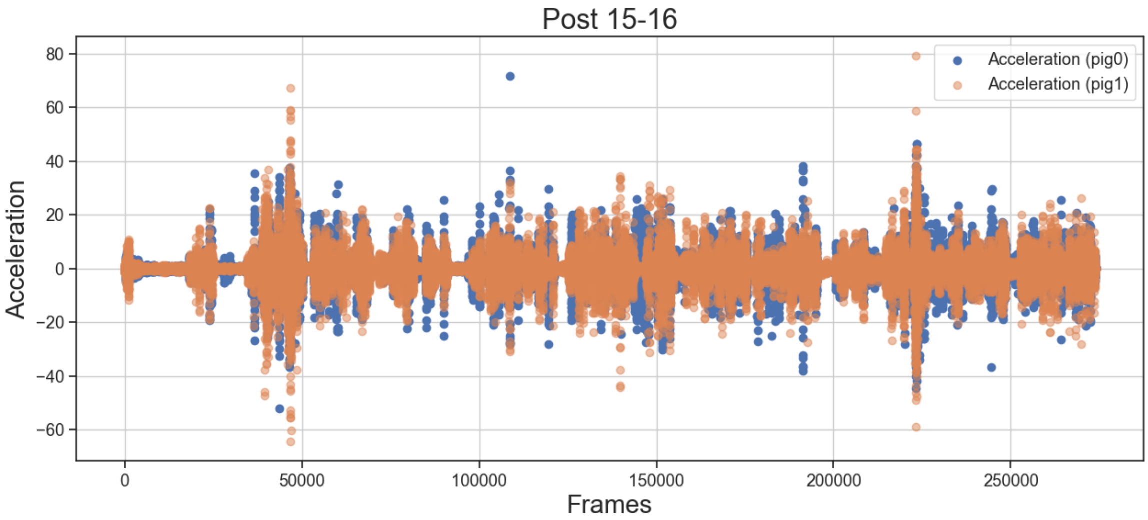

Figure F.5 *Scatter plot illustrating the snout acceleration data for the two pigs in group post 15-16 across frames.*

G | Acceleration across distance

The following appendix contains scatter plots illustrating the relationship between the distance between the snout nodes of the two pigs in each pen and the corresponding accelerations.

On figures G.1 to G.5 scatter plots of the snout acceleration across distances between the snouts of the two pigs for the remaining groups are presented. On all plots, the x-axis represents the distance between the snout nodes, while the y-axis indicates the acceleration of the snout nodes on the individual pigs.

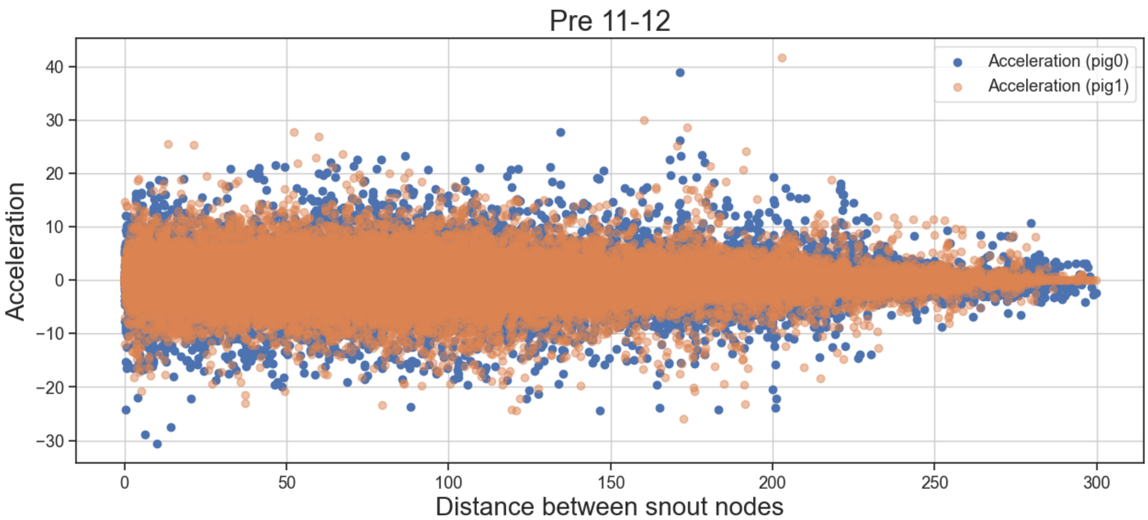

Figure G.1 *Scatter plot illustrating the distance between the snout nodes of the individual pigs in group pre 11-12 and the corresponding accelerations.*

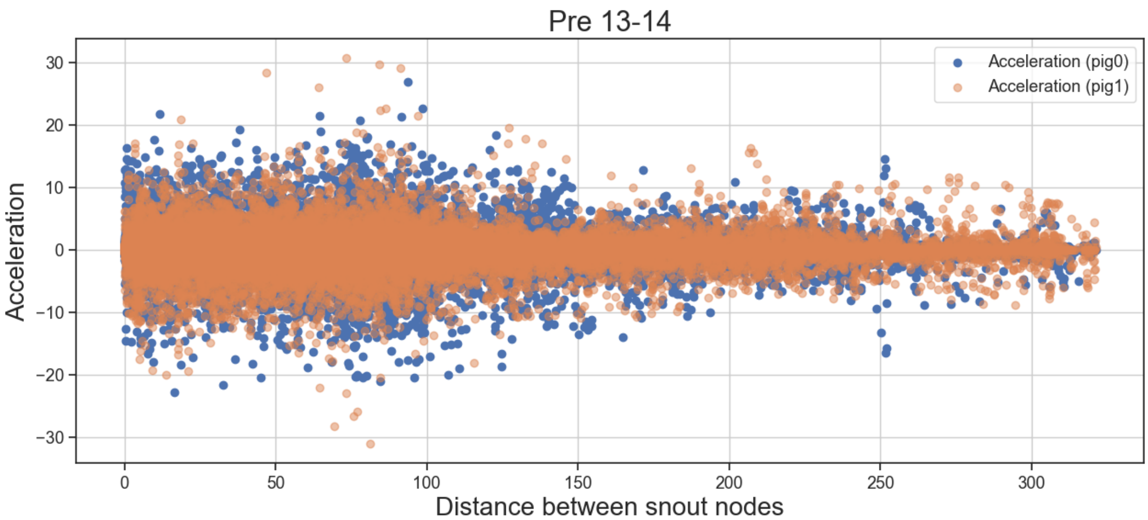

Figure G.2 *Scatter plot illustrating the distance between the snout nodes of the individual pigs in group pre 13-14 and the corresponding accelerations.*

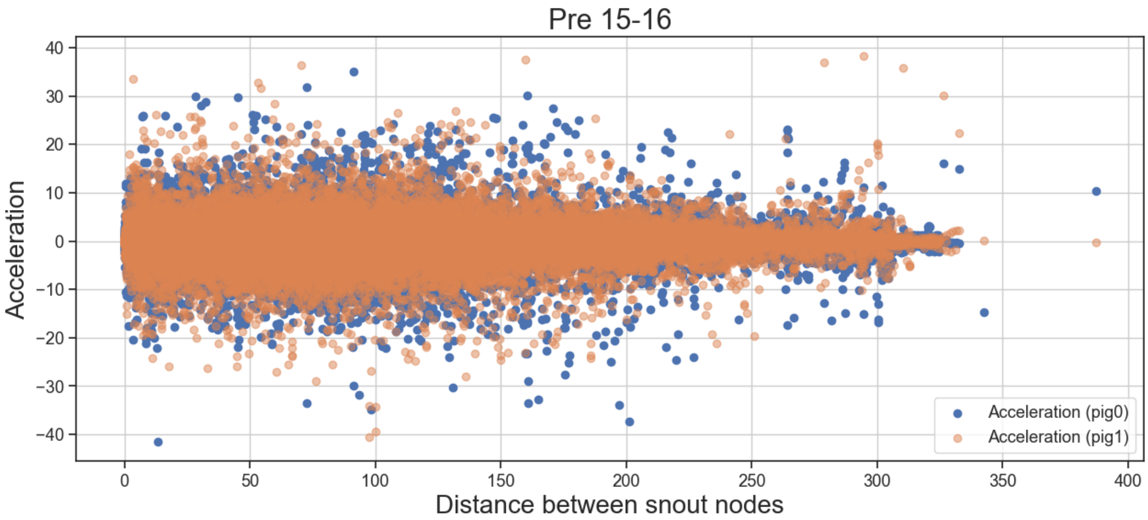

Figure G.3 *Scatter plot illustrating the distance between the snout nodes of the individual pigs in group pre 15-16 and the corresponding accelerations.*

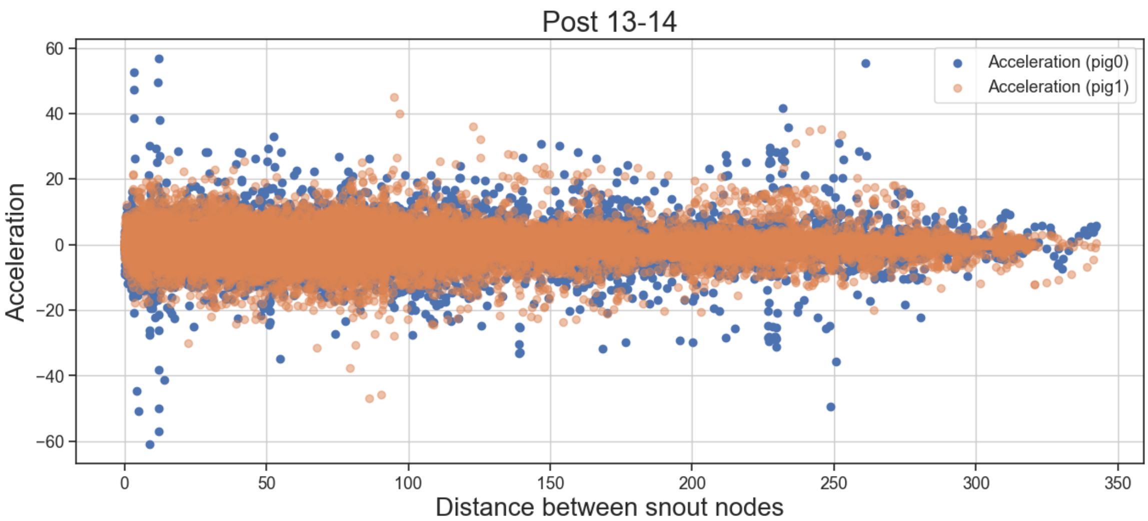

Figure G.4 *Scatter plot illustrating the distance between the snout nodes of the individual pigs in group post 13-14 and the corresponding accelerations.*

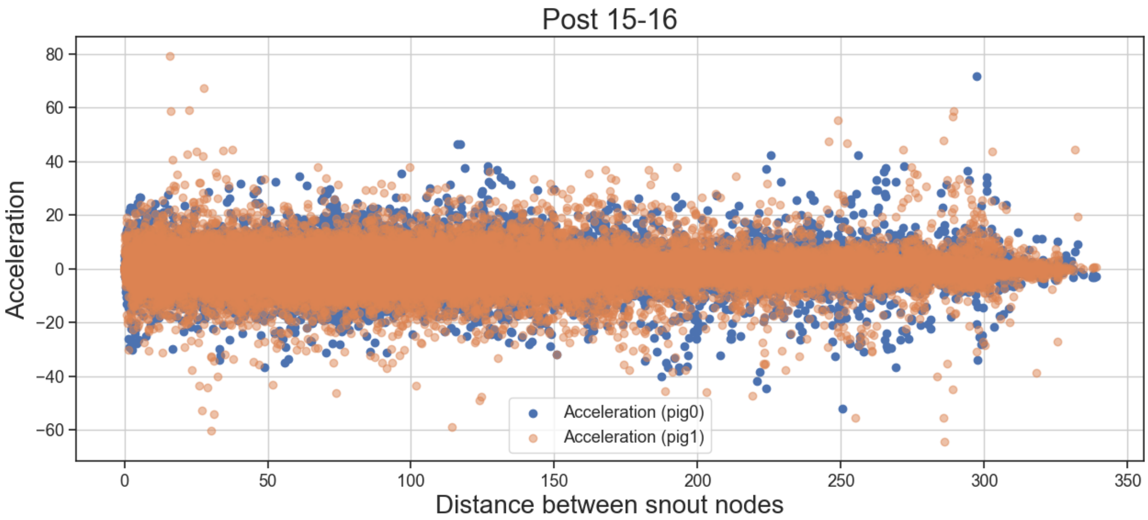

Figure G.5 *Scatter plot illustrating the distance between the snout nodes of the individual pigs in group post 15-16 and the corresponding accelerations.*

H | Post-clustering analysis

In the following appendix parts of the results of the post-clustering analysis are presented. The results consist of an overview of the descriptive statistics for the remaining features as well as distribution of data points within the clusters for each group.

H.1 Descriptive statistics

In tables H.1 to H.5, the descriptive statistics for the remaining features are provided in the following order: acc2, d1, d2, d3, and d4. The descriptive statistics include values of the mean, median, standard deviation, variance, minimum, maximum, and quartiles (Q1, Q3) for each feature.

Cluster	Mean	Std	Min	25%	50%	75%	Max
0	0.010	0.724	-26.959	-0.076	0.000	0.079	35.076
1	0.001	1.371	-42.692	0.173	0.000	0.174	45.565
2	-0.012	1.286	-43.923	-0.148	0.000	0.139	40.552

Table H.1 *Table presenting the descriptive statistics for the acc2 feature, representing acceleration of the shoulder node.*

Cluster	Mean	Std	Min	25%	50%	75%	Max
0	-0.570	0.414	-1.164	-0.882	-0.568	-0.324	3.654
1	1.958	0.916	-1.161	1.316	1.920	2.582	5.171
2	0.386	0.386	-1.162	0.009	0.327	0.721	4.261

Table H.2 *Table presenting the descriptive statistics for the d1 feature, representing the distance between snout nodes of the two pigs.*

Cluster	Mean	Std	Min	25%	50%	75%	Max
0	-0.615	0.364	-1.263	-0.869	-0.658	-0.424	2.784
1	1.917	0.695	-1.257	1.422	1.935	2.447	5.652
2	0.502	0.654	-1.262	0.009	0.402	0.919	4.508

Table H.3 *Table presenting the descriptive statistics for the d2 feature, representing the distance between shoulder nodes of the two pigs.*

Cluster	Mean	Std	Min	25%	50%	75%	Max
0	-0.614	0.320	-1.421	-0.845	-0.638	-0.388	2.240
1	2.107	0.711	-1.293	1.550	1.994	2.581	5.872
2	0.415	0.477	-1.417	0.091	0.337	0.744	3.843

Table H.4 *Table presenting the descriptive statistics for the d3 feature, representing the distance between snout node of the reference pig and shoulder node of the non-reference pig.*

Cluster	Mean	Std	Min	25%	50%	75%	Max
0	-0.524	0.465	-1.779	-0.795	-0.471	-0.210	2.598
1	1.915	0.903	-1.642	1.296	1.858	2.458	6.510
2	0.303	0.698	-1.774	-0.156	0.287	0.789	4.353

Table H.5 *Table presenting the descriptive statistics for the d4 feature, representing the distance between snout node of the reference pig and tail node of the non-reference pig.*

H.2 Proportions within clusters

In table H.6, the values of the calculated proportions of data from the individual groups in the respective clusters are provided.

Group	Cluster	Proportion [%]
Pre 11-12	0	55,36%
	1	13,58%
	2	31,05%
Pre 13-14	0	70,67%
	1	6.60%
	2	22,71%
Pre 15-16	0	51,81%
	1	17,27%
	2	30,90%
Post 11-12	0	50,67%
	1	12,37%
	2	36,94%
Post 13-14	0	77,92%
	1	6,87%
	2	15,20%
Post 15-16	0	56,60%
	1	15,91
	2	27,48

Table H.6 *Table presenting the proportion of observations within the three clusters for all groups.*