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INTERFACING THE PIG CORTEX- TOWARDS A TRANSLATIONAL LARGE ANIMAL MODEL OF LTP-LIKE PAIN

BY
TAHA AL MUHAMMADEE JANJUA

DISSERTATION SUBMITTED 2021

AALBORG UNIVERSITY
DENMARK

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by

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AALBORG UNIVERSITY
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CV

Taha holds a BE in Mechatronics Engineering from the National University of Science and Technology, Rawalpindi, Pakistan, and an M.Sc. in Biomedical Engineering from the National University of Science and Technology, Islamabad, Pakistan. He enrolled at Aalborg University as a Marie Skłodowska Curie FRESCO PhD student under the supervision of Winnie Jensen and co-supervision of Thomas Gomes Nørgaard dos Santos Nielsen as a part of the Neural Engineering and Neurophysiology group at the Department of Health Science and Technology (HST). Taha's PhD project was supported by the Horizon 2020 research and innovation programme (Grant no. 754465) as well as the Center for Neuroplasticity and Pain (CNAP), which was supported by Danish National Research Foundation (DNRF121).

As a researcher, Taha has designed and fabricated upper limb prostheses and processed electromyography signals recorded from the anterior tibialis muscle after the chiropractic intervention during his undergraduate and master's programs, respectively. He has experience in Computer-Aided Drawing (CAD), 3D printing, design, structural analysis of exoskeletons and prostheses, bio-signal processing.

The PhD program allowed Taha to develop his skills as a neuroscientist. He has a license to work with animals (EU Function ABD) and has a firm grip on neurosurgery and neurophysiological signal processing. Apart from cortical signal processing, Taha has been involved in teaching and supervising projects linked to the brain-computer interface and designing and fabricating a low-cost 3D-printed exoskeleton for stroke rehabilitation in collaboration with Mads Rovsing Jochumsen.

ENGLISH SUMMARY

Animal models are instrumental in investigating neurophysiological disorders because they allow the use of invasive measurements from the brain in a relevant disease model. Pigs as translational pain models may be valuable because translational pain research, conducted using rodents, remains unsuccessful in translating entirely to humans, likely due to differences between rodents and human physiology. Hence, there is an increasing need to develop alternatives to overcome species-specific issues. The primary aim of the Ph.D. work was to, therefore, establish a translational pig pain model, i.e., the *LTP-like pain model*.

Long term potentiation (LTP) is a phenomenon characterised by increased synaptic strength and is related to pain. LTP can be artificially induced using high-frequency electrical stimulation (HFS) and has been used to establish a pain model in both humans and rodents. The *LTP-like pain model* has been studied in humans using pain ratings combined with objective measures like electroencephalography (EEG). Rodent studies have used more invasive techniques such as penetrating microelectrode arrays (MEA) that offer a higher temporal and spatial resolution than EEG. A relatively less invasive technique that is popular in rodents and humans is electrocorticography (ECoG) or micro electrocorticography (µECoG). While µECoG and EEG have been compared, no direct comparison has been made so far in terms of cortical information overlap between µECoG and MEA, which was the secondary aim of the present work.

Three studies were designed to address the thesis objectives. **Study I** analysed the spike activity in the primary somatosensory cortex (S1), detectable only by the MEA, to demonstrate the cortical effect of HFS on the ulnar nerve. The results demonstrated an increase in cortical excitability due to HFS. In **Study II**, the S1 response to HFS on the ulnar nerve was evaluated using event-related potentials and spectral analysis. The results reflected the MEA's ability to capture local field potentials and showed pig as a valid model to study LTP-like pain. **Study III** compared evoked cortical responses recorded from a µECoG array and an MEA. The study focused on the signal quality and information content, quantified by the power, in specific frequency bands, of the recorded signal from the two electrodes and compared each electrode based on ease of the surgical procedure.

In conclusion, electrophysiological changes following peripheral electrical stimulation were assessed using the µECoG and the MEA in the pig. The MEA reflected cortical information when inducing an LTP-like pain model in pigs. Additionally, the MEA was compared with a µECoG array in terms of invasiveness and power in commonly used frequency ranges.

DANSK RESUMÈ

Anvendelse af dyremodeller kan understøtte studier af neurofysiologiske lidelser, fordi det er muligt at optage signaler direkte fra hjernen med brug af invasive teknikker i en relevant sygdomsmodel. Brug af grise som en translatorisk smerte model er sandsynligvis vigtig fordi translatorisk smerte forskning baseret på gnavere, stadig fejler når de overføres til kliniske forsøg. Det primære formål med Ph.D. arbejdet var derfor at etablere en translationel smerte model i grise, dvs. *LTP-lignende smerte model.*

Long term potentiation (LTP) er et fænomen der er karakteriseret ved øget synaptisk styrke og er relateret til smerte. LTP kan kunstigt induceres ved brug af høj-frekvent elektrisk stimulation (HFS) og har derfor været brugt i etableringen af en smerte model i både mennesker og gnavere. Effekten af den *LTP-lignende smertemodel* er blevet undersøgt hos mennesker ved hjælp af smertevurderinger kombineret med objektive mål som elektroencefalografi (EEG). I gnavere har man brugt mere invasive teknikker såsom det penetrerende mikroelektrode array (MEA) der har en højere tidsmæssig og rumlig opløsning. En relativt mindre invasiv teknik, der ofte er brugt i gnavere og mennesker, er elektrokortikografi (ECoG) eller mikro elektrokortiografi (µECOG). Mens µECoG og EEG er blevet sammenlignet, er der hidtil ikke foretaget nogen direkte sammenligning med hensyn til kortikal informationsoverlapning mellem µECoG og MEA, hvilket var det sekundære formål med dette arbejde.

Tre studier blev gennemført for at adressere afhandlingens mål. **Studie I** analyserede spike aktiviteten i det primære somatosenoriske cortex (S1), som kun kan optages af MEA, for at demonstrere den kortikale virkning af HFS på ulnar nerven. Resultaterne viste en stigning i kortikal excitabilitet efter HFS. I **Studie II** blev S1 responset på HFS på ulnar nerven evalueret ved hjælp af *event-related potentials og spektralanalyse*. Resultaterne afspejlede MEA's evne til at optage LFP signaler (*(local field potentials)* og viste at grise kan bruges som model for LTP-lignende smerter. **Studie III** sammenlignede evokerede kortikale responser optaget fra et µECoG-array og et MEA array. Signalkvaliteten og informationsindholdet blev sammenlignet, (kvantificeret ved at beregne energien i specifikke frekvensbånd) og elektrodenes praktiske anvendelse i kirurgiske procedurer blev vurderet.

Som konklusion blev elektrofysiologiske ændringer efter perifer elektrisk stimulering vurderet ved hjælp af µECoG og MEA i grisen. MEA afspejlede kortikal information, når man inducerede en LTP-lignende smertemodel hos grise, idet man fremhævede grise som translationelle modeller. Undersøgelserne fremhævede fordelene ved µECoG i forhold til MEAs med hensyn til niveauet af invasivitet og energien i almindeligt anvendte frekvensområder for hver elektrode.

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I dedicate my PhD to my father, Professor Naeem Ul-Hassan Janjua (late). He would say at this point, “Life is a collection of moments of happiness and joy. This is one such moment”. Thank you for always believing in me. Here is to a promise fulfilled.

I want to thank my PhD supervisor, Winnie Jensen. Without her constant encouragement, support, and confidence in me, this would not have been possible. I give my thanks to my co-supervisor, Thomas Gomes Nørgaard dos Santos Nielsen, for his valuable feedback and insight on all my ideas. A special thanks to Felipe for being my go-to person for all my rants and troubleshooting each challenge with me. I want to thank Suzan for always being empathetic and open to discussions about the project and otherwise. I could not have asked for better teammates in this project. I owe my gratitude to the animal lab technicians, specially Torben, for their consistent and reliable help with the experiments.

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CHAPTER 1. INTRODUCTION

Neurological disorders accounted for 16.8 % of worldwide deaths in 2015 (Feigin et al., 2017). An essential part of biomedical research of neurological diseases is developing a surrogate model of the disease in animals. A surrogate model allows a better understanding of mechanisms of neurophysiological diseases, where it is vital to assess intracortical changes in response to the induced model of research. These intracortical responses can be measured invasively, often in rodents, by penetrating multi-electrode arrays (MEA) into the cortex. However, the results from these studies do not necessarily translate into human subjects during clinical trials (Ballantyne, 2010). In the USA, out of the 56 billion USD allotted to preclinical research, up to 50% (28 billion USD) is unsuccessful in translating animal models to human models every year (Freedman et al., 2015),(Keen, 2019). A possible cause for this difference might be that human physiology differs from rodent models used to demonstrate the disorder (Yezierski & Hansson, 2018). Other probable reasons include the differences in cortical structural complexity (Cooke & Bliss, 2006), the overall grey matter to white matter ratio (Mota et al., 2019) and neuronal molecular differences (Kalmbach et al., 2018). Therefore, there is a need for an animal model that resembles the human condition to a higher degree.

Monkeys have become famous for neurological research since their brain resembles the human brain anatomically and physiologically (Gray & Barnes, 2019). However, monkeys are expensive to use as research models and are not abundantly available for research (Friedman et al., 2017). Pigs are an alternative that is already effectively being used in research on cardiology (Crick et al., 1998), pulmonary diseases (Judge et al., 2014), and skin research (Summerfield et al., 2015). The cortical structure and response of the pigs have been studied, demonstrating a similarity with the human brain in terms of brain development and grey matter to white matter ratio (Sauleau et al., 2009). Pigs offer a level of complexity closer to the human central nervous system (CNS) and the ability to record invasively, as done in rodents.

Pigs as translational pain models are valuable because translational pain research conducted using rodents remains unsuccessful in translating entirely to humans (Barrot, 2012). Despite the robust preclinical efficacy demonstrated by many drugs, these compounds failed during clinical trials due to differences between rodents and human physiology (Akhtar, 2015). Hence, there is an increasing need to offer alternatives to overcome species-specific issues (Mao, 2012). Additionally, pain researchers believe that many forms of clinical pain cannot be mimicked in animal models due to poor match with human pain symptoms and considerable experimenter bias in pain assessment (Mogil et al., 2010). Thus, there is a need to develop non-subjective and non-behavioural measurements for nociception to 'backwards' translate findings in human studies.

Therefore, the focus of the present thesis was to investigate the feasibility of using pigs as a translational pain model.

CHAPTER 2. STATE-OF-THE-ART

2.1 ANIMAL MODELS IN BIOMEDICAL RESEARCH

Over the years, animals have played a pivotal role in medical research. Animal models improve our understanding of human physiology by comparing animal and human physiological responses to an induced model of a disease. This improved understanding of disease helps develop countermeasures in the form of vaccines, drugs and, therapy to prevent and recover from similar diseases. One of the most exceptional examples is the Nobel-prize winning discovery of insulin using dogs as animal subjects (Banting et al., 2007). Similarly, monkeys were used as test subjects for polio vaccination before clinical tests (Baicus, 2012). Another example is the use of rabbits for atherosclerosis research (Fan et al., 2015).

Amongst all animal subjects, rodents are the most extensively used research animals because of their relative ease of maintenance, short lifespans, and the ability to modify them genetically (Bryda, 2013). The high reproduction rate allows genetically modified subjects to isolate a specific phenotype to respond to the treatment, e.g., a cortical reaction to light stimulus on the neurons (Serrano Cardona & Muñoz Mata, 2013). Mice, for instance, have been instrumental in understanding hearing loss (Ohlemiller, 2019). Mice are also used in cancer research to create an animal model similar to the human condition (W. Zhang et al., 2011). New therapeutic methods for spinal injuries have been discovered using rats (Minakov et al., 2018).

Despite the genetic similarity between rodents and humans, there is a lack of molecular, immunological and cellular similarities that would allow successful translation of a rodent study to a human model, e.g. in cancer research (Mak et al., 2014). Clinical trials highlight a failure to translate drug effects with human subjects during drug development, possibly due to the difference in complexity between rodents and humans (Freedman et al., 2015). The human brain is far more complex than the rodent brain (Hodge et al., 2019),(Semple et al., 2013). Some differences in neurophysiology exist in the proportion of neuronal regions, laminar distributions, morphology, which might explain the lack of reproducibility in neurophysiological research on humans (Hodge et al., 2019). This gap between rodent and human studies paved the way to using large animal models as translational models such as monkeys, pigs, horses, and sheep (Gigliuto et al., 2014).

2.2 TRANSLATIONAL ANIMAL MODELS

All non-rodent mammalian animal species used for translational research are considered large animal models (Ziegler et al., 2016). Large animal models are advantageous compared to rodent models in terms of pathology and surgical

approaches (Sorby-Adams et al., 2018),(Elsayed et al., 2019). These large animals have already been used to understand stroke and traumatic brain injury (Sorby-Adams et al., 2018) and gastrointestinal pathology (Ziegler et al., 2016). For example, sheep have been used for cardiovascular research (Singh et al., 2009) and postoperative pain research (Häger et al., 2017).

Monkeys are another example of successfully adopted translational models. These nonhuman primates' similarity to human physiology allows them to be fruitful in neurophysiological research, particularly Parkinson's Disease, Alzheimer's Disease, stroke, and spinal cord injury (Goldberg, 2019). Even though nonhuman primates (monkeys) play an essential role in medical research, they are expensive to use. A standard quad-cage for indoor-housed monkeys can range from 8500 USD to 1 million USD (Harding, 2017). Compared to rodent studies, special care is required to house monkeys, contributing to the high cost of research with nonhuman primates. Additionally, considerable ethical and legal aspects in Europe ensure that research with monkeys is strictly regulated (Akhtar, 2015),(SCHEER, 2017).

2.3 PIGS AS TRANSLATIONAL ANIMAL MODELS

The porcine model offers a suitable alternative while maintaining a closer anatomical and physiological link with human studies (Lind et al., 2007).

2.3.1 PIGS IN BIOMEDICAL RESEARCH

Pigs have a life span of 12-15 years (Lind et al., 2007). They grow fast, attaining puberty 5-6 months after birth (Lind et al., 2007). Furthermore, pigs offer closer anatomical and physiological properties to humans regarding their cardiovascular system, immune response, pulmonary system, and skin structure. Pigs are also the species of choice for pharmaceutical research because of their high metabolism and growth rate (Swindle et al., 2012). This similarity makes pigs a popular choice as a research model in cardiology (Sider et al., 2014), respiratory pathology research (Judge et al., 2014), skin research (Pierpaolo Di Giminiani et al., 2014)(Marro et al., 2001). Pigs are abundantly available due to modern farming practices and the demand for pigs for nutrition (Roth & Tuggle, 2015). Even though the cost of pigs remains higher than rodent-based experiments, the translational ability of pigs in recent studies is encouraging for overcoming this gap in the long run.

2.3.2 PIGS IN NEUROPHYSIOLOGICAL RESEARCH

The pig brain has a similar white matter to grey matter ratio as humans (Ryan et al., 2018). Pigs also have gyri and sulci similar to what is found in the human brain (Verena Schmidt, 2013). Figure 2.1 compares the human brain with a pig brain (Clouard et al., 2012). The large size of the pig brain allows more accessible surgical

procedures compared to rodents (Hoffe & Holahan, 2019). Moreover, porcine surgical procedures are like human brain surgical procedures.

In the last decade, there was an increase in the use of pigs for research in modelling human brain disorders (Clouard et al., 2012), such as Alzheimer's Disease (Lind et al., 2007) and traumatic brain injury (Roth & Tuggle, 2015). The pig brain's functional mapping has been researched (Sauleau et al., 2009; Uga et al., 2014) since the pig brain's size allows easier identification of cortical structures using imaging measures. Recently, pigs have been used to demonstrate a healthy brain-computer interface by Neuralink (Crane, 2020).

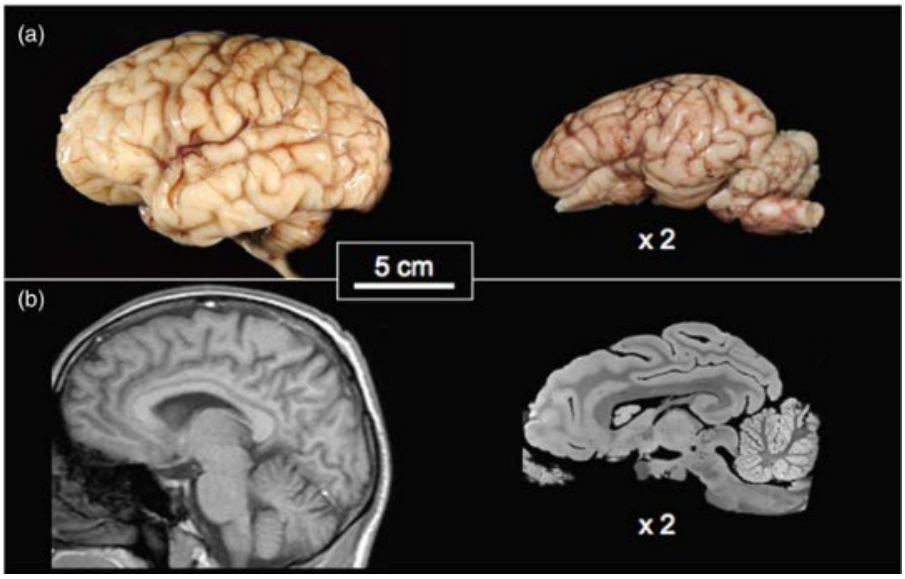

Figure 2.1 (with permission from Clouard et al.). Comparison of the pig brain (right) and the human brain (left). (a) Ex-vivo anatomical brain and (b) magnetic resonance brain images.

2.3.3 PIGS IN PAIN RESEARCH

Pain research in pigs initially focused on pain relief for farm animals (Noonan et al., 1994). Later, the pig model became famous as a translational disease model (Swindle et al., 2012). Amongst the pain models used for research with pigs, the nerve growth factor (NGF) injection model has been used most frequently (Obreja et al., 2011),(Hirth et al., 2013),(Rukwied et al., 2010). Rukwied et al. demonstrated the effect of NGF one week after injection (Rukwied et al., 2010). The researchers reported that NGF resulted in thermal, mechanical, and chemical peripheral sensitisation. Another inflammatory pain model was the ultraviolet irradiation pain model that radiated UV-B light source (1 J/cm^2) on the pigskin and observed

mechanical and thermal sensitivity (P. Di Giminiani et al., 2014). Di Giminiani et al. reported decreased withdrawal latencies and thresholds upon thermal and mechanical stimulation 24 and 48 hours after UV-B irradiation (P. Di Giminiani et al., 2014).

These pain models reflect how pigs are a valuable model for pain research. Additionally, similar assessment techniques adopted in pigs and human models of peripheral neurophysiology directly compare human and porcine pain models (Rukwied et al., 2010), (Meijs et al., 2021). These assessment techniques include: measuring evoked potentials, peak alpha frequency and power in frequency bands (Meijs et al., 2021).

2.4 LTP-LIKE PAIN MODEL

Long term potentiation (LTP) is a phenomenon characterised by increased synaptic strength (Jürgen Sandkühler & Gruber-Schoffnegger, 2012). LTP in the spinal nociceptive pathways can be induced using high intensity, short duration, high-frequency stimulation (HFS) (J. Zhang et al., 2016) (Ruscheweyh et al., 2011). Spinal LTP is a form of LTP that develops in the dorsal horn of the spinal cord due to HFS on a peripheral nerve (Yang et al., 2014). LTP offers stimulus specificity; the stimulated nerve used to induce HFS is the only one affected by the resulting potentiation in the CNS (Kirk et al., 2010).

Since spinal LTP is typically induced by noxious input, spinal LTP is hypothesised to contribute to acute postoperative pain and forms of chronic pain that develop from a painful event, neuropathy or peripheral inflammation (Ruscheweyh et al., 2011). Drdla-Schutting et al. reported that spinal LTP is induced by abrupt opioid withdrawal, making it a possible mechanism of some forms of opioid-induced hyperalgesia (Drdla-Schutting et al., 2012). These studies concluded that preventing LTP induction may help prevent the development of amplified postoperative pain. Furthermore, successful reversal of an established LTP may help treat patients with an LTP component to their chronic pain.

2.4.1 LTP IN RODENTS

Spinal LTP has been induced in rodents using high-intensity HFS on the sciatic nerve to demonstrate changes in the measured C-fibre evoked potentials in the superficial dorsal horn of the spinal cord (Liu & Sandkühler, 1997) (Schouenborg, 1984). LTP has also been proven to develop due to peripheral inflammation (Ikeda et al., 2006) and mechanical nerve injury used for neuropathic pain (H.-M. Zhang et al., 2004),(Jurgen Sandkühler & Liu, 1998). Furthermore, a positron emission topography (PET) scan showed that HFS results in acute hypermetabolism in the S1 (Hjornevik et al., 2008), highlighting the cortex's role in spinal LTP.

2.4.2 LTP-LIKE PAIN IN HUMANS

A numerical rating scale (NRS) was used in human experiments to confirm changes due to HFS induced spinal LTP (Lang et al., 2007). The results showed a 50% increase in the normalised pain ratings after HFS. Van den Broeke et al. used a visual analogue scale (VAS) combined with event-related potentials in response to pinprick stimulus to signify the effect of HFS on human subjects (Emanuel N. Van Den Broeke et al., 2010). The same group similarly used pinprick stimulation response and electroencephalography (EEG) response to study the effect of secondary hyperalgesia induced via HFS (Emanuel N. Van Den Broeke et al., 2017). The researchers reported an increase in low-frequency neuronal oscillations followed 64 and 96 mN pinprick stimulation after HFS on the forearm. Using EEG, the group demonstrated LTP to induce hyperalgesia, providing an opportunity to develop non-subjective biomarkers of pain in humans.

2.4.3 LTP IN PIGS

LTP as a pain model has been well-explored in rodents and humans (including EEG studies in humans), but the cortical responses to LTP remain comparatively less explored in rodents. LTP can be studied in pigs since pigs have already proven useful for neurophysiological research using various pain models (Herskin & Di Giminiani, 2018). Studying LTP in pigs can be fruitful since pigs allow invasive intracortical measures to be made, similar to rodents, while also exploring the translational aspect of LTP.

2.5 INTERFACING THE CORTEX

Cortical areas involved in pain processing mechanisms include, but are not limited to, the somatosensory cortex (S1), the anterior cingulate cortex (ACC), the prefrontal cortex (PFC) and the insula (Garland & Ph, 2013),(Lu et al., 2016). Due to the anatomical similarity between pigs and humans, S1 can easily be identified and accessed via neurosurgery (Sauleau et al., 2009).

Over the years, cortical information has been assessed in various ways. More recently, non-invasive techniques such as functional magnetic resonance imaging (fMRI), functional near infra-red spectroscopy (fNIRS) have become popular but traditional measures such as EEG are still commonly used when extracting neural information. A disadvantage of these techniques is that, due to volume conduction, the information becomes clustered with irrelevant data (Rutkove, 2007). Hence, the cortical state must be estimated using source separation algorithms on the measured signals (Ma et al., 2016),(Baillet et al., 2001).

Alternatively, invasive measures such as using penetrating microelectrode arrays (MEAs) and electrocorticography (ECoG) provide a closer look into the cortical

processes since the electrodes are placed in proximity with the neurons while the brain responds to peripheral stimuli (Buzsáki et al., 2016). MEAs can detect a broader range of frequency components than all other techniques, which explains their popularity in animal research (Szostak et al., 2017). However, despite their advantages, invasive measures of cortical analysis are susceptible to infection in chronic experiments with large animals and cannot easily be used with human subjects due to ethical concerns.

In recent years EEG and ECoGs have been compared to assess the advantage of one technique over another (Petroff et al., 2016), (Im & Seo, 2016), (Buzsáki et al., 2016). Compared to EEG, ECoGs have a higher signal to noise ratio, less susceptibility to artefacts and improved temporal and spatial resolution; hence, they are increasingly used for brain-computer interfaces (Jeremy Hill et al., 2012). However, EEG remains the most commonly used technique in humans because of the non-invasive nature of the recording setup (Im & Seo, 2016). On the other hand, MEAs are frequently used in animal studies for recording spike activity and local field potentials in vivo and in vitro experiments (Kellis et al., 2016), (Herreras, 2016),(Buzsáki et al., 2012). The development of microelectrode grids (Brodnick et al., 2019), (Rogers et al., 2019), (Rubehn et al., 2009) has allowed researchers to discover a more localised nature of the µECoG electrodes (Dubey & Ray, 2019).

To our knowledge, no direct comparison in terms of power in different frequency ranges has been made between the MEA and the µECoG electrodes. This comparison may be helpful in chronic experiments in large animals when damage to blood vessels while implanting the electrode could lead to infections. µECoGs also hold a particular advantage over MEAs since the µECoG array is placed on the brain's surface while MEAs need to be inserted. This electrode positioning ensures that the brain remains relatively undisturbed by the recording procedure.

2.6 PROCESSING CORTICAL INFORMATION

Data recorded via MEAs is traditionally processed by removing the low-frequency components to identify spike activity using peri-stimulus time histograms (PSTHs) (Weille, 2006). PSTHs are typically made to demonstrate changes in the firing rate of neurons within a specified time window (1 to 10 ms bin size) (Shimazaki & Shinomoto, 2007). The area under the curve (AUC) of the PSTH represents the most number of spikes detected by the MEA within a time window, and the calculated latency of the peak spike (peak latency) represents the time taken for the cortex to respond to a stimulus by increasing the spike activity to the highest value (Ghazanfar & Nicolelis, 1999). This spike activity reflects the firing rate of the neurons as they process the somatosensory information (Brown et al., 2004). Figure 2.2 summarises the process of computing PSTHs from spike activity.

MEA also offer the ability to extract cortical information regarding the local field potentials (Herreras, 2016). The raw data can be filtered from 0 to 300 Hz to compute

event-related potentials (Murray et al., 2008) as well as neuronal oscillations in predefined frequency bands, namely, alpha (8 to 13 Hz), beta (18 to 25 Hz), low-gamma (30 to 70 Hz), high-gamma (70 to 150 Hz), delta (0.5 to 3 Hz), and theta (3.5 to 7 Hz) (Ploner et al., 2017). µECoG does not offer the firing rate like the MEAs since the brain tissue acts as a low pass filter and prevents the µECoG array from capturing this information (Dubey & Ray, 2019). Another explanation for this is that the larger size and lower impedance of µECoGs prevent the capture of short-duration action potentials produced by cortical neurons compared to MEAs. However, µECoGs can still provide information in the time domain through evoked responses and topographical changes in the recorded cortical signals (Jeremy Hill et al., 2012).

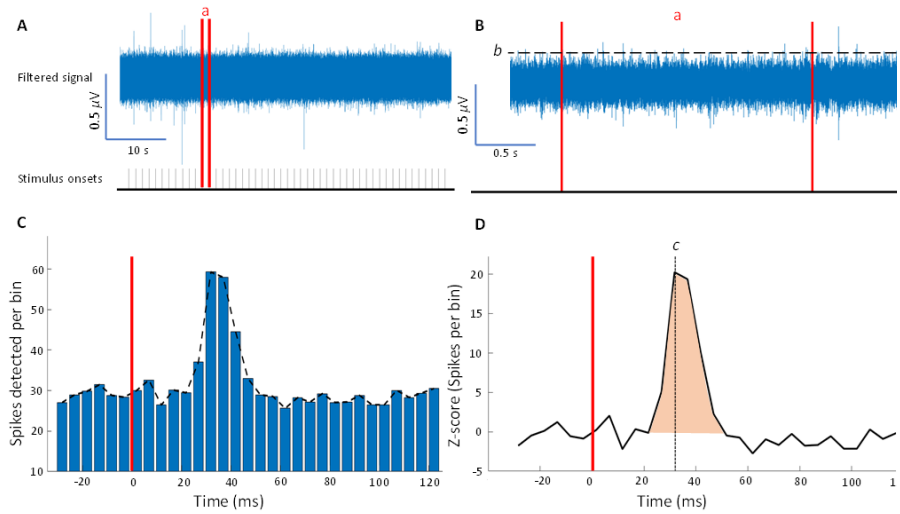

Figure.2.2. Illustration of how PSTHs were computed. A) An example of the filtered signal along with stimulation onsets B) A magnified view of the spikes and the spike detection threshold labelled 'b' C) PSTH constructed using a 5 ms bin size D) Using the PSTH to measure the AUC and the peak amplitude labelled 'c'.

Changes in the amplitude of the event-related potentials (ERPs) demonstrate a joint synchronisation of local field potentials in response to peripheral input(s) (Herreras, 2016). ERPs can be computed by averaging across a specified number of trials ranging from 50 to 100 (peripheral stimulus-locked time windows). An example of how an ERP is computed is shown in Figure 2.3.

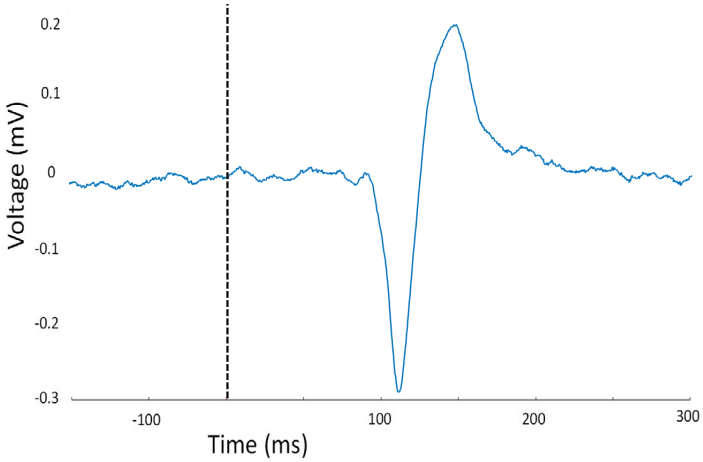

Figure 2.3. An example of the ERP of one channel, from the MEA, averaged across 50 trials. The dotted line represents the stimulus onset.

CHAPTER 3. OUTLINE OF THE PH.D. WORK

The porcine model offers an alternative translational animal model that can overcome the monkey model's high costs and ethical concerns while maintaining a close link with human studies. The similarity between pig and human brain sizes also allows one to compare different electrode types used in animal research (Sauleau et al., 2009). To our knowledge, there has not been a direct comparison, in terms of energy in recorded frequency bands, between the MEA and µECoG electrode types, like there has been for µECoGs and EEG electrodes (Petroff et al., 2016), despite their critical role in the brain research (Im & Seo, 2016).

3.1 THESIS AIMS

The primary aim was to establish a translational pig pain model by implementing an LTP-like pain model. The secondary aim was to compare recording methods for assessing intracortical processes by comparing two commonly used methods of intracortical signal recording in animals, namely, µECoG array and the MEA.

3.2 SPECIFIC RESEARCH QUESTIONS

To address the thesis aims, three specific research questions were designed.

Q1. Can S1 spike activity, detected by the MEA, capture the effect of peripherally induced HFS?

Study I aimed to establish whether the MEA could capture cortical signals from the S1. The recorded signals from the MEA were analysed, demonstrating the effect of peripherally driven HFS on the pig cortex. The effect of HFS induced spinal LTP was validated by measuring the changes in the spike activity in the S1. The work is described in the following publication:

Study I: Modulation of intracortical S1 responses following peripheral nerve high-frequency electrical stimulation in Danish Landrace pigs – Journal of Brain Research (under review).

Q2. Can local field potentials from S1 reveal the effect of LTP in pigs?

Study II focused on changes in the local field potentials recorded from the S1 using the MEA. This study filtered out the high-frequency components and measured changes in the neuronal oscillations and event-related potentials after HFS. The work has resulted in the following publication:

Study II: The effect of peripheral high-frequency electrical stimulation on the primary somatosensory cortex in pigs – IBRO Neuroscience Reports DOI: 10.1016/j.ibneur.2021.08.004.

Q3. Is there an advantage of using MEAs for extracting cortical information compared to µECoGs?

In study III, the µECoG array was placed on the S1 surface and compared the recorded signals to the recorded data from the MEAs in Study I and Study II. The analysis was performed using power spectral density on the raw signal extracted from both arrays. The power in a range of frequency bands was also compared between the two types of arrays. Comparing the arrays would allow future animal researchers to assess the usefulness of using MEAs, which are comparatively more invasive than µECoGs. The work is described in the following publication:

Study III: Why so invasive? A micro ECoG and microelectrode array comparison for assessing peripherally driven cortical response – IEEE Transactions in Biomedical Engineering (in preparation).

3.3 SOLUTION STRATEGY

Several methodological choices were made to address the research questions.

3.3.1 METHODOLOGICAL CHOICE: S1 AS THE CORTICAL REGION FOR RECORDING

Located on the postcentral gyrus, S1 is a primary receptor of somatosensory information (Bushnell et al., 1999), (Hu et al., 2014). S1 is activated by touch, pressure and even auditory and visual stimuli and is also one of the central regions involved in pain processing mechanisms studied in rodents and humans (Borich et al., 2015), (Frot et al., 2013). S1 is divided into sub-regions that correspond to sensation on the forelimb of pigs (Orlowski et al., 2019), (Bjarkam et al., 2004), making it ideal for placing electrodes for recording the cortical response to peripheral stimulation and spinal LTP-like neuroplasticity. In pigs, the S1 is easily accessible via cortical surgery. Hence, S1 was selected to record cortical responses to peripheral stimulation.

3.3.2 METHODOLOGICAL CHOICE: µECOG VS MEA

The µECoG electrode was placed in the same position as the MEA in separate animals, ensuring that the µECoG array was the same size and orientation as the MEA.

For subdural recordings, a 32 channel µECoG (Neuronexus Probes, USA) was placed on the surface of S1 during the experiment. The µECoG, shown in Figure 3.1, was 7 mm by 4 mm wide with a contact diameter of 200 µm and a 1 mm interelectrode distance.

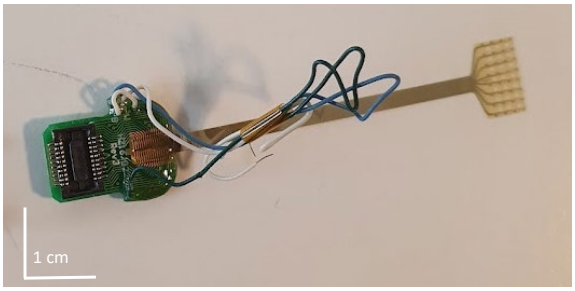

Figure 3.1. 32-channel Neuronexus µECoG used for recording from S1.

A 16-channel Microprobes MEA (Microprobes Inc., Gaithersburg, MD, USA) was selected for intracortical recording signals from S1. The MEA, shown in figure 3.2, had 1 mm between adjacent electrodes, which consisted of 2 mm long shafts with only the tip exposed for recording. The electrode was inserted 2 mm into the S1 using a Kopf micromanipulator.

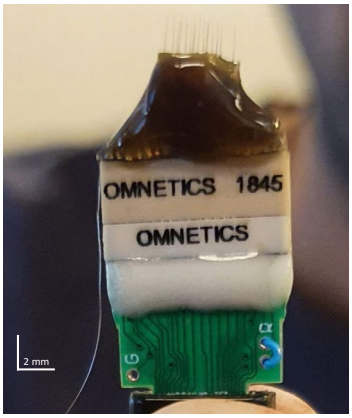

Figure 3.2. 16-channel Microprobes MEA connected to an Omnetics adapter (for compatibility with the TDT recording setup).

3.3.3 METHODOLOGICAL CHOICE: LTP AS A SURROGATE MODEL OF PAIN

A vital part of this research was to highlight that the pig cortex has a similar neurophysiological response compared to humans. The induced pain model had to be one that was tested in both rodents and humans so the human neurophysiological responses could be 'backwards' translated while rodents based invasive measures could substantiate the pain model in pigs. LTP-like pain model was one such pain model. LTP mimics a state of hyperalgesia as reported in human studies (Jürgen Sandkühler, 2007). Additionally, HFS induced on the forearm demonstrated a state of hyperalgesia in humans (Jurgen Sandkühler & Liu, 1998). Klein et al. reported increased pain ratings 20 min after HFS was induced (Pfau et al., 2011).

Spinal LTP has been induced using HFS on the sciatic nerve in rodents. The changes recorded on the spine showed increased excitatory post-synaptic potentials' amplitude (Ranclic et al., 1993). Furthermore, LTP in rodents resulted in hypermetabolism in S1 and increased evoked potential in the thalamus in rodents (González-Hernández et al., 2013),(Hjornevik et al., 2008). In most rodent studies, The LTP induction using HFS was by using 100 Hz, four sweeps of 10 times the motor threshold of the animal (Sanoja et al., 2013),(Hansen et al., 2007). Hence, LTP in pigs was induced similarly to ensure that the recorded cortical response was comparable to rodents and humans.

CHAPTER 4. METHODOLOGICAL APPROACHES

4.1 STUDY DESIGN

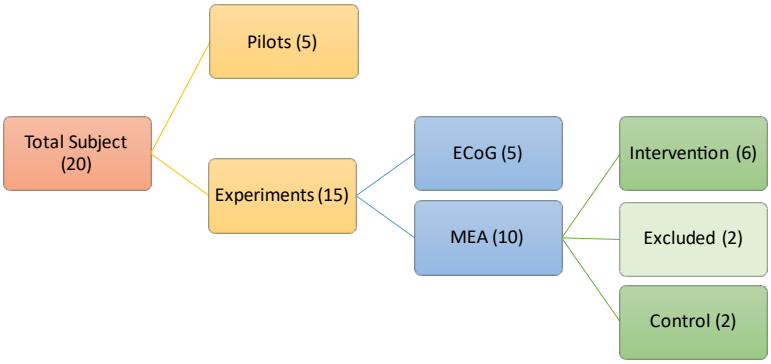

Figure 4.1. Summary of the 20 animals' selection and allotment for each group.

All experiments were designed and conducted following the guidelines of protocol number 2017-15-0201-01317 of the Danish Veterinary and Food Administration under the Ministry of Environment and Food of Denmark.

Twenty female pigs weighing 33 kg ± 3.5 kg (mean ± standard deviation) were used for the three planned studies. As shown in Figure 4.1, five animals were selected as pilots to assess the cortical response to peripheral stimulation and determine whether the surgical procedure was possible to perform within the time frame of the animal laboratory. Out of the 15 remaining subjects, five were used for µECoG-based analysis and the rest for MEA-based analysis. The animal selection was randomised by ensuring that the surgeon performing on the experiment day was blinded to the animal conditions before selecting the animal for the group allotment.

In the MEA-based study, one animal did not respond to anaesthesia; therefore, the experiment had to be terminated, and noise interference was experienced in the second experiment, so the data had to be excluded from further analysis. Of the remaining eight subjects, two were used for the control group, and six were allotted to the intervention group.

4.2 METHODOLOGICAL DEVELOPMENT

The first challenge while performing the pilot experiments was the lack of a stereotaxic frame to stabilise the pig in position while performing the surgery. The stereotaxic frame had to be compatible with Kopf micromanipulators available in the animal laboratory. It was imperative to construct a stereotaxic frame capable of performing the surgery on a range of pig weights since pig head diameters grew substantially within weeks, ranging from 16 cm to 33 cm (ear to ear length). Thus, a stereotaxic frame was designed in Solidworks (Solidworks, USA). The stereotaxic frame was constructed using a combination of acrylic sheets (20 mm thick) and polylactic acid (PLA)-based 3D printed parts. The 3D printed parts played a critical role in ensuring compatibility between the stereotaxic frame walls and the Kopf micromanipulators, as shown in Figure 4.4.

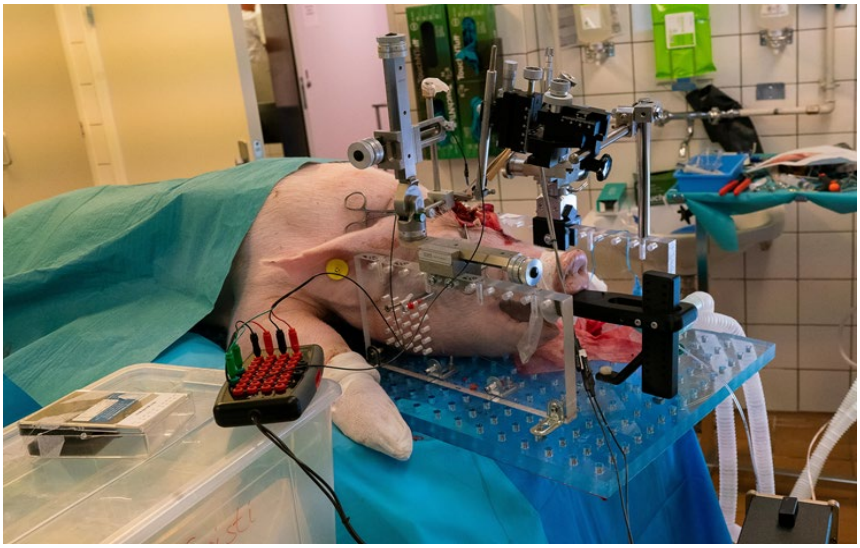

Figure 4.2 Example of a pig experiment with a custom-built stereotaxic frame and Kopf micromanipulators

4.2.1 THE STEREOTAXIC LOCALISER BOX

The localiser box shown in Figure 4.2 consisted of a rectangular base plate (750 mm length by 400 width mm by 20 mm thickness) onto which two side walls (300 mm length by 150 mm width by 20 mm thickness) were mounted using stainless steel corner braces. Each side wall had a five-by-five array of six mm diameter holes (inter-hole distance of ten mm) placed to insert aluminium skull screws used to fix the subject's head in the localiser box. Figure 4.3 demonstrates a CAD model of the complete assembly of the localiser box.

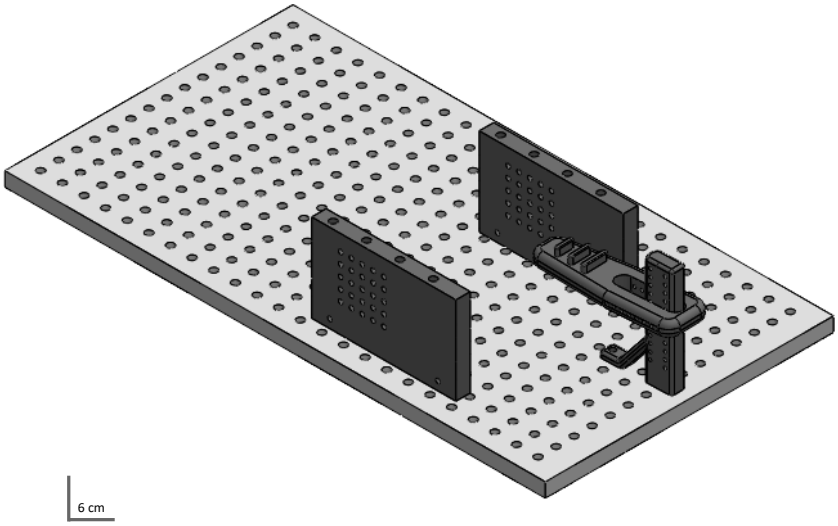

Figure 4.3 CAD model of the stereotaxic frame

4.2.2 MESH OF HOLES IN THE BASEPLATE

The baseplate had a mesh of holes for two reasons:

1. Temperature regulation: The animal body temperature is constantly monitored during an experiment since it affects the neurophysiological response of the subject. A thick base plate can inhibit proper temperature regulation. Using a meshed base plate allowed a relatively improved temperature regulation using an air blanket (Mistral-Air Plus, MA1100-EU).

2. The animal's head size can vary depending on the subject's weight. Pigs head sizes can vary from 16 cm to 30 cm ear to ear width depending on their weight and age (Bollen et al., 2010). This difference in animal head sizes was compensated by moving and securing the side walls across the whole meshed base plate and placing them using corner braces according to the experiment's requirement.

4.2.3 MOUTHPIECE

The assembly also contained a 3D printed adjustable mouthpiece that was needed to secure the snout in position. The mouthpiece was mounted on the base plate using two screws (10 mm diameter), shown in figure 4.4A, had three mm protrusions five mm apart to ensure that the subject's front teeth could be firmly secured. All components of the mouthpiece were made from PLA.

4.2.4 MICROMANIPULATOR ADAPTER (3D PRINTED) FOR KOPF MODEL 1760

A micromanipulator adapter was designed, and 3D printed to mount the Kopf Model 1760 micromanipulator on the sidewall. The design specifications are shown in figure 4.4.

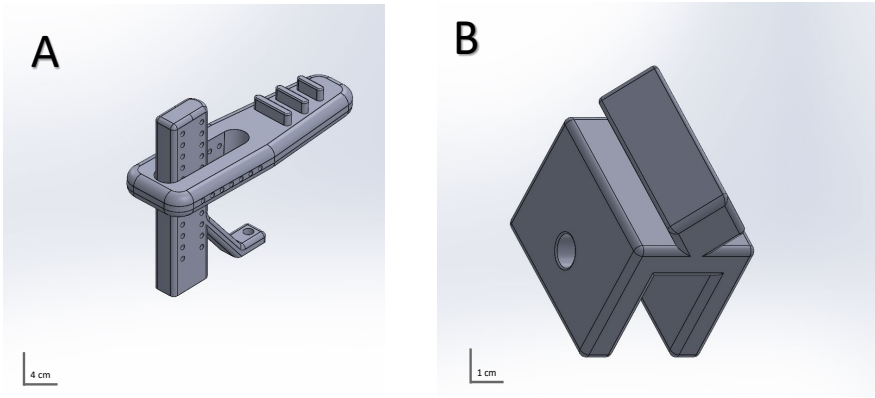

Figure 4.4 A) CAD model of the 3D printed mouthpiece. B) Design of the 3D printed micromanipulator adapter for Kopf Model 1760

4.2.5 DIRECT WALL MOUNT

The localiser box had eight mm in diameter and 50 mm deep holes in the walls to allow mountable micromanipulators onto the sidewall (example shown in Figure 4.2). Furthermore, eight mm holes were made into the side walls to mount the Kopf slider assembly (Kopf Model 1760-61) on the sidewall, as shown in Figure 4.5.

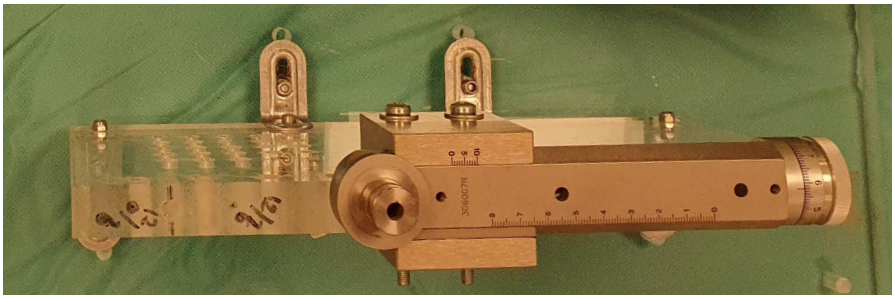

Figure 4.5 Kopf Slider assembly for the micromanipulator mounted on the sidewall of the assembly

4.3 EXPERIMENTAL SETUP

On the day of the surgery, a pig was sedated using an intramuscular injection (Zoletil mix for pigs – contains: tiletamine, zolazepam, xylazine, ketamine and butorphanol). Once sedated, the pig was placed on the operating table in a supine position while intubating it with oxygen and air mixture (1:1 ratio). Hydration was ensured using a consistent flow of saline-infused through the jugular vein. For the anaesthesia, 1.5 to 2.5% minimum alveolar concentration (MAC) was administered with propofol (2 mg/h/kg IV) and fentanyl (10 µg/h/kg IV). Signs of stress were monitored using physiological measures recorded every 15 min. Upon any abnormality, the pig was stabilised by adjusting the anaesthetic parameters. The pig was euthanised after the experiment by overdosing pig with pentobarbital (intravenously infused).

All the recording equipment was purchased from Tucker Davis Technologies (TDT) (Alachua, FL, USA.). After the surgery, the electrodes were connected to a ZIF clip connected to the SI-8 Neurodigitizer. The SI-8 was connected to the RZ2 processor via optic fibres. The RZ2 processor was connected to an RS4 data streamer to store unfiltered data. The RZ2 was also connected to the WS-8 workstation for online streaming while recording the cortex using the Synapse software. The recording setup is illustrated in Figure 4.6.

Both arrays (MEA and µECoG) were placed in the S1 to record intracortical changes due to HFS on the ulnar nerve. The electrodes were placed at the same cortical position (S1), so the recorded intracortical information could be compared to demonstrate the strengths and weaknesses of each technique.

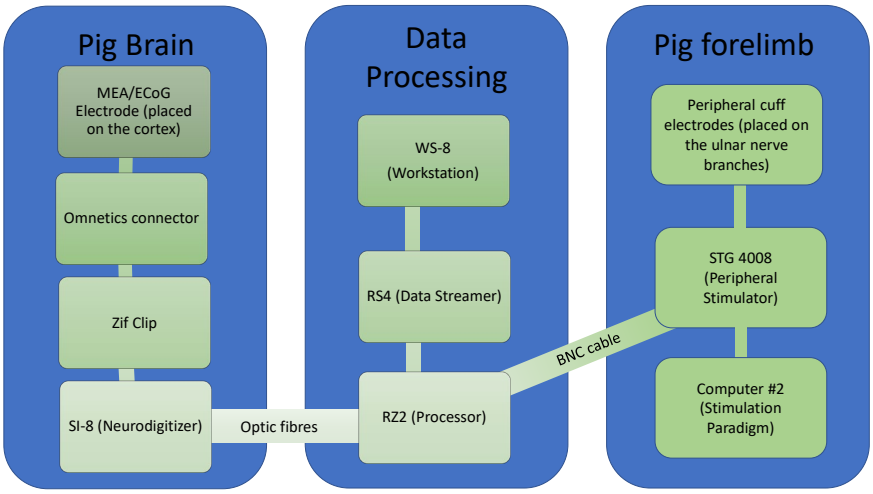

Figure 4.6 Illustration of the recording setup.

4.3.1 SURGICAL PROCEDURE

While the pig was in the supine position, the peripheral surgery was performed where two ulnar nerve branches were exposed. Custom-made tripolar cuff electrodes (contact size of 1 mm) were placed around each branch and secured using three sutures on each cuff. Saline was injected into the cuff electrodes to ensure contact and humidity, and saline-dipped gauze was placed in the wound before closing it using sutures.

At this point, the pig was flipped to a prone position and fit into a custom-built stereotaxic frame. Two eight mm diameter screws were used to fix the pig against the sidewalls of the stereotaxic frame by pushing each screw against each cheekbone. Subsequently, craniotomy was performed in which an electrosurgical knife was used to expose the skull. Two holes were drilled down to the dura for the ground and reference screws. A three cm by five cm window, centred around the bregma, was made to expose S1. The skull was drilled using a rotatory tool (Dremel 8220, Dremel, US). After removing the skull, the dura was flushed with saline before starting durotomy. A syringe needle was bent to form a hook used to pull the dura and cut using micro scissors. The dura was held two mm above the cortex using micro forceps while exposing the brain to minimise bleeding. The brain was regularly flushed with saline throughout the surgery and during recording. An example of the exposed cortex is shown in Figure 4.7, highlighting the targeted S1.

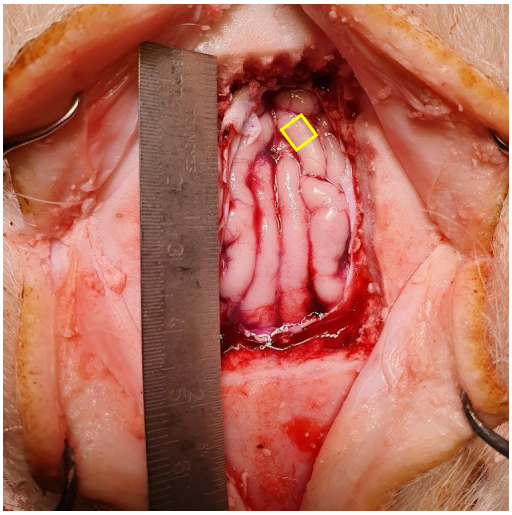

Figure 4.7 Picture of the brain exposed. The yellow box indicates where the MEA was inserted or the µECoG was placed on the S1.

4.3.2 PERIPHERAL STIMULATION PARADIGM

The ulnar nerve was stimulated by a programmable stimulator, STG 4008 (Multichannel Systems, Reutlingen, Germany). The stimulator was also connected to the TDT system to synchronise the triggers with the recording system. One of the cuff electrodes was used to stimulate the ulnar nerve for probing the brain, while HFS was induced using both cuff electrodes. A 1 mA amplitude, 50 µs pulse duration stimulation was used for probing. The HFS was induced using a 15 mA, 1 ms pulse duration, four sweeps at 100 Hz. Figure 4.8 illustrates the peripheral stimulation paradigm along with the recording protocol.

4.3.3 RECORDING PROTOCOL

The recording and stimulation protocol was divided into three phases, shown in Figure 4.8.

T0 phase (Pre-LTP): During the T0 phase, three sets of 50 peripheral stimulations (1 mA, 500 µs) were administered to the ulnar nerve at 0.5 Hz with 12 min breaks between each set.

Intervention (LTP phase): During the intervention, both ulnar nerve branches were targeted. A 15 mA, 1 ms pulse width, 100 Hz sweep was induced four times. No stimulation was done during this phase in the control group.

T1-T3 phases (Early, Mid, Late LTP phases): After an intervention, during the phases T1 to T3, the same stimulation protocol was adopted for the rest of the experiment. Each phase represents 45 min of the experiment for illustration.

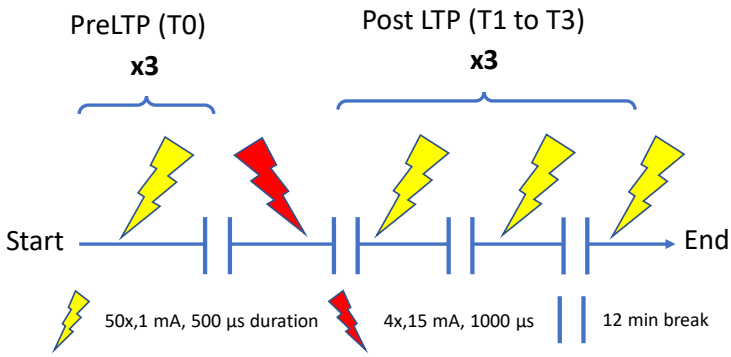

Figure 4.8 A summary of the experimental protocol

4.4 SIGNAL PROCESSING METHODS

Data, recorded at a sampling frequency of 24 kHz from the MEA and the µECoG array, was analysed within the time and frequency domains. In the time domain, the event-related potentials (ERP) and the peri-stimulus time histograms (PSTHs) were computed from the MEA. The data from the MEA was also assessed in the frequency domain, where frequency band oscillations were analysed, using power spectral density (PSD) to denote the effect of the intervention. Frequency band power was also used to compare the MEA and the µECoG array.

4.4.1 PRE-PROCESSING

Power line noise was removed from the recorded data using an FIR 8th order notch filter at 50 Hz in the pre-processing phase. The respective harmonics of the 50 Hz noise up to 500 Hz were also removed from the raw data. After this, malfunctioning channels were visually identified and removed from the analysis before further analysis. For the MEA analysis, the data were bandpass filtered (FIR 8th Order) between 0.3 Hz to 300 Hz to identify changes in the local field potentials and from 500 Hz to 9000 Hz (FIR 8th Order – bandpass) to isolate the spike activity detected by the MEA for calculating PSTHs. Additionally, the data was windowed into epochs of -500 ms to 1500 ms for illustration.

4.4.2 DATA ANALYSIS

ERPs were computed from the MEA and µECoG to demonstrate the effect of probing the CNS using peripheral electrical stimulation on the ulnar nerve. ERPs were calculated by averaging the 50 trials to minimise detected cortical activity non-locked to the stimulation. ERPs across all channels were pooled to represent the overall changes in the S1 in response to LTP.

PSTHs from MEAs were computed using the data filtered between 500 Hz to 9000 Hz. A five ms bin size was used to detect peaks using the "*findpeaks*" function in MATLAB (MathWorks, Inc., Natick, MA, USA). A threshold of four times the standard deviation of the 300 ms pre-stimulus baseline was set for spike detection. The spikes detected per bin were converted to Z-score by subtracting the spikes during baseline and dividing the obtained value by the standard deviation of the spikes during the pre-stimulus period. The area under the curve (AUC) of the PSTH was used to denote the change in spikes due to peripheral electrical stimulation following HFS. Peak latency was also measured to identify the effect of the intervention on the response time of the S1 neurons.

In the frequency domain, neuronal oscillations were isolated according to predefined frequency bands: delta, theta, alpha, beta, low-gamma, and high-gamma. Each channel was windowed into epochs from 75 ms to 200 ms after stimulus using a

Hamming window. A periodogram of the same length as the epoch was then applied to calculate the band power in each frequency range. This technique was implemented using the "*bandpower*" function in MATLAB (MathWorks, Inc., Natick, MA, USA). The comparison between the µECoG and MEA also used the "*bandpower*" function for comparing power captured by each electrode type within different frequency ranges. The bandpower comparison was made within the 500 ms time window (post-stimulus) and across the 500 ms time window before stimulus and 100 ms post-stimulus time window.

4.5 STATISTICAL ANALYSIS

For all three studies, the normality of the data was assessed through residual analysis via QQ-plots and histogram plots. A Wilcoxon signed-rank test was used for the ERP-based and PSTH-based analyses to test changes in the three phases (T1-T3) compared to T0. Bonferroni correction was applied for multiple comparisons with a significance level of 0.05 adopted before the Bonferroni correction. Changes in the frequency band power were statistically analysed using a Friedman test on each intervention group and control group. Upon a significant difference between the time-phases, a Mann-Whitney U test was performed to signify the time phases where the difference between each intervention group and its respective control group was significant. In the third study, a Wilcoxon rank-sum exact test was used to assess the ERP magnitude between the MEA and the µECoG. The Friedman test assessed the differences in the frequency bands power within the µECoG and the MEA. A Mann-Whitney U test was used to compare the power in each electrode across frequencies.

CHAPTER 5. SUMMARY OF THE MAIN FINDINGS

This chapter summarises the three studies introduced in Chapter 3: Outline of the Ph.D. work. A brief overview of each study's main results and how each one addressed the respective research questions are given below.

5.1 SUMMARY STUDY I

Study I aimed to investigate changes in the S1 spike activity following HFS using the MEA. In this study, the ulnar nerve was probed 50 times using a 1 mA, 500 µs stimulation pulse every 2s ± 250 ms. A 12 min break followed this before repeating the process two more times. The overall 45 min recording was categorised as a single recording set, namely, the T0 phase. The T0 phase was followed by an intervention for six pigs, where HFS (16 mA, 1000 µs, four sweeps) was used to induce spinal LTP-like neuroplasticity. The control group, consisting of two pigs, was not subjected to HFS but immediately moved on to the next phase. T1 to T3 phases followed the intervention phase where the ulnar nerve was probed like the T0 phase.

The recorded data was filtered from 500 Hz to 9000 Hz to isolate spike activity for computing PSTHs using a 5 ms bin size. PSTHs demonstrated that the AUC increased in the T1 phase, which became significantly higher than the T0 phase during the T2 phase ($p < 0.01$). The AUC then decreased slightly, returning to the T1 phase level in the T3 phase. The peak latency, signifying the latency of the highest peak of the PSTH, was also compared across the different time phases. However, the peak latency remained unaffected by the intervention showing no difference across the three phases.

The AUC of the PSTH demonstrated that the excitability of the S1 increased, likely due to LTP-like neuroplasticity induced via HFS. Since the peak latency was not affected by HFS, it was deduced that the response time of S1 was either not affected by HFS or that the response was not captured using the 5 ms bin size.

5.2 SUMMARY STUDY II

Study II aimed to use the MEA to investigate the effect of HFS on the S1 local field potentials. The study was conducted on the signals recorded for Study I; hence, the methodology for extracting cortical data was the same. However, data processing in Study II was performed by removing line noise and its harmonics using a notch FIR filter followed by a bandpass filter between 0.3 Hz and 300 Hz to capture local field potentials. The filtered data was analysed by computing ERPs and changes in the

power of neuronal oscillations. The ERP analysis involved using the first negative peak post-stimulus (labelled N1) and determining changes in this peak across the different time phases (T0 to T3). Furthermore, power in neuronal oscillations (alpha, beta, delta, theta, gamma, and high gamma) was computed in this study.

The results highlighted a significant increase ($p < 0.01$) in N1 amplitude during the T2 phase 45 min after the intervention. This increase in N1 amplitude decreased in the T3 phase. Power in neuronal oscillations highlighted a similar trend in frequency bands due to HFS. These results were consistent with the change in the PSTH data found in Study I.

These findings signified that the changes due to peripheral HFS did not only affect the excitability of the S1 neurons, as demonstrated in Study I but also resulted in increased synchronised action potentials across neurons in the S1 due to HFS. The neuronal oscillations demonstrating a similar trend in power illustrates that the effect of HFS can be measured using power in specific frequency oscillations in peripherally driven evoked potentials.

5.3 SUMMARY STUDY III

The third study was designed to compare µECoGs to MEAs with respect to large animal studies. Ten pigs, evenly divided into two groups of MEA and µECoG, were used for this study. All animals were subjected to three sets of 50, 1 mA, 500 µs duration electrical stimulations on the ulnar nerve while recording the cortical response from the pig S1 using an MEA. The peak-to-peak amplitude of the cortical response from each electrode and the power across the conventionally used set of frequency ranges (alpha, beta, gamma, delta, theta, high gamma), as well as higher frequency ranges (150 Hz to 300 Hz, 300 Hz to 500 Hz, 500 Hz to 1 kHz, 1 kHz to 5 kHz, 5 kHz to 10 kHz), were extracted and compared. The two electrode types were evaluated based on each frequency band's post-stimulus to pre-stimulus power ratio.

The ERP analysis showed that the MEA based peak-to-peak amplitude was significantly greater than the µECoG ($p < 0.01$). The spectral analysis demonstrated that the MEA had more power in the conventional frequency ranges than the µECoG ($p < 0.01$). The MEA also had higher power in the higher frequency ranges compared to the µECoG ($p < 0.01$). Furthermore, the conventionally used frequency ranges showed a higher post-stimulus to pre-stimulus power ratio in both electrodes ($p < 0.01$). The MEA outperformed the µECoG in the post-stimulus to pre-stimulus power ratio within these frequency ranges ($p < 0.01$).

The comparison between MEA and µECoG was essential because of the extensive use of both electrodes in animal studies (Fekete & Pongrácz, 2017), (Brodnick et al., 2019), (Foffani et al., 2004), (Kim et al., 2018). Power across frequency bands has not been compared between the two electrode types despite the abundance of use in

animal experiments. The MEA offers the ability to detect changes in the firing rate of neurons and the local field potentials (Brette, 2015), (Kim et al., 2018). The proximity of the MEA electrode to neurons allows a high signal to noise ratio. On the other hand, the µECoG array offers a relatively less invasive option of recording cortical information. The µECoG array can detect local field potentials and has become famous for brain-computer interfaces (Seymour et al., 2017), (Gierthmuehlen et al., 2011), (Volkova et al., 2019).

A direct comparison between these two electrodes may allow a better choice to be made for chronic large animal experiments where implanted electrodes are more susceptible to damage due to the subject's movement. This study demonstrated that both electrodes could be used to assess local field potentials and showed that despite the surgical ease and minimal damage to the subject offered by the µECoG, the MEA maintained a higher signal to noise ratio in all frequency ranges.

Table 5.1 (next page) reviews all three studies' research questions, techniques, and results.

	Study I	**Study II**	**Study III**
Research Question	**Q1** Can S1 spike activity, detected by the MEA, capture the effect of peripherally induced HFS?	**Q2** Can local field potentials from S1 reveal the effect of LTP in pigs?	**Q3** Is there an advantage of using MEAs for extracting cortical information compared to µECoGs?
Main Technique	- Insert the **MEA** into the **S1** - Analyse the **spike activity** by computing PSTHs - Calculate the **AUC** and **peak latency** of the PSTHs - Assess changes in the AUC and peak latency due to HFS	- Insert the **MEA** into the **S1** - Analyse the **local field potentials** by computing **ERPs** and **band power** in standard **neuronal oscillations** - Assess changes in ERP and neuronal oscillations due to HFS	- Place the µECoG on the surface of the **S1** and insert MEA into the S1. - Compute **power spectral density** of each electrode - Assess changes in power in frequency bands across frequency ranges during evoked activity
Result	- Significantly increased AUC 45 min after intervention - No effect on the peak latency	- Significantly increased N1 45 min peak after intervention - A similar trend found in neuronal oscillations	- µECoG detected LFP like MEA. - The power of MEA was higher than µECoG in all frequency ranges

CHAPTER 6. DISCUSSION

This thesis investigated the feasibility of using pigs as a translational model of LTP-like pain and compared the cortical responses from the MEA and µECoG to peripheral stimulation on the ulnar nerve. The first two studies suggested that pigs are viable for translational research on cortical processes. In these studies, a pain model was induced that had been tested in rodent models and human studies to identify similarities or differences in cortical processing of LTP-like neuroplasticity between the two species. The porcine cortical response was analysed in terms of changes in N1 peak and neuronal oscillations and changes in the spike activity using PSTHs, a technique widely implemented in rodents because of the invasive nature of MEAs.

6.1 MEA RECORDINGS IN RESPONSE TO LTP-LIKE NEUROPLASTICITY

The use of MEAs to detect changes due to HFS allowed a direct comparison between methods used in human cortical processing experiments and rodent studies of pain processing using an LTP-like pain model.

6.1.1 SPIKE ACTIVITY

Spike activity represents the neuronal firing rate during cortical processing. Each “spike” represents an action potential used by neurons to communicate (Brette, 2015). The firing rate can be defined over time, neurons, or trials (Brette, 2015).

In Study I, changes in the S1neurons’ excitability were investigated using an MEA placed 2 mm into cortex targeting layer 4 of the S1. PSTH was calculated based on the recorded spike activity using the MEA, and changes in the AUC and peak latency of the PSTH were used to identify the S1 response to HFS on the ulnar nerve. The PSTH's AUC represented the number of spikes detected by the MEA following peripheral electrical stimulation. The greater the AUC, the more neurons fired in response to peripheral stimulation. On the other hand, peak latency measured the delay in the response of the maximum number of neurons after peripheral electrical stimulation. The results of this research displayed a pattern similar to what is found in rodents' thalamus response to LTP induced on the sciatic nerve (González-Hernández et al., 2013),(Sanoja et al., 2013). Hernandez et al. reported increased neuronal excitability in the posterior triangular nucleus of the Wistar rat’s thalamus in response to spinal LTP, induced via HFS on the sciatic nerve (González-Hernández et al., 2013). The observed changes were detected via PSTHs. A similar setup for spinal LTP induction, using HFS on Sprague Dawley rats' sciatic nerve highlighted a gradual increase in spike activity of thalamic neurons in the ventro-posterolateral nucleus that

became significantly greater than the baseline 60 min after intervention (Sanoja et al., 2013).

6.1.2 LOCAL FIELD POTENTIALS

Local field potentials (LFPs) denote brain activity that reflects dynamic information flow across neurons (Herreras, 2016). An increase in stimulus amplitude may not necessarily reflect an increase in the amplitude of the LFP (Herreras, 2016). In EEG studies, LFP has been shown to contain signals from other brain regions due to volume conduction that must be removed using computational algorithms such as source separation (Ma et al., 2016). In this study, LFP has been used to reflect ERP changes and neuronal oscillations, similar to how human studies analyse cortical activity while inducing a pain model (Michail et al., 2016), (Emanuel N. Van Den Broeke et al., 2010).

Cortical signal analysis in the second study showed that the N1 peak amplitude increases 45 min after HFS and remains significantly higher than the T0 phase during the T3 phase ($p < 0.01$). This increase in N1 is comparable to the N100 peak measured in human studies (Kirk et al., 2010),(Liang et al., 2016). The effect of HFS was reported 20 to 40 min after intervention in human studies where the N100 ERP amplitude and reported pain ratings were combined. (Klein et al., 2006),(Hjornevik et al., 2008).

Synchronised neuronal activity is also reflected by power in frequency bands such as alpha, beta, theta, delta, and gamma (Ploner et al., 2017). Sensory activation can lead to evoked oscillations that correspond to the stimulus (Başar, 2013). Study II measured these neuronal oscillations to compare the pig neuronal oscillations to the human brain's pain response. Michail et al. reported increased gamma and theta oscillations in S1 while processing pain in human subjects (Michail et al., 2016). The group also reported a significant decrease in the alpha frequency range when the subjects experienced a painful stimulus. Results from Study II concluded that neuronal oscillations more than doubled power in delta, high gamma, and theta frequency ranges 45 min after HFS. This similarity was used to deduce that the S1 response to peripheral electrical stimulation was like the human brain response when LTP induced central sensitisation causes hyperalgesia in the subjects (Jürgen Sandkühler, 2007).

Interestingly, Study II showed an increase in alpha-band power after the intervention, unlike the results from human experiments. Michail et al. suggested a correlation between attention and suppression of alpha-band oscillations, as also reported by Babiloni et al. (Babiloni et al., 2006). This trend was not seen in the results of this thesis because the subjects were anaesthetised during the experiment.

6.2 TYPE OF INFORMATION CARRIED BY µECOG AND MEA

Studies I and II established that pigs could be used as a translational model of LTP-like pain using MEAs. Study III focused on analysing whether MEA was suitable for cortical data extraction and assessment compared to a µECoG array. Researchers in neurophysiological research have used various subdural electrodes, including custom-built designs, to capture cortical information (Brodnick et al., 2019). These custom designs are later batch manufactured to meet the growing popularity of brain-computer interface and brain research (Wodlinger et al., 2011). However, MEAs remain a popular choice in animal research (Kim et al., 2018).

MEAs are extensively used because these electrodes are in proximity of neurons in consideration (Kajikawa & Schroeder, 2011). In animal research models MEAs are widely used to pinpoint changes in the cortex in response to a disease model such as the SNI model of pain (Toettrup et al., 2020), (Mahmud & Vassanelli, 2016). The challenge with MEAs remains in the surgical procedure of exposing the brain and inserting the electrodes into the cortex without damaging any blood vessels. Additionally, the oscillatory movement of the pig brain means that the MEA is unable to pick up signals without motion artefact.

ECoG arrays offer a solution to the oscillatory movement of the pig brain. Since the electrode array must be placed on the brain's surface, it moves along the brain oscillations and is unaffected by the motion artefact. Furthermore, µECoG arrays do not damage the blood vessels because they are not inserted into the cortex to record cortical signals. µECoG arrays can also record cortical activity above the dura surface (Toda et al., 2011). This ease of electrode placement and prevention of blood vessel damage makes µECoG arrays ideal for chronic experiments.

Study III illustrated the range of frequencies accessible using µECoG, demonstrating that the effect of LTP-like pain shown in Study II can be replicated using µECoGs instead of MEAs. The study demonstrated the time series representation of the brain responses using ERPs and compared the power across different frequency domains between the µECoG and the MEA. This comparison also highlighted if helpful information could be extracted from the brain beyond the typical 0.3 Hz to 300 Hz range (Im & Seo, 2016). The surgical ease and placement of the µECoG array on the brain surface could help ensure fewer complications in a chronic setup while maintaining a similar cortical information assessment setup.

6.3 METHODOLOGICAL CONSIDERATIONS

The motivation behind using pigs as translational animal models stemmed from the understanding that even with the diverse use of animals in biomedical research, animal experimentation results do not always translate into clinical trials. More recently, pigs have become popular since they offer a closer neurophysiological system to humans,

see, e.g. (Meijs et al., 2021), (Caste et al., 2016), (Burrell et al., 2019). As Schmidt et al. demonstrated in their work, pigs' brains' anatomical features such as sulci and gyri are also very close to human brains (Verena Schmidt, 2013). Therefore, it was critical to understand if the pig brain physiology is also like humans in terms of neuroplasticity.

Several other considerations were made while conducting the experiments to ensure the reproducibility of the data. One of the challenges was to ensure that the anaesthetic protocol minimally affected cortical processing. This problem was addressed by dropping the infusion rate of sevoflurane to 0% MAC 30 min before starting the recording protocol. A lot of bone bleeding was experienced by the pig during the surgery, so bone wax was used to prevent any bone bleeds during the recording. Care was taken while removing the dura, so no blood vessels on the brain's surface got ruptured. It was also a challenge to locate the S1 sometimes when the S1 cortex was found running parallel to the brain's midline. It was later decided to exclude these experiments from the analysis. A significant difference between MEAs and µECoGs was that the brain oscillations that affected the MEAs did not have the motion artefact in µECoGs since they were placed on the brain's surface while recording. For all the studies, the average of all the individual channels was used to denote the effect on the S1. Although this averaging removed spatial information from individual channels, it provided insight into the overall cortical processing of the cortical region (S1).

Furthermore, it was assumed that spinal LTP was induced using HFS since changes in the spinal cord following HFS were not recorded. Even so, it was ensured that HFS was induced using the same parameters used in rodents to induce LTP while recording from the spine to see the development of spinal LTP (J. Zhang et al., 2016). This technique has also been used in the human LTP-like pain model (E N Van Den Broeke et al., 2021).

Non-nociceptive stimulation was induced above the motor threshold but below the C-fiber activation threshold while probing the brain response through peripheral stimulation on the ulnar nerve. Thus, the animal did not experience nociceptive stimuli, and therefore, the resulting changes in cortical response were due to hyperalgesia.

The stereotaxic frame was an essential part of the experimental setup, which proved viable for recording cortical information using MEAs and µECoGs. The interface could securely position the pig head during surgery, and the 3D printed adaptor proved especially useful when selecting the optimal electrode placement in/on the cortex.

6.4 FUTURE PERSPECTIVES

It can be deduced from this thesis that the µECoG array offers similar cortical information to the MEA while reducing the risk of infection in pigs, making it a suitable choice for chronic studies using large animals such as pigs. Moreover, due to the extended features of the stereotaxic frame like multi-micromanipulator compatibility, multi-electrode recording can be explored in future experiments with large animals in which multiple cortical regions can be recorded simultaneously. These areas could be the ACC, S1 and the PFC, which are known for their role in pain processing (Luo & Wang, 2009), (Tøttrup et al., 2021), (Cardoso-Cruz et al., 2013).

CHAPTER 7. CONCLUSIONS

This Ph.D. thesis investigated the feasibility of using the pig as a translational model of LTP-like pain and used the porcine brain to compare MEA and µECoG arrays as tools for accessing cortical information. A neuroplastic effect was detected in S1 following HFS induced spinal LTP. This effect was highlighted in studies I and II, where the local field potentials' amplitude and spike activity increased significantly after intervention. However, towards the end of the experiment, the effect decreased. This phenomenon needs to be explored in future studies where the chronic impact of LTP can be targeted.

The comparison between µECoG and MEAs in Study III demonstrated the usefulness of µECoGs in obtaining cortical information like the MEA. The study showed the ability of µECoGs to capture local field potentials like the MEA. Additionally, since µECoG arrays are relatively less invasive, they may be ideal for many large animal studies. The study emphasised the advantage of using MEA since they offer a higher signal to noise ratio, reflected by the power in all frequency bands, compared to the µECoG.

After this project, some unanswered questions include the role of other pain processing areas such as ACC, PFC, and the insula in the LTP-like pain model. Furthermore, peripheral HFS induced LTP-like neuroplasticity in the pig may be validated through spinal recordings that can be made in future experiments.

The work in this PhD has started to answer questions on which electrodes to use while recording from the brain in an animal model of research. It has also shown the importance of pigs as a translational model of research in neuroplasticity using the LTP-like pain model as one example of how pigs can be used to understand cortical processing mechanisms.

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