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The effect of peripheral high-frequency electrical stimulation on the primary somatosensory cortex in pigs

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ABSTRACT

This study implements the use of Danish Landrace pigs as subjects for the long-term potentiation (LTP)-like pain model. This is accomplished by analyzing changes in the primary somatosensory cortex (S1) in response to electrical stimulation on the ulnar nerve after applying high-frequency electrical stimulation (HFS) on the ulnar nerve. In this study, eight Danish Landrace pigs were electrically stimulated, through the ulnar nerve, to record the cortically evoked response in S1 by a 16-channel microelectrode array (MEA). Six of these pigs were subjected to HFS (four consecutive, 15 mA, 100 Hz, 1000 μs pulse duration) 45 min after the start of the experiment. Two pigs were used as control subjects to compare the cortical response to peripheral electrical stimulation without applying HFS. Low-frequency components of the intracortical signals (0.3–300 Hz) were analyzed using event-related potential (ERP) analysis, where the minimum peak during the first 30–50 ms (N1 component) in each channel was detected. The change in N1 was compared over time across the intervention and control groups. Spectral analysis was used to demonstrate the effect of the intervention on the evoked cortical oscillations computed between 75 ms and 200 ms after stimulus. ERP analysis showed an immediate increase in N1 amplitude that became statistically significant 45 mins after HFS ($p < 0.01$) for the intervention group. The normalized change in power in frequency oscillations showed a similar trend. The results show that the LTP-like pain model can be effectively implemented in pigs using HFS since the cortical responses are comparable to those described in humans.

1. Introduction

Long-term potentiation (LTP) is a well-documented phenomenon widely investigated in pain research, learning, and memory (Sandkühler, 2007). As a pain model, LTP can be induced by high-frequency and high-intensity electrical stimulation over a short period of time, leading to increased synaptic strength (Zhang et al., 2016). To date, LTP has been extensively studied using human as well as rodent subjects (Clapp et al., 2012).

Most studies with human subjects on LTP-like pain are based on pain ratings. Characteristic changes, due to LTP-like pain, are the facilitation of C-fiber and Aδ-fiber pathways and include increased perceived burning and stinging sensation, respectively, in response to peripheral electrical stimuli (Hansen et al., 2007). Klein et al. reported the development of dynamic mechanical allodynia in eight out of 13 subjects after high-frequency stimulation (HFS). All participants developed punctate hyperalgesia immediately after HFS, reaching the maximum level 40–60 min after HFS. The reported increase in pain ratings in response to pinpricks was up to 300% (Klein et al., 2006). This descriptive analysis, in the form of pain ratings, is often paired with electroencephalography (EEG) to identify objective biomarkers based on cortical activity (van den Broeke et al., 2012, 2017; Sun et al., 2021; Iannetti et al., 2013). Studies pertaining to the effect of HFS on the event-related potentials (ERPs) reported an increase in amplitude 30 min after intervention (van den Broeke et al., 2012, 2013; van den Broeke and Mouraux, 2014).

In studies where LTP-like plasticity was induced through high-frequency auditory stimuli, the auditory evoked potentials demonstrated an increase in N1 peak due to the intervention (Kirk et al., 2010). A similar change was reported in the N1-b component in response to photic stimuli applied after photic tetanus (Kirk et al., 2010). The identified change in N1 is known to last at least an hour after the stimulation. In such studies, LTP-like plasticity was induced either in the

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lateral geniculate body of the midbrain or in the visual cortex, giving rise to increased ERPs similar to those evoked by electrical stimulation applied to the skin (van den Broeke et al., 2012; Kirk et al., 2010).

In rodents, cortical changes in response to spinal LTP show an increase in the amplitude of evoked responses in the thalamus of rats (González-Hernández et al., 2013). Thalamus plays a vital role in relaying sensory impulses to the cerebral cortex. This information is processed in the primary somatosensory cortex (S1), which plays an essential part in pain processing mechanisms (Bushnell et al., 1999). Hjornevik et al. demonstrated that spinal LTP induced in rats using HFS resulted in acute metabolic response in the S1 (Hjornevik et al., 2008). However, to our knowledge, no animal research has been carried out so far that studies the electrophysiological effect of LTP on S1.

Despite the invasive nature of rodent-based experiments that offer a close-up focus on neuronal activity in response to a pain model, there is a need for animal models that better represent the cortical response to nociception since the rodent brain is in many ways different from the human brain (Sjöstedt et al.; Castel et al., 2016). Our suggested alternative is using pigs. Pigs have sulci and gyri, similar to the human brain (Sauleau et al., 2009); moreover, the porcine brain is divided into subregions, like the human brain, which performs specific tasks with coherence (Schmidt, 2015). Therefore, because of the anatomical and physiological similarities between the pig brain and the human brain (Schmidt, 2015), pigs may serve as a better translational model for investigating pain mechanisms.

Apart from evoked potentials, where Broeke et. al. reported an increase in N1-P2 peak to peak amplitude in the S1 after HFS (Van Den Broeke et al., 2010), frequency band oscillations have also been used to demonstrate the cortical effect of a pain model in humans. For example, Michail et al. investigated the neural oscillations that allow human subjects to process pain and touch using EEG (Michail et al., 2016). The results showed an increase in alpha, theta, and gamma-band activity due to pain perception. Ploner et al. reported changes in frequency bands 150–400 ms after stimulation (Ploner et al., 2017). The results showed increased oscillations in gamma, delta, theta, and alpha frequency bands and highlighted a complex spectral-temporal-spatial pattern of frequency oscillations that occurs in response to pain (Ploner et al., 2017).

In this study, we aimed to establish the spinal LTP-like pain model in pigs using HFS. We studied the effect of HFS by analyzing ERPs and frequency band powers, in S1, up to three hours after the intervention.

2. Methodology

This study was conducted in accordance with the Danish Veterinary and Food Administration under the Ministry of Environment and Food of Denmark (protocol number: 2017–15–0201–01317).

2.1. Experimental procedures

Eight healthy female Danish Landrace pigs weighing 33.2 ± 3.4 kg (mean ± standard deviation) were included in the study. Six were subjected to HFS, and two pigs were allocated to the control group. In the case of the control group, the pig was surgically operated on, and peripheral electrical stimulation was applied, but HFS was not applied. The subjects were randomly selected before the day of the surgery by an animal technician blinded to the group allocation of the animal.

Before the surgery, the pigs were acclimatized for two weeks at the animal laboratory. Their diet and health were monitored. The pigs were given a rich environment with treats and toys daily, and they were housed together to prevent social deprivation. The room was maintained at ~24 °C with a 13:11 h light-dark cycle. The pigs fasted overnight before the surgery to ensure that the pigs did not aspirate stomach contents while under anaesthesia; water was not restricted during this time.

2.2. Surgical procedures

The subject was anesthetized in the home-pen with an intramuscular injection of the zoletil mix for pigs (which included zoletil (5 ml solution containing: tiletamine 25 mg/ml and zolazepam 25 mg/ml), 6.25 ml xylazine (20 mg/ml), 1.25 ml ketamine (100 mg/ml), and 2.5 ml butorphanol (10 mg/ml)). The subject was initially placed in a supine position during the surgery and intubated with a 1:1 oxygen and air mixture. A constant infusion of isotonic saline was administered via the jugular vein. Anaesthesia was maintained with sevoflurane (1.5–2.5% MAC), propofol (2 mg/h/kg) and fentanyl (10 ug/h/kg). Physiological parameters were monitored every 15 min during the experiment. If the subject showed abnormal signs of stress e.g., increase in end-tidal CO_2 beyond normal values, abrupt and change in heart rate and blood pressure, it was attempted to stabilize the pig by adjusting the anaesthetic parameters. Alternatively, a shot of atropine (0.5 ml) or pentobarbital (100 mg) was given as a rescue intervention to stabilize the pig. If these methods were ineffective, the experiment was terminated. The temperature was measured through a thermocouple probe placed in a catheter inserted into the bladder.

To ensure depth of anaesthesia, we monitored the following during surgery: temperature, end-tidal CO_2, oxygen saturation, heart rate, respiration rate, blood pressure, and jaw tone (Swindle). The animals were mechanically ventilated at 15 cycles per minute. The temperature was maintained using a temperature-controlled air blanket (Mistral-Air Plus, MA1100-EU) placed under the pig.

During the surgery, the pig's femoral artery was cannulated for a precise measure of arterial blood pressure. At the same time, the ulnar nerve was accessed via an incision in the forelimb. Two branches of the nerve were carefully isolated, and two tripolar stimulation cuff electrodes (10 mm long, 1.8 mm inner diameter, platinum-iridium ring electrodes with a 3 mm centre-to-centre distance) were placed around each branch. The cuffs were fixated with ligatures. Subsequently, the pig was flipped into a prone position.

For the brain surgery, the animal was fixed into a custom-built stereotaxic frame via screws drilled in the zygomatic arch. Then, during craniotomy, a three by five cm rectangle opening was made centred around bregma using a rotary tool (Dremel 8220, Dremel, US). Two holes were drilled next to the rectangle to place the ground and reference points (stainless steel screws). Then, the dura was removed using surgical scissors and micro forceps, making sure no blood vessels were ruptured over the exposed cortex. Fig. 1 shows the view of the cortex after craniotomy.

Once the surgery was complete, sevoflurane was slowly decreased to 0% before placing the electrodes into the cortex. At the same time, the flow rate of propofol and fentanyl was increased by 100% while carefully monitoring the vital signs. Motivation for this was 2-fold. Firstly, sevoflurane is known to have a depressive effect on the central nervous system in general. Secondly, both sevoflurane and propofol have a secondary effect on NMDA receptors (Wada et al., 2018; L et al., 2014) which are essential to induce LTP at the spinal level (Sandkühler and Gruber-Schoffnegger, 2012).

At the end of the experiment, the pig was euthanized by an overdose of pentobarbital administered intravenously.

2.3. Electrophysiological Recordings

A 16-channel microelectrode array (MEA) was used to record signals from S1 (Part# MEA-PI-A3-00-16-0.6-2.0-3-1.0-1.0-1-1SS-1, Microprobes Inc., Gaithersburg, MD, USA). The square grid array had 2 mm long shafts, 1 mm distance between adjacent electrodes. S1 was located using the brain's anatomical structure compared with Schmidt et al. (Schmidt, 2015) and Sauleau et al. (2009). The S1 was expected to run diagonally to the midline, anterior to the visual cortex, which was seen as the cortex's bulged part posterior to the bregma. The electrode array was inserted 2 mm into the cortex and suspended using a

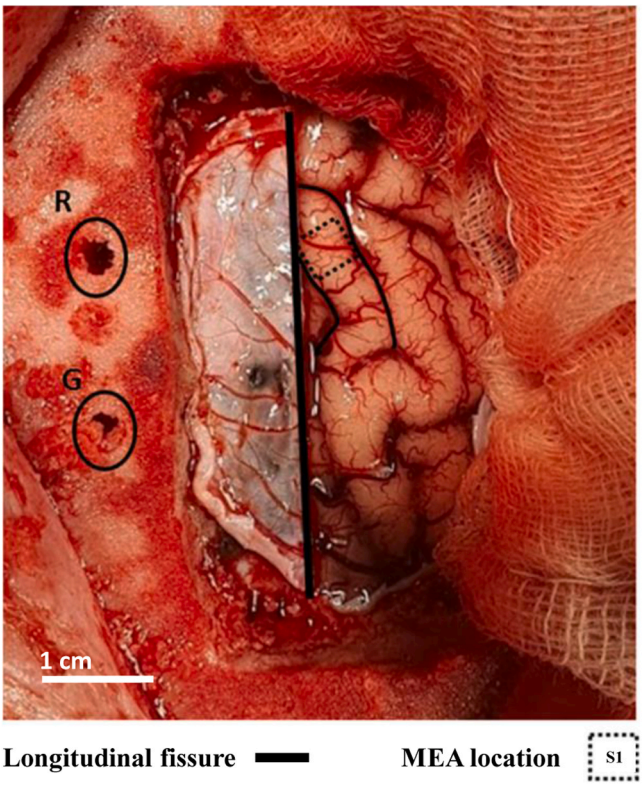

Fig. 1. View of the cortex after exposure. The locations R and G refer to holes drilled for the reference and ground screws respectively. The black line indicates the longitudinal fissure of the brain. The dotted square denotes the primary somatosensory cortex (S1) where the microelectrode array (MEA) was inserted.

micromanipulator (Kopf Instruments, USA) mounted on the stereotaxic frame walls. Fig. 1 illustrates the top view of the brain after craniotomy, highlighting the location of S1 where the MEA was inserted. To ensure good signal quality and a low signal-to-noise ratio, the cortex was regularly flushed with saline to prevent any blood clots on S1.

The MEA was connected to a ZIF-Clip through an Omnetics adapter (Omnetics Connector Corporation, Minneapolis, USA). The ZIF-Clip was connected to a preamplifier (SI-8; input voltage range: ±500 mV) that transferred the data to the processor (RZ2) through optic fibres. Unfiltered data was sampled at 24,414.06 Hz and stored in a data streamer (RS4). Synapse suite, a software for neural data recording and online processing, was used to filter and visualize the recorded data in real-time during the experiment. All the recording equipment and software was from Tucker-Davis Technologies, Alachua, FL, USA.

2.4. Experimental protocol

Recordings from the MEA started 30 min after the array was suspended in the cortex. A baseline recording of 30 s was obtained before and after each set of stimulations. The experimental protocol is summarized in Fig. 2.

2.4.1. Phase I (Pre-LTP)

Before the intervention, 50 stimulations at 1 mA and 500 μs pulse-width were applied to the ulnar nerve. The parameters selected for the peripheral stimulation are non-nociceptive, i.e., the stimulation was supramaximal for motor units but presumably subthreshold for C-fibres. A-beta fibres assumedly mediated the cortical response observed in S1, based on the distance from the forelimb and the selected non-noxious parameters for peripheral stimulation.

To avoid habituation, an inter-pulse interval was kept on average 2000 ms with a pseudo-random interval of 250 ms. This set of stimulations was repeated three times with 12 min breaks in-between.

2.4.2. Phase II (LTP)

In the intervention group, both branches of the ulnar nerve were simultaneously electrically stimulated four times, with an intensity of 15 mA and 1000 μs pulse duration at 100 Hz. A 10 s interval was made between each train of stimulations. In the control group, no stimulation was induced during this phase.

2.4.3. Phase III (Post-LTP)

After the intervention, the protocol from Phase I was repeated nine times before concluding the experiment.

Twelve recording sets were made based on these three phases; three sets were recorded during Pre-LTP while stimulating the ulnar nerve. The remaining nine were recorded Post-LTP.

2.5. Signal Processing

The recorded data were processed using MATLAB 2020a (MathWorks, Inc., Natick, MA, USA). The processed data was exported into R (Team R Development Core, 2018) for graphical illustration and statistical analysis.

The recorded signal was first filtered from 0.3 H to 300 Hz (8th order Butterworth bandpass filter). Also, a 50 Hz notch filter was used to remove power line noise from the signal. Faulty channels were removed based on visual inspection of the data. Baseline correction was done using 500 ms before stimulation in each epoch.

The filtered signal was windowed into epochs of 1500 ms (500 ms pre-stimulus; 1000 ms post-stimulus). The epochs were averaged over every 50 trials that represented each recording set.

For illustration, the data was divided into four sections: *T0 (Pre-LTP)* – representing the first 45 min of the experiment; *T1 (Early-LTP)* – representing the first 45 min after intervention or control; *T2 (Mid-LTP)* – representing the next 45 min of the experiment after T0; and *T3 (Late-LTP)* – representing the last 45 min of the protocol.

The N1 component represents the first depolarization peak following stimulation (30–50 ms after stimulation) in the S1. The N1 component seen at 50 ms likely correlates to the N1 or N2 peak observed in pain

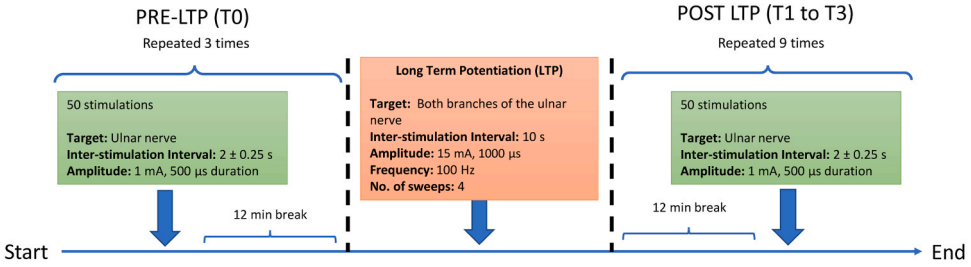

Fig. 2. Summary of the experimental protocol. Pre-LTP (T0) phase had 3 sets of 50 stimulations on the ulnar nerve. After this LTP was induced in the intervention group. This was followed by phases T1 to T3, each representing 45 min of recording time after LTP.

studies in humans within the first 100 ms, which in humans represents the greatest amplitude after peripheral electrical stimulus (Kirk et al., 2010; Liang et al., 2016). This component has been used in this study to demonstrate the effect of the intervention on the S1. Fig. 3 shows an example of how N1 is measured from an ERP averaged across 50 trials on a single channel.

The data were normalized by dividing the recorded N1 with the average N1 during the T0 phase to show the change in N1 relative to the average T0-N1. The obtained values were compared across the 12 recording sets, signifying the different phases of the experiment (i.e., T0-T3).

The data from each recording set was also analyzed for changes in specific frequency bands, namely, alpha (8–13 Hz), beta (18–25 Hz), delta (0.5–3 Hz), theta (3.5–7 Hz), low-gamma (30–70 Hz) and high-gamma (70–150 Hz). The signal from each channel was windowed into epochs to capture information from 75 ms to 200 ms after stimulus. The average power in each frequency band during this time window was calculated by applying a Hamming window on the extracted epochs and applying a periodogram of the same length on the signal. This was accomplished by using the *bandpower* function in MATLAB.

The power was then normalized with respect to the relative change in the power of each frequency band before intervention/control (T0 phase).

2.6. Statistical analysis

For the ERP analysis, a Wilcoxon signed-rank test was performed on the three phases (T1-T3) compared to the reference (T0). Bonferroni correction was applied for multiple comparisons. The significance level adopted before the Bonferroni correction was 0.05.

The statistical analysis for the frequency band power was done by applying a Friedman test on the intervention group and control group respectively of each frequency band. Upon a significant difference between the time-phases, a Mann-Whitney *U* test was performed to identify the time phases where the difference between the control and intervention groups was significant. The normality of the data was assessed through residual analysis via QQ-plots and histogram plots.

3. Results

3.1. Event-related potentials

Results of the ERP analysis, illustrated in Fig. 4, showed an increase in N1 in the intervention phase. Statistical analysis showed a significant increase (relative changes compared to T0 Phase) in N1 following the intervention in the T2 ($p < 0.01$) and T3 phases ($p < 0.01$) compared to T0. Fig. 4 demonstrates that there is a decrease in N1 in T3 (Late-LTP)

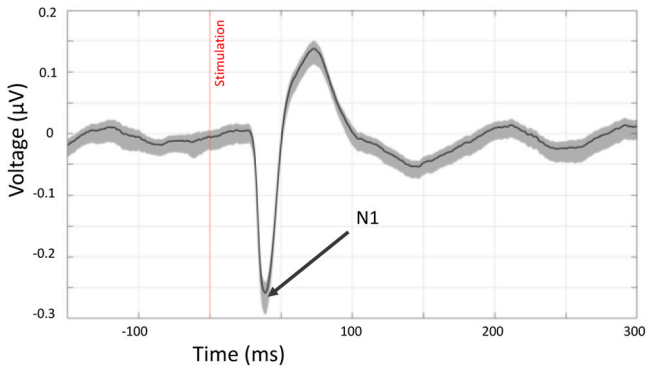

Fig. 3. Example of an Event Related Potential from a single channel of the MEA averaged across 50 stimulations in one recording set (one animal). N1 represents the first negative peak after stimulation respectively. The shaded region represents the standard error due to 50 stimulations.

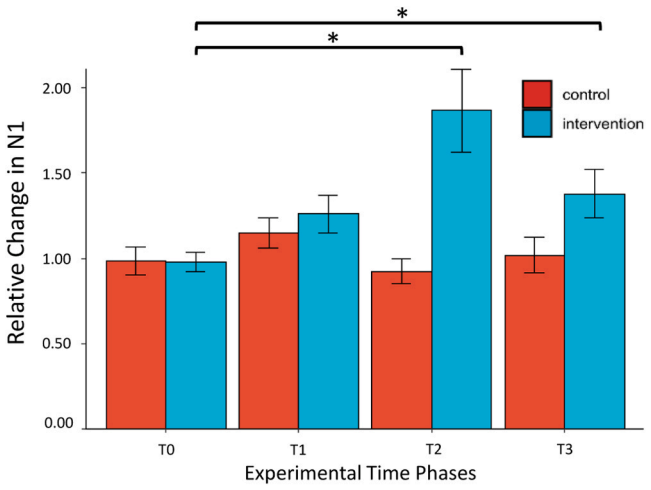

Fig. 4. Relative change in N1 in the control and intervention group. The error bars represent a 95% confidence interval. Experimental time phases (T0–T3) represent the time phases Pre LTP, Early LTP, Mid LTP, and Late LTP.

even though it remains significantly greater than T0. No significant changes were identified in the control group. Table 1 summarizes the mean values and confidence intervals for the N1.

3.2. Spectral activity

Results from the frequency band oscillations analysis are shown in Fig. 5. During the T1 phase, the intervention group showed a significant decrease in gamma oscillations compared to the control group ($p < 0.01$). This was followed by an increase in all frequency bands in T2 ($p < 0.01$). This increase in power was more prominent in alpha, theta, delta, and high-gamma oscillations where the band-power was observed to be more than twice the baseline (T0), as well as the corresponding T2 mean value in the control group. By the end of the experiment (T3), all frequency band powers drop to the same level as T1. However, low-gamma power was significantly lower than the corresponding control group power at T3 ($p < 0.01$). Table 2 summarizes the mean values and 95% confidence interval for each frequency band in the control and intervention groups.

4. Discussion

This study investigated the cortical changes in the pig S1in response to electrical stimulation on the ulnar nerve after HFS. The change in N1 due to HFS is similar to the effect of HFS observed in humans, where the N1-P1 peak to peak amplitude significantly increased due to spinal LTP (Van Den Broeke et al., 2010).

In animals, peripheral electrical HFS induced on the sciatic nerve resulted in changes in the thalamus (González-Hernández et al., 2013). Hjornevik et al. demonstrated the effect of spinal LTP induced through HFS on the sciatic nerve in rats (Hjornevik et al., 2008). The authors observed changes in the spinal cord 20 min after HFS as well as the contralateral S1 in the acute phase. In human experiments, ERP analysis

Table 1
Summary of mean values [95% confidence intervals] of N1 in the control and intervention group.

	Condition	
Phase	Control group	Intervention group
T0	0.985 [0.906,1.06]	0.979 [0.925,1.03]
T1	1.15 [1.06,1.23]	1.26 [1.15,1.37]
T2	0.923 [0.851,0.995]	1.87* [1.63,2.11]
T3	1.02 [0.915,1.12]	1.38* [1.23,1.52]

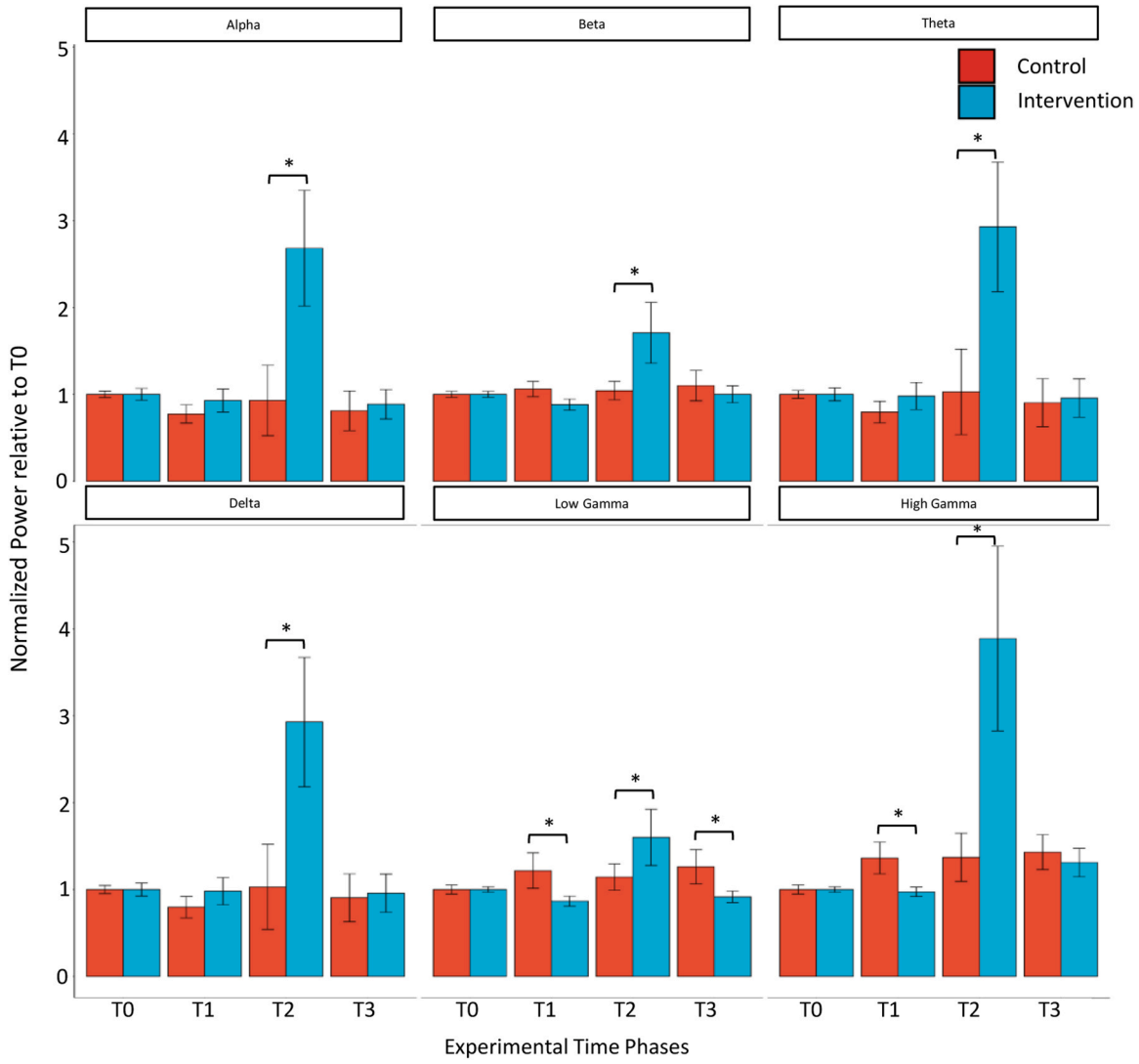

Fig. 5. Relative frequency band power in each time phase compared between the control and intervention group. Error bars represent the 95% confidence interval. Experimental time phases (T0-T3) represent the four phases of the experiment in the control and intervention groups.

Table 2
Summary of the mean [95% confidence intervals] of each frequency band power in all the time phases (T1-T3).

	Condition					
	Control group			Intervention group		
Frequency band	T1	T2	T3	T1	T2	T3
α	0.775 [0.670, 0.880]	0.931 [0.527, 1.34]	0.812 [0.584, 1.04]	0.928 [0.796, 1.06]	2.68* [2.02, 3.35]	0.886 [0.717,1.06]
β	1.06 [0.974, 1.15]	1.04 [0.937, 1.15]	1.10 [0.925, 1.28]	0.883 [0.818, 0.947]	1.71* [1.36, 2.06]	1.00 [0.904, 1.10]
δ	0.797 [0.673, 0.920]	1.03 [0.538, 1.52]	0.904 [0.628, 1.18]	0.981 [0.823, 1.14]	2.93* [2.18, 3.67]	0.958 [0.738, 1.18]
θ	0.797 [0.673, 0.920]	1.03 [0.538, 1.52]	0.904 [0.628, 1.18]	0.981 [0.823, 1.14]	2.93* [2.18, 3.67]	0.958 [0.738, 1.18]
Low γ	1.22 [1.01, 1.42]	1.14 [0.993, 1.29]	1.26 [1.06, 1.46]	0.866* [0.808, 0.923]	1.60* [1.28, 1.92]	0.915* [0.848, 0.982]
High γ	1.36 [1.18, 1.55]	1.37 [1.09, 1.65]	1.43 [1.23, 1.63]	0.973* [0.920, 1.03]	3.89* [2.83, 4.95]]	1.31 [1.15 1.48]

combined with verbal feedback from the subjects indicates that HFS may be used as a technique to induce LTP-like sensitization (van den Broeke et al., 2012, 2013, 2010).

In our study, the increased cortical excitability induced through HFS was likely due to spinal LTP since the parameters selected for peripheral ulnar stimulation were the same as those used in rodent experiments inducing spinal LTP after sciatic nerve stimulation (Zhang et al., 2016). However, it cannot be excluded that a form of LTP or other neuroplastic changes have occurred elsewhere in the central nervous system.

It is worth noting that the change in N1 amplitude takes some time to

become significantly greater than the baseline (T0). This is consistent with human studies, where a significant change in pain ratings after LTP was reported, peaking 20–45 min after intervention (Klein et al., 2006; Hjornevik et al., 2008). Similarly, human EEG studies showed significant differences post- compared to pre-intervention 20 min after LTP (Klein et al., 2006).

In our work, there is a visible trend in the effect of LTP from T2 to T3 where the cortical amplitude decreases significantly ($p < 0.01$). Although it is still significantly higher than the T0 phase, which implies that the effect of LTP remained persistent in the T3 phase, the magnitude of the effect decreased, which is in line with decrease in pain ratings after 4 h of LTP (Klein et al., 2006).

Along with changes in the N1 amplitude, we have also observed changes in the frequency band power in the intervention group. This increase in frequency band power in response to peripheral electrical stimulation denotes the same trend seen in human experiments. For example, Michail et al. (2016) demonstrated how gamma and theta oscillations show a significantly higher power of 150–300 ms after a painful stimulation. The authors also reported, however, a significant decrease in alpha band power, which was associated with attention to the induced pain. This trend in alpha power was not seen in our experiments, most likely because the animal was anaesthetized. Nevertheless, there is a similarity between painful cortical responses in humans to symptoms shown in pig experiments that point towards the possibility of LTP-like plasticity induced by HFS.

The control group reflects the N1 amplitude and frequency oscillations power changes when HFS was not applied to the ulnar nerve. The observed values remained relatively consistent throughout the experiment.

It is worth noting that, in the control group, the power in the high gamma band slowly increased over the duration of the experiment and became significantly higher than the T1 phase by T3 ($p < 0.01$). The intervention group, on the other hand, showed an increase in the N1 amplitude as well as the theta, gamma, and alpha band power 45 mins after the intervention. These results could signify that the effect of HFS on the brain is not immediate, similar to human-based experiments (van den Broeke et al., 2012). Graphical representation of the time phases in Figs. 4 and 5 showed an increase in cortical change in the T1 phase (immediately after intervention); however, the trend was not significant ($p > 0.01$). This effect is in line with the work by Van den Broeke et al., where the increase in ERP amplitude occurred 30 min after HFS was induced (van den Broeke et al., 2012).

In human experiments, hyperalgesia in response to electrical stimuli and mechanical allodynia, induced due to HFS, have been observed (Klein et al., 2004). Human studies have the advantage of feedback during pain induction, but they are not invasive; therefore, it is difficult to get a clearer image of cortical responses. By successfully implementing a model of LTP-like pain in pigs, we offer a promising translational model of pain. Furthermore, pigs can offer a chance to study LTP on a more comprehensive level, focusing on the high-frequency components that can be recorded via invasive means. So far, the low-frequency component-based analysis is similar to what has been reported in human studies.

Klein et al. indicated a 30% increase in pain ratings in response to electrical test stimuli after LTP-like plasticity was induced using HFS (Klein et al., 2004). Van den Broeke et al. studied the effect of HFS on the human skin based on reported pain intensity in response to electrical stimulation and measured the corresponding ERPs (van den Broeke et al., 2012). The same group reported an increase in the N1 peak due to HFS application (Van Den Broeke et al., 2010). In our results, we saw a similar trend in N1, but the effect diminished slightly towards the end of the experiment (T3 phase). Nevertheless, the increase in N1 amplitude remained greater than baseline during this phase. This result signifies that the increase in N1 amplitude, observed in T2 and T3 phases, could be the result of hyperalgesia induced due to HFS.

There is a dissociation between increased N1 relative to T0 and the oscillatory activity at T3 phase, most likely because neuronal oscillations and ERP represent different characteristics of the brain state due to their different biophysical origins (Edwards et al., 2009).

According to the International Association for the Study of Pain, pain is a subjective experience; therefore, it cannot be studied without feedback (Treede, 2018). However, animal models of pain are still widely used to investigate healthy and pathological mechanisms of pain. identify biomarkers for nociception. Translational studies also require investigating those mechanisms in human studies. There is now enough evidence supporting that the S1 plays a vital role in pain processing mechanisms along with four other areas, namely: the secondary somatosensory cortex (S2), the prefrontal cortex (PFC), anterior cingulate cortex (ACC), and insula (Zhuo, 2011).

It is essential to highlight that S1 is affected, not only by nociceptive input but also by cognitive factors such as attention (Bushnell et al., 1999). Therefore, we have used a control group to ensure that the changes in the S1 represent the cortical response to the peripheral stimulation after applying HFS rather than any other possibly nociceptive inputs at the time of the experiment.

4.1. Methodological considerations

One of the challenges of the study was to place the MEA in the same position for all animals. Due to the inter-subject variability of the brain anatomy and the complexity of the surgery, S1 was, on occasion, found running parallel to the midline of the brain. The surgery occasionally resulted in bone bleeding and accidental rupture of blood vessels while accessing the S1.

Owing to the complex surgical procedure performed in a limited time, the changes in the spinal cord in response to HFS were not recorded. We believe adding spinal recording to the experimental setup can substantiate the effect of LTP, observed in S1.

A low dose of ketamine was given to anesthetize the subject approximately 5 h before HFS was applied. Since the ketamine half-life is 45 min, it is not expected to influence the induction of LTP.

5. Conclusion

In this study, we aimed to establish the LTP-like pain model in pigs. We accomplished this by applying HFS on the ulnar nerve that resulted in increased N1 amplitude after intervention as well increased alpha, beta, delta, theta, and gamma power during the T2 phase. In conclusion, we have reported on a first step to reverse translate human models of LTP-like pain to pigs, showing that pigs display similar cortical patterns as humans. This is a first step towards using pigs as translational models for investigating pain processing mechanisms.

Despite the significant role of S2, ACC, PFC, and the insula in pain processing, only S1 has been explored in this study. Future studies may also consider the latency and topographical changes that may occur due to HFS.

CRediT authorship contribution statement

Taha Al Muhammadee Janjua: Conceptualization, Methodology, Visualization, Investigation, Software, Validation, Formal Analysis, Data curation, Writing – original draft. **Thomas Gomes Nørgaard dos Santos Nielsen**: Conceptualization, Supervision, Resources, Software, Investigation, Methodology, Project administration, Writing – review & editing. **Felipe Rettore Andreis:** Software, Visualization, Investigation, Formal analysis, Writing – review & editing. **Suzan Meijs**: Conceptualization, Visualization, Investigation, Validation, Writing – review & Editing. **Winnie Jensen:** Conceptualization, Supervision, Project administration, Investigation, Writing – review & editing, Resources.

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References

van den Broeke, E.N., de Vries, B., Lambert, J., Torta, D.M., Mouraux, A., 2017. Phase-locked and non-phase-locked EEG responses to pinprick stimulation before and after experimentally-induced secondary hyperalgesia. Clin. Neurophysiol. 128, 1445–1456.

Van Den Broeke, E.N., Koeslag, L., Arendsen, L.J., Nienhuijs, S.W., Rosman, C., van Rijn, C.M., Wilder-Smith, O.H.G., van Goor, H., 2013. Altered cortical responsiveness to pain stimuli after high frequency electrical stimulation of the skin in patients with persistent pain after inguinal hernia repair. PLoS One 8 (12), e82701. https://doi.org/10.1371/journal.pone.0082701.

van den Broeke, E.N., Mouraux, A., 2014. High-frequency electrical stimulation of the human skin induces heterotopical mechanical hyperalgesia, heat hyperalgesia, and enhanced responses to nonnociceptive vibrotactile input. J. Neurophysiol. 111, 1564–1573.

van den Broeke, E.N., van Heck, C.H., Ceelen, L.A.J.M., van Rijn, C.M., van Goor, H., Wilder-Smith, O.H.G., 2012. The effect of high-frequency conditioning stimulation of human skin on reported pain intensity and event-related potentials. J. Neurophysiol. 108, 2276–2281.

Van Den Broeke, E.N., Van Rijn, C.M., Manresa, J.A.B., Andersen, O.K., Arendt-Nielsen, L., Wilder-Smith, O.H.G., 2010. Neurophysiological correlates of nociceptive heterosynaptic long-term potentiation in humans. J Neurophysiol. 103, 2107–2113.

Bushnell, M.C., Duncan, G.H., Hofbauer, R.K., Ha, B., Chen, J.I., Carrier, B., 1999. Pain perception: Is there a role for primary somatosensory cortex? Proc. Natl. Acad. Sci. USA 96, 7705–7709.

Castel, D., Sabbag, I., Brenner, O., Meilin, S., 2016. Peripheral neuritis trauma in pigs: a neuropathic pain model. J. Pain 17 (1), 36–49. https://doi.org/10.1016/j.jpain.2015.09.011.

Clapp, W.C., Hamm, J.P., Kirk, I.J., Teyler, T.J., 2012. Translating LTP from animals to humans: a novel method for non-invasive assessment of cortical plasticity. Biol. Psychiatry 71, 496–502.

Edwards, E., Soltani, M., Kim, W., Dalal, S.S., Nagarajan, S.S., Berger, M.S., Knight, R.T., 2009. Comparison of time-frequency responses and the event-related potential to auditory speech stimuli in human cortex. J. Neurophysiol. 102, 377–386.

González-Hernández, A., Martínez-Lorenzana, G., Rojas-Piloni, G., Rodríguez-Jiménez, J., Hernández-Linares, Y., Villanueva, L., Condés-Lara, M., 2013. Spinal LTP induced by sciatic nerve electrical stimulation enhances posterior triangular thalamic nociceptive responses. Neuroscience 234, 125–134.

Hansen, N., Klein, T., Magerl, W., Treede, R.D., 2007. Psychophysical evidence for long-term potentiation of C-fiber and Aδ-fiber pathways in humans by analysis of pain descriptors. J. Neurophysiol. 97, 2559–2563.

Hjornevik, T., Jacobsen, L.M., Qu, H., Bjaalie, J.G., Gjerstad, J., Willoch, F., 2008. Metabolic plasticity in the supraspinal pain modulating circuitry after noxious stimulus-induced spinal cord LTP. Pain 140, 456–464.

Iannetti, G.D., Baumgärtner, U., Tracey, I., Treede, R.D., Magerl, W., 2013. Pinprick-evoked brain potentials: a novel tool to assess central sensitization of nociceptive pathways in humans. J. Neurophysiol. 110, 1107–1116.

Kirk, I.J., McNair, N.A., Hamm, J.P., Clapp, W.C., Mathalon, D.H., Cavus, I., Teyler, T.J., 2010. Long-term potentiation (LTP) of human sensory-evoked potentials. Wiley Interdiscip. Rev. Cogn. Sci. 1 (5), 766–773. https://doi.org/10.1002/wcs.62.

Klein, T., Magerl, W., Hopf, H.-C.C., Sandkühler, J., Treede, R.-D.D., 2004. Perceptual correlates of nociceptive long-term potentiation and long-term depression in humans. J. Neurosci. 24, 964–971.

Klein, T., Magerl, W., Treede, R.D., 2006. Perceptual correlate of nociceptive long-term potentiation (LTP) in humans shares the time course of early-LTP. J. Neurophysiol. 96, 3551–3555.

Liang, M., Lee, M.C., O'Neill, J., Dickenson, A.H., Iannetti, G.D., 2016. Brain potentials evoked by intraepidermal electrical stimuli reflect the central sensitization of nociceptive pathways. J. Neurophysiol. 116, 286–295.

Lapébie, F.-X., Kennel, C., Magy, L., Projetti, F., Honnorat, J., Pichon, N., Vignon, P., François, B., 2014. Potential side effect of propofol and sevoflurane for anesthesia of anti-NMDA-R encephalitis. BMC Anesthesiol. 14, 5. https://doi.org/10.1186/1471-2253-14-5 (no pagination).

Michail, G., Dresel, C., Witkovský, V., Stankewitz, A., Schulz, E., Alexandre, C., Israel, B., Valentini, E., 2016. Neuronal oscillations in various frequency bands differ between pain and touch. Front. Hum. Neurosci. 10, 1–8.

Ploner, M., Sorg, C., Gross, J., 2017. Brain rhythms of pain. Trends Cogn. Sci. 21, 100–110.

Sandkühler, J., 2007. Understanding LTP in pain pathways. Mol. Pain 3, 1–9.

Sandkühler, J., Gruber-Schoffnegger, D., 2012. Hyperalgesia by synaptic long-term potentiation (LTP): an update. Curr. Opin. Pharmacol. 12, 18–27.

Sauleau, P., Lapouble, E., Val-Laillet, D., Malbert, C.H., 2009. The pig model in brain imaging and neurosurgery. Animal 3, 1138–1151.

Sjöstedt E., Zhong W., Fagerberg L., Karlsson M., Mitsios N., Adori C., Limiszewska A., Hikmet F., Huang J., Du Y., Lin L., Dong Z., Yang L., Liu X., Jiang H., Xu X., Wang J., Yang H., Bolund L., Mardinoglu A., Zhang C., Von Feilitzen K., Lindskog C., Pontén F., Luo Y., Hökfelt T., Uhlén M. , Mulder J. An atlas of the protein-coding genes in the human, pig, and mouse brain.

Sun, G., Wen, Z., Ok, D., Doan, L., Wang, J., Chen, Z.S., 2021. Detecting acute pain signals from human EEG. J. Neurosci. Methods 347, 108964.

Swindle M.M. Pain Control & Pain Research in Swine.

Team R Development Core, 2018. A language and environment for statistical computing. R Found. Stat. Comput. 2. ⟨https://www.R-project.org⟩.

Treede, R.D., 2018. The International Association for the Study of Pain definition of pain: as valid in 2018 as in 1979, but in need of regularly updated footnotes. Pain Reports 3, 3–5.

Schmidt, Verena, 2015. Comparative anatomy of the pig brain - An integrative magnetic resonance imaging (MRI) study of the porcine brain with special emphasis on the external morphology of the cerebral cortex. VVB Laufersweiler Verlag 183.

Wada, N., Tashima, K., Motoyasu, A., Nakazawa, H., Tokumine, J., Chinzei, M., Yorozu, T., 2018. Anesthesia for patient with anti-N-methyl-D-aspartate receptor encephalitis. Medicine 97 (50), e13651. https://doi.org/10.1097/MD.0000000000013651.

Zhang, J., Hoheisel, U., Klein, T., Magerl, W., Mense, S., Treede, R.D., 2016. High-frequency modulation of rat spinal field potentials: effects of slowly conducting muscle vs. skin afferents. J. Neurophysiol. 115, 692–700.

Zhuo, M., 2011. Cortical plasticity as a new endpoint measurement for chronic pain. Mol. Pain 7, 54.