Modulation of Intracortical S1 Responses Following Peripheral Nerve High-Frequency Electrical Stimulation in Danish Landrace Pigs

Janjua, Taha Al Muhammadee; Nielsen, Thomas Gomes Nørgaard dos Santos; Andreis, Felipe Rettore; Meijs, Suzan; Jensen, Winnie

Published in:
11th International IEEE/EMBS Conference on Neural Engineering, NER 2023 - Proceedings

DOI (link to publication from Publisher):
10.1109/ner52421.2023.10123841

Publication date:
2023

Document Version
Accepted author manuscript, peer reviewed version

Modulation of Intracortical S1 Responses Following Peripheral Nerve High-Frequency Electrical Stimulation in Danish Landrace Pigs

1st Taha Al Muhammadee Janjua
Center for Neuroplasticity and Pain (CNAP), Department of Health Science and Technology, Aalborg University
Aalborg, Denmark
taha@hst.aau.dk

2nd Thomas Gomes Nørgaard dos Santos Nielsen
Center for Neuroplasticity and Pain (CNAP), Department of Health Science and Technology, Aalborg University
Aalborg, Denmark
thnn@hst.aau.dk

3rd Felipe Rettore Andreis
Center for Neuroplasticity and Pain (CNAP), Department of Health Science and Technology, Aalborg University
Aalborg, Denmark
fran@hst.aau.dk

4th Suzan Meijs
Center for Neuroplasticity and Pain (CNAP), Department of Health Science and Technology, Aalborg University
Aalborg, Denmark
smeijs@hst.aau.dk

5th Winnie Jensen
Center for Neuroplasticity and Pain (CNAP), Department of Health Science and Technology, Aalborg University
Aalborg, Denmark
wj@hst.aau.dk

***Abstract*— Long-term potentiation (LTP) has been extensively studied with rodents and human subjects to understand pain mechanisms. This phenomenon remains relatively less explored in pigs, even though pigs present a suitable translational model for neurophysiological research. This study aimed to investigate changes in the spike activity in the primary somatosensory cortex (S1) in response to spinal LTP-like neuroplasticity induced by high-frequency electrical stimulation (HFS) in Danish landrace pigs. Six animals were investigated (two controls and four interventions). A 16-channel multi-electrode array was implanted into the S1. A tripolar cuff was placed around the ulnar nerve. HFS (15 mA, 100 Hz, 1 ms) was induced on the ulnar nerve branches to induce LTP-like neuroplasticity, followed by non-nociceptive stimulation to probe the S1 response. Peristimulus time histograms (PSTHs) were constructed based on the neuronal spikes detected from S1. For the intervention group, the PSTH showed a significant increase in the area under the curve (AUC) 45 min (T2 phase) after applying the HFS. These results were in line with findings based on local-field potentials, i.e., the cortical excitability increased immediately after intervention and became significantly greater during the T2 phase. The result of this study is believed to be an essential contribution to developing a translational, large-animal model of LTP-like pain to bridge research between animal models and clinical applications.**

Keywords- Long-term potentiation, LTP-like neuroplasticity, primary somatosensory cortex, animal model

I. INTRODUCTION

Pain research in animals is greatly debated in the literature since many medical compounds have shown to be promising in the preclinical phase but failed due to differences between rodent and human physiology. The porcine model may be a suitable alternative due to the anatomical and physiological resemblance to humans [1]. The focus of this study was to establish a large animal model of LTP-like pain.

Cortical regions known for their role in pain processing include the primary somatosensory cortex (S1), anterior cingulate cortex (ACC), prefrontal cortex (PFC), and insula [2]. S1 plays an essential role in encoding the intensity and duration of noxious stimuli and is commonly targeted to record neuronal signals in human and animal studies [2]-[3].

In humans, the advantage of studying pain mechanisms often lies in obtaining verbal feedback from the subjects. On the other hand, a more comprehensive picture of the cortical processes can be provided by invasive animal pain models. For examples the spared nerve injury model [4], and spinal long-term potentiation (LTP) of pain [5].

LTP is characterized by increased synaptic strength that leads to enhanced signal transmission between neurons [6]. LTP in the spinal dorsal horn is proposed as a cellular mechanism underlying hyperalgesia [7]. An increase in synaptic strength in the dorsal horn of the spinal cord is categorized as spinal LTP and can be artificially generated by high-frequency, high-intensity, short-duration (HFS) electrical stimulation on a peripheral nerve [6]. HFS-induced spinal LTP is a pain model that appropriately correlates human profiles of primary hyperalgesia, secondary hyperalgesia and mechanical allodynia [7].

In rats, HFS resulted in an increase in the amplitude of evoked responses in the thalamus [8]. Since sensory information is relayed from the thalamus to S1, this reported increased evoked response amplitude was expected to affect S1 [9]. Sanoja et al. reported increased excitability of the thalamic sensory neurons after spinal LTP in rats [10]. Similarly, Hjornevik et al. demonstrated that spinal LTP resulted in acute hypermetabolic response in the S1 [11]. As such, this study highlighted the role of S1 in the perception of nociceptive input in rats.

In humans, HFS resulted in increased reported pain ratings [12] and the amplitude of the evoked cortical responses [13]. However, human pain models have certain limitations, *i.e.*, cortical signal recording techniques used for human subjects are primarily non-invasive. Electroencephalography (EEG) recorded from the scalp has attenuated high-frequency components due to the skull and brain tissue acting as a low-pass filter [14]. On the other hand, animal models allow high-frequency characteristics of intracortical processing to be identified which can offer more isolated temporal and spatial information.

We have previously investigated changes in local field potentials recorded by intracortical electrodes in response to HFS [15]. We analyzed the modulation of the first post-stimulus negative peak (N1) over time across the intervention and control groups. The analysis showed an immediate increase in N1 amplitude that became statistically significant 45 mins after HFS for the intervention group. The normalized change in power in frequency oscillations showed a similar trend. This study's aim was to investigate if this information was retained in the higher frequency components (100-5000 Hz) to analyze the changes in neuronal spike activity.

Our previous work focused on the lower frequency range (0.5 Hz to 150 Hz) to align our findings with human experiments, but this study focuses on the similarity between rodent models of LTP-like pain and the pig model.

II. Methods

This study was conducted following the Danish Veterinary and Food Administration under the Ministry of Food, Agriculture and Fisheries (protocol number 2017-15-0201-01317). This study included six female pigs (Danish Landrace) that weighed 33.2 ± 3.4 kg (mean and standard deviation). Four animals were allocated to the intervention group, and two pigs were allocated to the control group. All animals were acclimatized for two weeks before the experiment to minimize the effect of stress. They were housed in pairs and given a rich environment with toys, treats, and a 13:11 hour light-dark cycle in a room maintained at ~24°C.

A. Surgical Procedures

Our previous study described the surgical procedures in detail [15]. Briefly, the pigs were anaesthetized using a 5 ml Zoletil mixture and maintained using 1.5-2.5% mean alveolar concentration (MAC) of sevoflurane, propofol (2 mg/hr/kg IV), and fentanyl (10 µg/hr/kg IV). A tripolar stimulation cuff electrode (10 mm long, 1.8 mm inner diameter, platinum-iridium ring electrodes with a 3 mm center-to-center distance) was placed around the two ulnar nerve branches in the forearm. Craniotomy and durotomy were performed to place a microelectrode array (MEA) into S1. Before the recording began, the sevoflurane was lowered to 0% MAC, and the propofol and fentanyl flow rates were doubled. At the end of the experiment, the pig was euthanized by an intravenous overdose of pentobarbital.

B. Electrophysiological Recordings

Intracortical signals from the S1 were recorded using a 16-channel MEA (2 mm long Tungsten shafts, 1 mm distance between shafts, Microprobes Inc., Gaithersburg, MD, USA). The MEA was placed 2 mm into the cortex using a micromanipulator (Kopf Instruments, USA). The S1 was located using anatomical features of the brain [16].

All the equipment and software used for recording were from Tucker-Davis Technologies, Alachua, FL, USA. As such, the MEA was connected to a Neurodigitizer (SI-8; input voltage range: ± 500 mV), where the raw data was sampled at 24414.06 Hz. The signals were transferred to a processor (RZ2) via fibre optic cables and stored in a data streamer (RS4). Synapse software was used for recording and online processing of the cortical signals.

C. Experimental Protocol

The experiment started with recording baseline signals from the S1 for 30 s. The remaining experimental protocol was divided into the following phases, each lasting 45 min (see Fig. 1).

- *Pre-LTP (T0).* 50 stimulations (1 mA and 500 µs pulse-width) were applied to one of the ulnar nerve branches. During this phase, muscle activity was observed while inducing electrical stimulation on the ulnar nerve branch. An inter-pulse interval of 2 s with a 250 ms pseudo-random interval was used to prevent habituation. The parameters defined for peripheral stimulation were non-nociceptive, i.e., the stimulation was subthreshold for C-fibres but supramaximal for motor units. The stimulation set was repeated thrice with 12 min between each set.
  Intervention. To induce LTP-like neuroplasticity in the intervention group, both ulnar nerve branches were simultaneously electrically stimulated four times (15 mA, 100 Hz, 1 ms pulse duration, 10 s interval between each train of stimulations). In the control group, no stimulation was induced during this phase.
- *Post-LTP (T1-T3).* The protocol from T0 was repeated three times and is referred to as the T1, T2 and T3 phases.

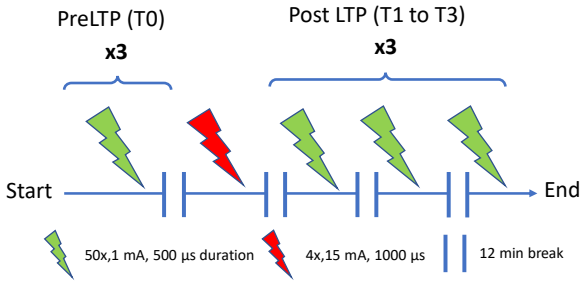

Fig. 1. Summary of the experimental protocol. The red color indicates the application of high-frequency electrical stimulation on the ulnar nerve branch for the intervention group.

D. Signal Processing

MATLAB 2020b (MathWorks, Inc., Natick, MA, USA) processed the recorded data. R was used for graphical illustration and statistical analysis [17].

The cortical signals were bandpass filtered between 100 Hz to 5000 Hz (8^{th} order Butterworth) and windowed into 2 s epochs (500 ms pre-stimulus to 1500 ms post-stimulus). Baseline correction was done by subtracting the mean value calculated in a time window of -300 ms to -5 ms prior to stimulation to improve signal quality and reduce the stimulation artefact.

Spike activity was identified by applying a threshold of 3.5x of the root mean square (RMS) (found for each channel in the 500 ms pre-stimulus time window) for each recording set (a recording set is defined as a 2s time window containing one stimulation) PSTH's with a bin size of 1 ms was created and normalized (Z-score calculation made by subtracting the

detected spikes during the 500 ms pre-stimulus time window and dividing the result by the standard deviation of the spikes detected during the 500 ms post-stimulus time window of each channel [18]). Only channels that showed a detected increase in peak PSTH amplitude greater than the 99% confidence interval were used for further analysis to ensure that noisy channels did not impact the study's results, and only regions of S1 demonstrating an increase in spike activity due to peripheral stimulation were represented. Afterwards, these channels were averaged to represent a grand average PSTH. The area under the curve (AUC) of the PSTH was used to quantify intracortical changes following HFS.

E. Statistical Analysis

Before statistical analysis of the results, the data were normalized by dividing the AUC by the average value during the T0 phase of the experiment. Outliers from the data were removed in R using a boxplot representation of the interquartile range. Then, QQ-plots and histogram plots were used to assess the normality of the data. Since the data were non-normally distributed, a statistical comparison between the AUC magnitude in the three phases (T1-T3) with the baseline (T0) was made using a Wilcoxon signed-rank test. A significance level of 0.05 was adopted, and Bonferroni correction was applied to correct for multiple comparisons. Upon finding significant differences in the time phase under consideration, a Mann-Whitney *U* test was performed to identify if the control and intervention groups were significantly different within that time phase.

III. Results

Results from the spike activity analysis in response to HFS in the intervention group can be seen in Fig. 2. The mean values and confidence intervals of AUC in all phases are displayed in Table 1. For the intervention group, we found a 20% increase in the mean AUC at T2 compared to T0 ($p < 0.01$), while T1 and T3 phases showed a 2% decrease ($p = 0.63$) and 9% decrease ($p = 0.31$), respectively. As such, the AUC in T3 returned to the value in the T1 phase. For the control group, we found a notable decrease in AUC over time; however, this change was not statistically significant.

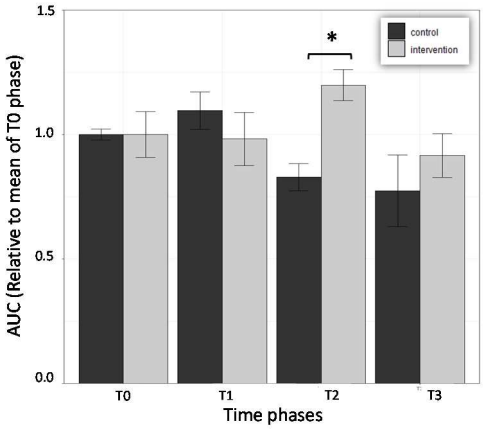

Fig. 2. Changes area under the curve (AUC) across the time phases T0-T3 relative to the average of the T0 phase for the control and intervention groups. The error bars represent the 95% confidence intervals, and the asterisk highlights a significant difference ($p < 0.01$).

Table 1. Mean area of under curve [95% confidence interval] relative to T0 phase in each control and intervention group phase. The asterisk highlights a significant difference ($p < 0.01$).

	AUC (Relative to mean of T0 phase)	
Phase	Control group	Intervention group
T0	1.00 [0.94,1.06]	1.00 [0.79,1.21]
T1	1.10 [0.90, 1.29]	0.98 [0.74, 1.23]
T2*	0.83 [0.69, 0.97]	1.20 [1.05, 1.34]
T3	0.77 [0.40, 1.14]	0.91 [0.71, 1.12]

IV. Discussion

HFS has been used to induce LTP-like sensitization in humans [19]; These studies reported an increased evoked-related potential and pain ratings 20 min after HFS [19], [20]. It is currently impossible to identify precise cortical changes in humans because of non-invasive recordings, preventing researchers from analyzing neuronal spike activity.

On the other hand, the observed changes in S1 spike activity were in line with the S1 metabolism changes in rodents [11]. A similar effect was seen in the thalamus in HFS-induced rats [10]. Furthermore, Hernandez *et al.* reported that induction of spinal LTP by HFS on the sciatic nerve enhanced the excitability of the posterior triangular nucleus of the thalamus [8]. This modulation, measured through PSTHs, demonstrated the effect of intervention 50 min after assumed LTP induction. Hence, the increased S1 excitability we observed was likely due to enhanced spinal and thalamic excitability. This was the reason for recording changes in the S1 excitability 12 min after intervention since expected changes occured at least 20 mins after intervention.

It is noteworthy that there is a difference in build-up time between our experiments and human studies. A possible explanation could be that the subjects are unanaesthetized in human studies. Hence, attention to stimulation likely played a role. We suspect that the increase in AUC in the control group, though non-significant, might be because of inter-subject variability.

Spinal LTP can be ensured by recording neuronal activity from the spinal cord. However, the complex surgical procedure involved in recording signals from the S1 meant that there was not sufficient time or resources to validate that LTP was induced using HFS by spinal recording. Furthermore, a single branch of the ulnar nerve was used for stimulation compared to both branches being stimulated for the intervention because the authors wanted to ensure that LTP was induced. The peripheral stimulation was not done on both nerves to ensure it did not become nociceptive due to the possible summation of the neuronal stimulations.

This study was focused on only those neurons that showed a change in spike activity due to peripheral stimulation. A topographical overview of S1 can represent changes throughout S1, including channels that are relatively non-responsive to peripheral electrical stimulation.

V. Conclusions

This study focused on quantifying the changes in the cortical spike activity in response to peripheral electrical stimulation after HFS. We showed a significant increase in AUC during the mid-LTP (T2) phase that signified an increase in excitability denoted by the number of detected neuronal spikes using PSTH analysis.

Our study is an important step toward establishing pigs as translational models in pain research. Future studies may be conducted to substantiate our findings that include additional pain processing areas or investigate spinal cord changes. Furthermore, simultaneous recordings from the thalamic, spinal and S1 neurons could improve our present LTP-like pain model by unravelling where changes occur and where they are relayed.

Acknowledgement

This work was funded by the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 754465 and the Danish National Research Foundation (DNRF121).

References

[1] J. J. Van Gompel et al., "Swine model for translational research of invasive intracranial monitoring," Epilepsia, vol. 52, no. 6, pp. e49–e53, Jun. 2011, doi: 10.1111/j.1528-1167.2011.03096.x.

[2] M. C. Bushnell, G. H. Duncan, R. K. Hofbauer, B. Ha, J. I. Chen, and B. Carrier, "Pain perception: is there a role for primary somatosensory cortex?," Proc. Natl. Acad. Sci. U. S. A., vol. 96, no. 14, pp. 7705–9, Jul. 1999, doi: 10.1073/pnas.96.14.7705.

[3] I. M. Devonshire, C. M. Greenspon, and G. J. Hathway, "Developmental alterations in noxious-evoked EEG activity recorded from rat primary somatosensory cortex," Neuroscience, vol. 305, pp. 343–350, Oct. 2015, doi: 10.1016/j.neuroscience.2015.08.004.

[4] S. Boccella et al., "Spared Nerve Injury as a Long-Lasting Model of Neuropathic Pain," in Methods in Molecular Biology, vol. 1727, 2018, pp. 373–378. doi: 10.1007/978-1-4939-7571-6_28.

[5] T. V. P. Bliss and S. F. Cooke, "Long-term potentiation and long-term depression: a clinical perspective," Clinics, vol. 66, no. SUPPL.1, pp. 3–17, Jan. 2011, doi: 10.1590/S1807-59322011001300002.

[6] E. N. Van Den Broeke, C. M. van Rijn, J. A. Biurrun Manresa, O. K. Andersen, L. Arendt-Nielsen, and O. H. G. Wilder-Smith, "Neurophysiological Correlates of Nociceptive Heterosynaptic Long-Term Potentiation in Humans," J. Neurophysiol., vol. 103, no. 4, pp. 2107–2113, Apr. 2010, doi: 10.1152/jn.00979.2009.

[7] J. Sandkühler, "Understanding LTP in pain pathways," Mol. Pain, vol. 3, pp. 1–9, 2007, doi: 10.1186/1744-8069-3-9.

[8] A. González-Hernández et al., "Spinal LTP induced by sciatic nerve electrical stimulation enhances posterior triangular thalamic nociceptive responses," Neuroscience, vol. 234, pp. 125–134, Mar. 2013, doi: 10.1016/j.neuroscience.2013.01.006.

[9] M. R. Borich, S. M. Brodie, W. A. Gray, S. Ionta, and L. A. Boyd, "Understanding the role of the primary somatosensory cortex: Opportunities for rehabilitation," Neuropsychologia, vol. 79, pp. 246–255, Dec. 2015, doi: 10.1016/j.neuropsychologia.2015.07.007.

[10] R. Sanoja, N. Taepavarapruk, E. Benda, R. Tadavarty, and P. J. Soja, "Enhanced Excitability of Thalamic Sensory Neurons and Slow-Wave EEG Pattern after Stimuli That Induce Spinal Long-Term Potentiation," J. Neurosci., vol. 33, no. 38, pp. 15109–15119, Sep. 2013, doi: 10.1523/JNEUROSCI.2110-13.2013.

[11] T. Hjornevik, L. M. Jacobsen, H. Qu, J. G. Bjaalie, J. Gjerstad, and F. Willoch, "Metabolic plasticity in the supraspinal pain modulating circuitry after noxious stimulus-induced spinal cord LTP," Pain, vol. 140, no. 3, pp. 456–464, 2008, doi: 10.1016/j.pain.2008.09.029.

[12] N. Hansen, T. Klein, W. Magerl, and R.-D. Treede, "Psychophysical Evidence for Long-Term Potentiation of C-Fiber and Aδ-Fiber Pathways in Humans by Analysis of Pain Descriptors," J. Neurophysiol., vol. 97, no. 3, pp. 2559–2563, Mar. 2007, doi: 10.1152/jn.01125.2006.

[13] E. N. van den Broeke and A. Mouraux, "High-frequency electrical stimulation of the human skin induces heterotopical mechanical hyperalgesia, heat hyperalgesia, and enhanced responses to nonnociceptive vibrotactile input," J. Neurophysiol., vol. 111, no. 8, pp. 1564–1573, Apr. 2014, doi: 10.1152/jn.00651.2013.

[14] A. Dubey and S. Ray, "Cortical Electrocorticogram (ECoG) Is a Local Signal," J. Neurosci., vol. 39, no. 22, pp. 4299–4311, May 2019, doi: 10.1523/JNEUROSCI.2917-18.2019.

[15] T. A. M. Janjua, T. G. N. dos S. Nielsen, F. R. Andreis, S. Meijs, and W. Jensen, "The effect of peripheral high-frequency electrical stimulation on the primary somatosensory cortex in pigs," IBRO Neurosci. Reports, vol. 11, no. September, pp. 112–118, Dec. 2021, doi: 10.1016/j.ibneur.2021.08.004.

[16] Verena Schmidt, 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, 1st ed. VVB Laufersweiler Verlag, 2015, 2015. [Online]. Available: https://books.google.dk/books/about/Comparative_Anatomy_of_the_Pig_Brain_An.html?id=hJgCjwEACAAJ&redir_esc=y

[17] Team R Development Core, "A Language and Environment for Statistical Computing," R Foundation for Statistical Computing, vol. 2. R Foundation for Statistical Computing, Vienna, Austria, p. https://www.R-project.org, 2018. [Online]. Available: http://www.r-project.org

[18] S. G. K. Patro and K. K. Sahu, "Normalization: A Preprocessing Stage," IARJSET, vol. 2, pp. 20–22, Mar. 2015, doi: 10.17148/IARJSET.2015.2305.

[19] E. N. van den Broeke, C. H. van Heck, L. A. J. M. Ceelen, C. M. van Rijn, H. van Goor, and O. H. G. Wilder-Smith, "The effect of high-frequency conditioning stimulation of human skin on reported pain intensity and event-related potentials," J. Neurophysiol., vol. 108, no. 8, pp. 2276–2281, Oct. 2012, doi: 10.1152/jn.00391.2012.

[20] D. B. Pfau, T. Klein, D. Putzer, E. M. Pogatzki-Zahn, R.-D. Treede, and W. Magerl, "Analysis of hyperalgesia time courses in humans after painful electrical high-frequency stimulation identifies a possible transition from early to late LTP-like pain plasticity," Pain, vol. 152, no. 7, pp. 1532–1539, Jul. 2011, doi: 10.1016/j.pain.2011.02.037.