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***Abstract*— In our laboratory, we have recently established a large animal model of LTP-like pain and extracted cortical features as objective measurements of nociception. We have previously reported an increase in the S1 cortical activity for both local-field potentials (LFP) and spike activity up to 90 min after induction of high-frequency stimulation. Our analysis so far has been based on averaging signals obtained from an intracortical array, thus losing any spatial information. The aim of this work was therefore to investigate spatio-temporal neural changes. Intracortical EEG recordings from pigs (n=7) were acquired using a 16-channel microelectrode array (MEA) placed in S1. To assess the cortical response, electrical stimulation was delivered to the ulnar nerve. Each experiment was divided into four blocks (T0-T3). The intervention group (n=5) received LTP between T0 and T1. We extracted the N1-P1 amplitude as a feature in the LFP signal range and the area under the curve (AUC) of the PSTH response as a feature to represent the spike signals. We found that LTP induced spatio-temporal changes in both the LFP and spike activity in the T2 and T3 phases, which is in line with our previous results [1]. However, in the present work, we additionally observed that the location of the maximal activity moved spatially between T0 and T2 (3/5 animals for LFP activity, 4/5 animals for spike activity).**

Also, we observed a cortical suppression in the T3 phase associated with long-term depression. A more detailed understanding of the cortical response and plasticity to nociception may potentially be a more suitable platform to investigate the efficacy of novel drugs to treat pain.

Keywords- LTP, spatio-temporal analysis, LFP, spike, pig

I. Introduction

Recorded brain signals are used to deduct information on the brain's response to e.g., a stimulus, behavior, or a disease state. In animal models, the neural activity is typically recorded using a microelectrode array (MEA).

Local field potentials (LFP) and spike activity represent different neural characteristics [2]. LFP activity (approx. 1-250 Hz) encodes the sum of neuron activation near the electrode, while the spike activity (approx. 250 Hz - 10 kHz) contains information about the firing of individual neurons [3]–[7]. Individual neurons or single channels are often studied in isolation, thus giving a limited picture of how neuronal populations give rise to sensation, behavior, or other complex brain processes. Moreover, the responses of single cells to external stimuli are often averaged over several trials to reduce the effects of neuronal variability. This results in a limited picture and use of data because the spatial aspect of the neural activity is removed by the averaging approach [2], [8]. Additionally, the evoked response analysis method is based on identifying the evoked responses in the time domain, which enables the possibility to look for neural information at a specific time [2]. However, the brain process information and takes decision-based on single events, making sense of the noisy response from individual neurons by evaluating the activity of large populations. Only a few studies have investigated neural responses using spatio-temporal analysis of multi-channel recordings.

The value of pain research in non-human animals is greatly debated in the literature since many medical compounds have been shown to be very promising in the preclinical phase but then failed due to differences between rodent and human physiology. The porcine, large animal model may be a suitable alternative due to the anatomical and physiological resemblance to humans. In our laboratory, we have focused on establishing a large animal model of LTP-like pain and extraction of cortical features as an objective measurement of nociception and chronic pain [9]. We have previously reported an increase in the S1 cortical activity for both LFP and spike activity 45 min after induction of high-frequency stimulation in pigs (see [1] and abstract prepared by Janjua et al. at this conference). The intracortical data was recorded using a 4x4 MEA array, and the analysis was based on averaging the data over the entire array, thus ignoring any spatial information. The aim of this study was therefore to investigate spatio-temporal neural changes to reveal possible novel patterns. A better understanding may potentially be used to investigate novel drugs to treat pain [9].

II. Methods

A. Experimental Procedures

The experimental procedures were approved by the Danish Veterinary and Food Administration (protocol 2017–15–0201–01317). Seven female pigs were included in the study (Danish Landrace, 33.2 ± 3.4 kg). The experimental procedures are described in brief, and details can be found in [1].

To deliver the peripheral electrical stimulation sequences a tripolar cuff electrode was placed around each of the ulnar nerve branches in the left forelimb. A 4x4 multi-electrode array (MEA-PI-A3-00-16-0.6-2.0-3-1.0-1.0-1-1SS-1, Microprobes Inc., shaft length = 2 mm, distance between shafts = 1 mm, depth = 2 mm from the pia surface) was then implanted in S1 right hemisphere, see Fig. 1. The recordings were initiated 30 min after array implantation.

The experiment was divided into four blocks; T0 (pre-LTP), T1, T2, and T3 (post-LTP). To evaluate the cortical response before and after induction of LTP-like neuroplasticity, somatosensory evoked potentials (SEP's) were recorded.

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As such, three sets of 50 electrical stimulation pulses (amplitude = 1 mA, duration = 500 µs, inter-pulse interval = 2±0.25s) were delivered within each block. To introduce LTP-like neuroplasticity in the intervention group (n = 5), an electrical stimulation sequence (amplitude = 15 mA, pulse duration = 1000 µs, frequency = 100 Hz) was delivered to the ulnar nerve (4 repetitions, 10 s between each stimulus sequence) between the T0 and T1 blocks. In the case of the control group (n=2), the same procedures were applied, except that no stimulation was delivered in the intervention phase. Data were collected and sampled at 25 kHz (Tucker-Davies Technologies, Alachua, FL, USA). At the end of the experiment, the animals were euthanized with an overdose of pentobarbital.

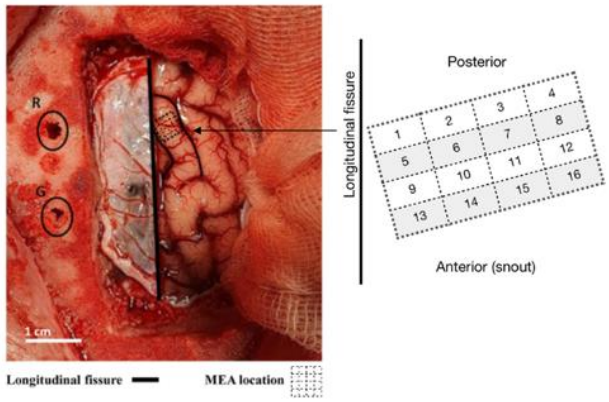

Fig. 1. Top view of the cortex (left), which visualizes the placement and orientation of the 4x4 microelectrode array (MEA) (right) in the primary somatosensory cortex (S1). Marked holes are ground (G) and reference point (R). The longitudinal fissure is marked by the black line.

B. *Signal Processing*

The data were processed offline using the Scientific PYthon Development EnviRonment (SPYDER 5.1.5) and filtered using the Scipy package (Scipy 1.7.1).

First, data was windowed into epochs of 1500 ms, with the stimulation pulses occurring at t = 500 ms. To remove low-frequency noise, the data were baseline corrected by subtracting the mean of the first 500 ms from each epoch and highpass filtered at 1 Hz (Butterworth filter). Additionally, a notch filter was used to remove power line noise. The data was then visually inspected to remove any broken channels. The signals were finally separated in LFP (1-250 Hz, 20th order highpass Butterworth filter) and spike activity (250 Hz – 10 kHz, 20th order bandpass Butterworth filter).

N1-P1 amplitude representing LFP activity

The chosen LFP feature for the time domain analysis was the peak-to-peak amplitude between N1 and P1 [10]. The LFP responses within one block were averaged (see Fig. 2A). The N1 and P1 were identified as the minimum and maximum activity, respectively, within a 100 ms window after electrical stimulation (see Fig. 2B). To allow comparison between channels and animals, Z-score normalization was applied relative to the T0 block.

Area Under the Curve (AUC) representing spike activity

To analyze the modulation in spike activity over time, the area under the PSTH curve was chosen as a representative feature. First, the single unit spike activity was detected (threshold = mean ± 2.5 std in a 100 ms window following electrical stimulation). A post-stimulus time histogram (PSTH) was then generated (bin size of 5 ms) and normalized (Z-score relative to the T0 block). Lastly, the AUC was found by discrete integration.

To retain the spatiotemporal element of the neural activity, the extracted features were interpolated in a gaussian 4x4 color map for each block (T0-T3), corresponding to the physiological placement of the MEA on S1, see Fig. 2C. To allow visual comparison between animals, a value representing the maximal activity (the highest 10%) or minimal activity (the lowest 10%) was plotted, see Fig. 3 and 4.

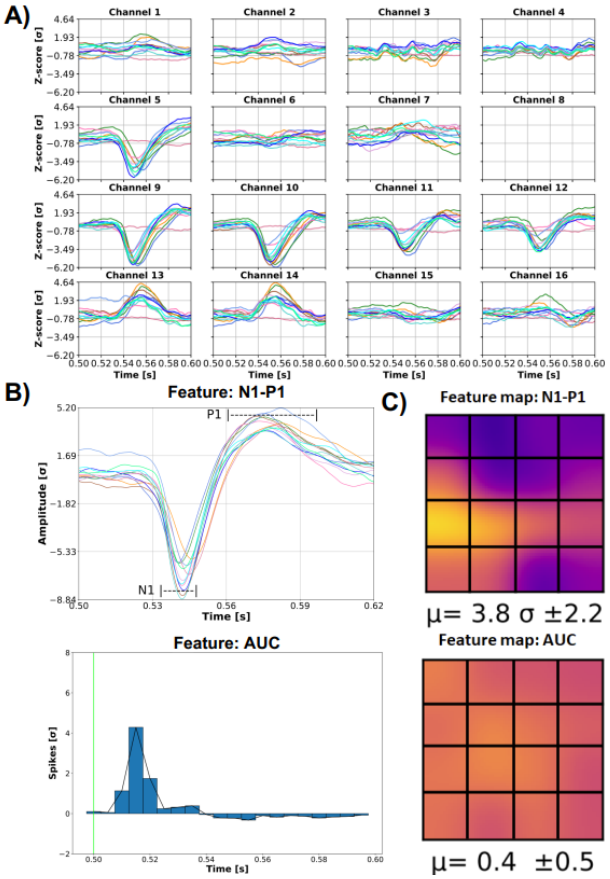

Fig. 2. Example data are shown from the T0 block in Exp #3. A) Evoked potentials. Data are averaged and normalized. B) Examples of N1-P1 features and the area under the curve (AUC) in the PSTH. C) Examples of feature maps representing the array activity, where brighter pixels indicate a higher value. The electrode placement is identical to the one shown in Fig. 1.

III. Results

A. *Spatio-temporal changes of the N1-P1 amplitude*

The results showed a spatio-temporal increase in maximal activity in T2 compared to T0 in three out of five animals in the intervention group, and for the control group, one out of two animals showed an increase (Fig. 3B, Table I). Additionally, a spatio-temporal decrease in the minimal activity was found between T0 and T3 for all animals in both the intervention and control groups (Fig. 3C, Table II).

The changes were found in different areas of the array for each animal. The analysis showed a mean spatio-temporal difference between T0 and T2 (Table I and Table II) that was higher for the intervention group compared to the control group.

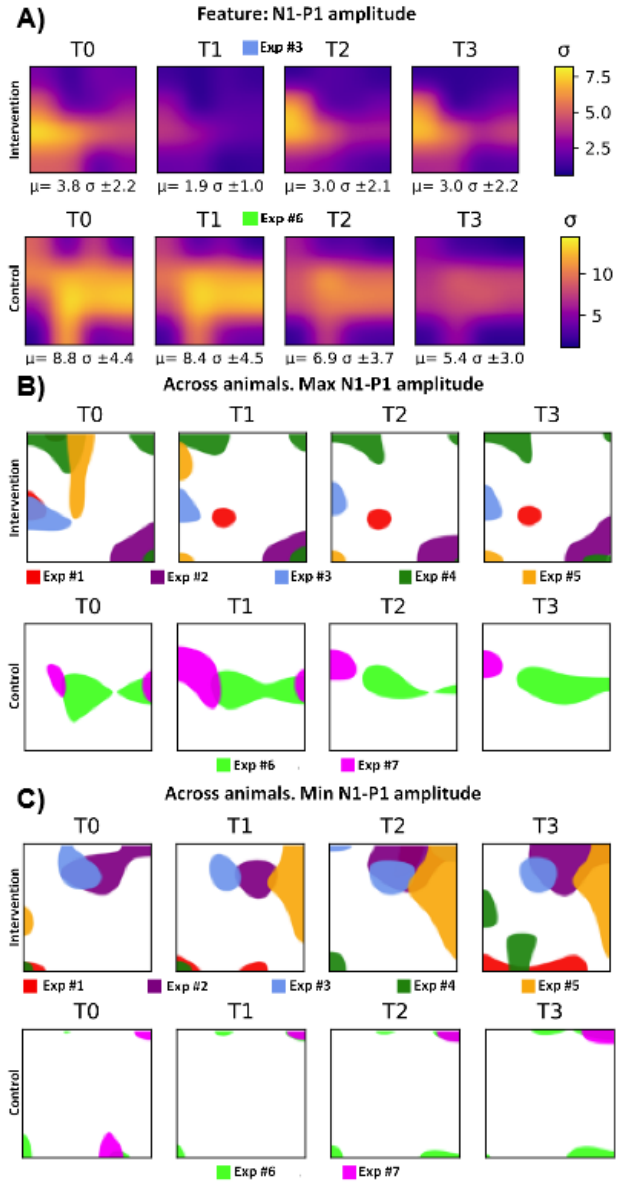

Fig. 3. A) The N1-P1 feature map from one intervention animal (Exp #3) and one control animal (Exp #6). B) Comparison of the Max N1-P1 amplitudes across animals in the intervention (Exp #1-#5) and control group (Exp #5-#6). C) Comparison of the Min N1-P1 amplitudes across animals

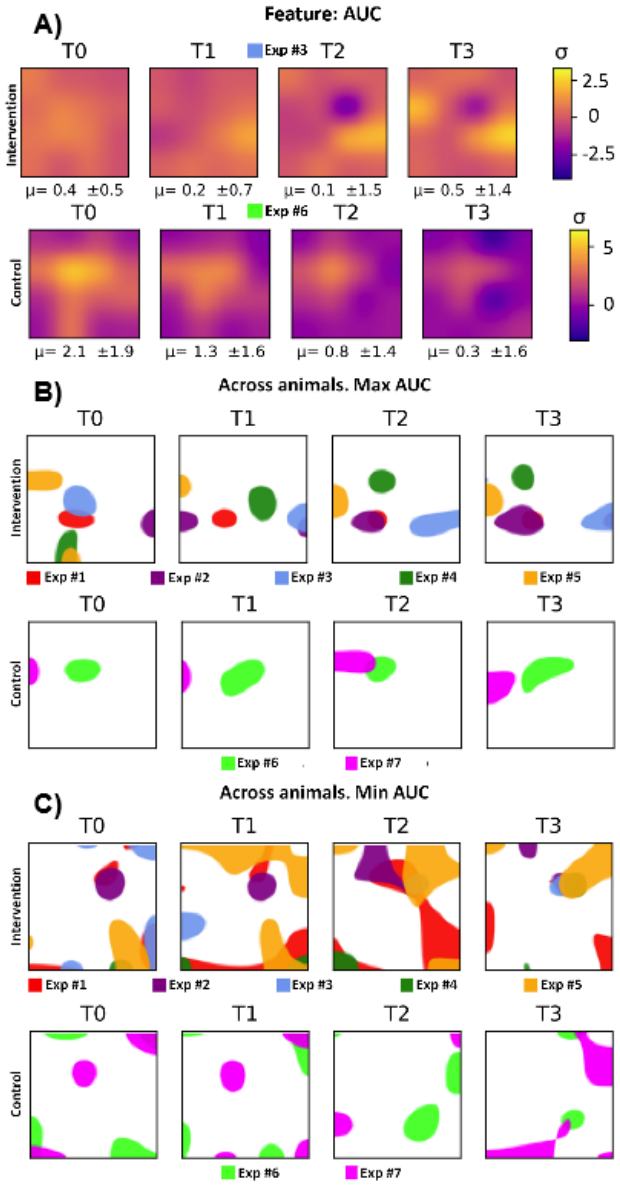

Fig. 4. A) The area under the curve (AUC) map from one intervention animal (Exp #3) and one control animal (Exp #6). B) Comparison of the Max AUC across animals in the intervention (Exp #1-#5) and control group (Exp #5-#6). C) Comparison of the Min AUC across animals

Table I. Evaluation of the spatial difference between the T0-T2 and T0-T3 Phases using the max feature value

Time domain max channel difference in T2-T0 and T3-T0											
Group		*Control*			*Intervention*						
Experiment		*C-1*	*C-2*	**Mean**	*I-1*	*I-2*	*I-3*	*I-4*	*I-5*	**Mean**	$\Delta(I-C)$
LFP (N1/P1)	$\Delta(T2_{Max}-T0_{Max})$	-2.0	5.0	**1.5**	3.0	2.9	-0.6	-1.7	22.7	**5.3**	**3.8**
	$\Delta(T3_{Max}-T0_{Max})$	-5.1	6.0	**0.5**	5.8	1.1	-0.6	-1.5	-5.0	**0.0**	**-0.5**
Spike (AUC)	$\Delta(T2_{Max}-T0_{Max})$	-1.7	-0.9	**-1.3**	0.7	1.6	1.6	-6.0	3.9	**0.4**	**1.7**
	$\Delta(T3_{Max}-T0_{Max})$	-3.5	-1.1	**-2.3**	0.1	0.3	2.1	1.1	2.0	**1.1**	**3.4**

Table II. Evaluation of the spatial difference between the T0-T2 and T0-T3 Phases using the min feature value

Time domain min channel difference in T2-T0 and T3-T0											
Group		*Control*			*Intervention*						
Experiment		*C-1*	*C-2*	**Mean**	*I-1*	*I-2*	*I-3*	*I-4*	*I-5*	**Mean**	$\Delta(I-C)$
LFP (N1/P1)	$\Delta(T2_{Min}-T0_{Min})$	-0.1	-1.4	**-0.8**	-0.4	0.2	-0.2	-0.1	-0.9	**-0.3**	**0.5**
	$\Delta(T3_{Min}-T0_{Min})$	-0.5	-2.1	**-1.3**	-0.4	-0.1	-0.5	-0.3	-2.5	**-0.8**	**0.5**
Spike (AUC)	$\Delta(T2_{Min}-T0_{Min})$	-0.9	-0.6	**-0.8**	-0.1	0.5	-3.9	-6.6	0.3	**-2.0**	**-1.2**
	$\Delta(T3_{Min}-T0_{Min})$	-3.1	0.2	**-1.5**	-0.6	-0.3	-2.6	-3.0	-0.4	**-1.4**	**0.1**

B. Spatio-temporal changes in AUC

Four out of five animals in the intervention group showed a spatiotemporal increase in maximum activity in T2 compared to T0 (Fig. 4B). Both animals in the control group showed a decrease in the maximal activity between T0 and T2 (Fig. 4C). Additionally, a spatio-temporal decrease in minimal activity between T0 and T3 was found for all animals in the intervention group (Fig. 4C). For the control group, one out of two animals showed a decrease. Similarly, to the N1-P1 amplitude, results showed a mean spatio-temporal difference between T0 and T2 (Table I and Table II).

IV. Discussion

A. Spatio-temporal changes of the N1-P1 amplitude

An increase in activity in N1 for the intervention group was found, which is in line with the results found in [1], where an increase was found in the N1/P1 peak-to-peak between T0 and T2 following LTP. Furthermore, the present results are consistent with van den Broeke et al. who found an increase in N1/P1 peak-to-peak amplitude measured using non-invasive EEG in humans following high-frequency stimulation (HFS) [10].

B. Spatio-temporal changes in spike activity

The observed change in the spatial response pattern caused by the LTP indicates that the spatio-temporal approach reveals novel patterns and information compared to a temporal approach. It may be relevant to further study the relationship between the clusters of neurons and the response of the entire neuron population, to reveal further information about how the brain propagates pain from individual neuron activity to population activity

C. Spatial suppression in T3

HFS has previously been used to induce long-term depression [11]. This may play a role in the spatio-temporal decrease in minimum activity in T3, which was observed for the intervention group for both LFP and spike activity. Janjua et al. (2021) also observed a significant decrease in the LFP activity between T2 and T3 [1], however, in this study, the activity in T3 remained above the baseline (T0). This is not in line with the findings in this study, where a spatial area in T3 was observed to decrease below the level of the baseline activity in T0 for all animals based on both observations from the LFP and spike activity. This indicates that a specific spatial area in the array may encode the suppression, which occurs following the LTP. This finding is consistent with [12], where a decrease in pain ratings four hours after LTP was found. The suppression found in T3 may therefore represent the onset of this effect. Additionally, Janjua et al. noted that the identified suppression in T3 may be a result of hyperalgesia induced by LTP [1]. It should be noted that the decrease in minimum activity in T3 was also observed in the control group for the LFP activity and one of two animals in the control group for spike activity, and the decrease may be an effect of an unknown mechanism in response to the repeated non-noxious stimuli. Further research is needed to explain the mechanisms behind the shifting spatial response pattern in the spike activity over time. For example, information theory may provide insight into how much information is encoded in the spike compared to the LFP activity. Decoding applications may benefit from the increased resolution in the spatio-temporal approach, to strengthen the prediction of the decoding algorithm.

V. Conclusions

This study investigated spatio-temporal neural changes to reveal novel patterns and information from the LFP and spike activity recorded in S1 in pigs in an animal model of LTP-like pain. The results indicated that the spatio-temporal approach can reveal spatial patterns and information from the LFP, and spike activity recorded. Especially, information was revealed from the spatial shift of the response pattern and an observed cortical suppression (T3 phase). Further research should investigate whether the spatio-temporal approach could add a new perspective to the propagation of nociception or pain in S1. A more detailed understanding of the cortical response and plasticity to nociception may potentially be a more suitable platform to investigate the efficacy of novel drugs to treat pain.

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