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Detection of Thermal Pain in Rodents through Wireless Electrocorticography

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Abstract— **In an effort to detect pain in an objective way, Electrocorticography (ECoG) signals were acquired from male Sprague-Dawley rats in response to thermally induced pain. A wearable, wireless multichannel system was utilized to acquire signals from freely-behaving animals during the experiments. ECoG signals were recorded before (baseline) and during the heat exposure for which animals withdrew their paws in response to the painful feeling. Analysis of the signals revealed a clear, high-amplitude peak at the moment of the paw withdrawal across all four recording channels in each test. Analysis in the frequency domain found the peaks coincided with an abrupt increase of delta rhythms (under 4 Hz). In the baseline, heating, and post-withdrawal segments, these rhythms were relatively low, indicating that the sharp increase in delta activity might be associated with pain. Theta, alpha, beta, and gamma rhythms were also measured, but no significant differences were found between each phase of the signals. These preliminary results are promising; however, more animal models will need to be tested to provide statistically significant results with high confidence.**

I. Introduction

Harmful stimuli to the skin or subcutaneous tissue (joints or muscle), are received by special receptors called nociceptors (thermal, mechanical, and polymodal). This received nociception travels to the dorsal horn of the spinal cord through Aδ and C fibers. There are five major paths from spinal cord to the brain, generally known as pain pathways [1], among which the spinothalamic tract is the most prominent. This ascending nociceptive pathway takes the pain signals to the thalamus. From the thalamus, the pain signals distribute to different brain areas including the somatosensory and motor cortices. These regions are located on the surface of the brain, and their signals can be recorded through electroencephalogram (EEG) from the surface of the skull or by electrocorticogram (ECoG), from the surface of the brain.

It has been shown that noxious stimuli cause changes in both the EEG and ECoG signals in humans [2] and in rodent models [3, 4]. Specifically, the effects of thermal stimuli upon EEG have previously been explored and determined to cause unique changes [4]. However, to our best knowledge, these studies have been conducted on either anesthetized or constrained animals; hence, the animals were under stress and the signals could not be directly associated to their behavioral perception of pain. Specifically there are evidences that anesthesia reduces the responses of the neural systems to noxious stimuli [5].

ECoG, which is an invasive procedure compared to EEG, involves the risks of surgery and recovery time [6]. However, ECoG provides a higher spatial resolution and broader bandwidths than those of EEG [7], perhaps deeming it better suited for a deeper understanding of nociceptive responses in animal models. This can be further verified, considering the numerous evidences that have used either ECoG or EEG signals to evaluate pain [4], [8-10]. One main issue for using ECoG on freely behaving animals has been the lack of a light-weight, wearable wireless system that is capable of acquiring signals from several areas in the brain. To address this issue, we have designed, fabricated, and successfully examined such a system for experiments in rodent models.

This preliminary study was performed to investigate alterations in ECoG brain rhythms produced by the cutaneous application of thermal stimuli. We employed a multi-channel communication device capable of wirelessly

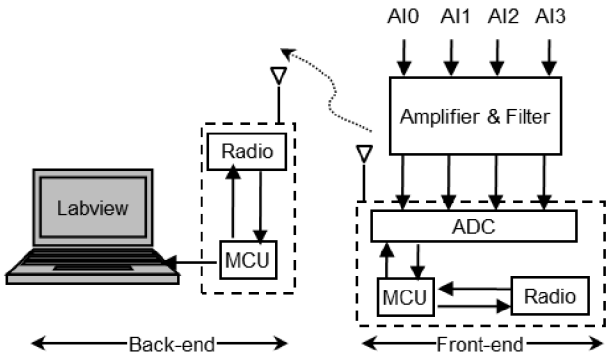

Fig. 1. The block diagram of the system to acquire Electrocorticograms.

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recording ECoG to examine nociceptive signals and their corresponding behaviors in freely-behaving rats. The recording system consists of a lightweight front-end, which can be worn by the animal transmitting recorded ECoG signals, up to a distance of 30 m, to a back-end receiver connected to a computer. The wireless acquisition of neurophysiological signals provides the added benefit of ease in studying awake, freely-behaving animal subjects to establish an objective measure for pain that is also associated with well-known nociceptive behaviors.

II. Methods and Materials

A. System Overview

A wearable, wireless module developed previously in our group [11] was utilized with modification in this study. This module is a platform that can be used for recording ECoG signals with changes in the signal conditioning (filter and amplifier designs), sampling rate and wireless transmission rate. The system is comprised of a front-end transmitter and a back-end receiver connected to a computer for data logging, display and restoration in a custom-made graphical user interface (GUI).

The front-end acquires the electrical signals from four implanted electrodes in an animal's brain. The digitized signals are sent through a 2.4-GHz transceiver. The back-end receives the radio data packets and feeds them through a universal asynchronous receiver/transmitter (UART) serial port to the GUI developed in LabVIEW (National Instrument). Figure 1 shows the block diagram of the system.

The front-end comprises an analog board to condition the signals, an analog to digital converter (ADC), a microcontroller unit (MCU) and a transceiver. The analog board amplifies the signal with a gain of 74dB, and filters it at a passband of 1–170 Hz. A low-power system-on-chip (nRF24LE1, Nordic Semiconductor [12]) that integrates ADC, MCU and the transceiver in a 5×5 mm^2 chip was used in this design. The signals were sampled at 1 ksps (kilo-samples per second) and digitized at 8-bit resolution before loaded into the data packets and wirelessly transmitted to the back-end. The back-end is comprised of another nRF24LE1 programmed as a receiver and an FTR232 adaptor, which converts the UART to the USB and loads the signals to the computer.

B. Animal Preparation

Four adult, male Sprague-Dawley rats were individually anesthetized with sodium pentobarbital (50mg/kg, i.p.) and secured into a stereotactic frame prior to surgery. Six ECoG screws (stainless steel, 1.58mm length, 0.53mm shaft diameter, Small Parts, Inc.) were implanted into each animal: two in the left and right somatosensory cortex of the hind limb (S1HL) (anterior–posterior (AP) −1.5 mm, medial–lateral (ML) 2.5 mm bilaterally from bregma), two in the motor cortex (M1) (AP 2 mm, ML 2.5 mm), and two serving as the ground and reference in the cerebellum (AP −11.5 mm, ML 1.5). An additional pair of larger screws (stainless steel, 1.58 mm length, 1.5 mm diameter, Plastics One Inc.) anchored the bone cement used to cover the electrodes. The ECoG screw electrodes were connected to a male socket embedded atop the cement. Animals were closely monitored and cared during a one-week healing period. Prior to testing, the rats were placed into a plastic chamber and lightly anesthetized with isoflurane (4–5%). Anesthesia was maintained through a mask delivering isoflurane (2–3%). A vest carrying the front-end of the wireless recording module was fitted onto the animal and connected to the exposed end of the implanted electrode socket. All surgical procedures and experimental protocols performed in this study were approved by the Institutional Animal Care and Use Committee at the University of Texas at Arlington.

III. Experimental Procedures and Results

A. Thermal Stimulation Testing

A Thermal Planter Test (Hargreaves' Method) apparatus (Harvard Apparatus) was used to deliver noxious stimuli. The rats were placed on the top of a glass pane and enclosed by a small plastic cage, allowed ample time to wake and acclimate to the setting. The receiver back-end was placed on the open ceiling of the cage, out of reach of the animal, and connected to a laptop computer via the adaptor.

A movable infrared source located under the glass pane was positioned under the targeted part of the animal (paw or tail) to be stimulated. Ten-second baseline signals were recorded prior to triggering the controller that simultaneously activated the infrared source and started a digital solid-state timer. The infrared source delivered heat at a constant power flux (60 I.R. intensity) until the animal withdrew its paw or flicked its tail upon feeling pain, upon which the heat was automatically switched off and the reaction timer was marked. Noxious stimuli were delivered in separate tests to the left hind paw, right hind paw and tail of each animal. Both paws and the tail were tested twice per animal and the corresponding reaction times were recorded. The retraction action was verified by two experimenters and distinguished clearly from possible random movements of the animal. A resting period was provided between the tests to allow the animals to calm prior to being subjected to the noxious stimulus again.

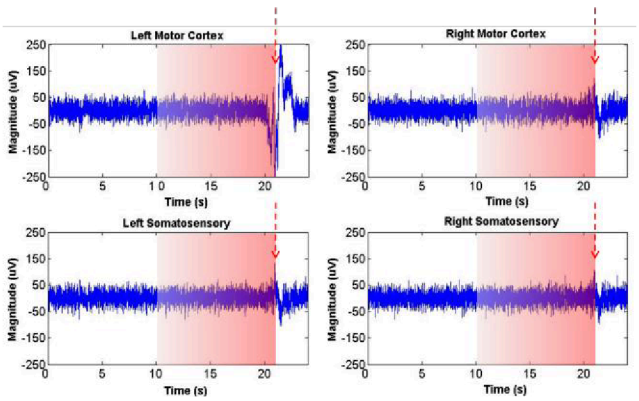

Fig. 2. A typical ECoG signal set recorded from left and right somatosensory and motor cortices during the test for thermal stimulus in the left paw. A baseline signal was recorded for 10 seconds prior to triggering the heat flux (marked with gradient red). The heating ceased upon the animal withdrawing its paw (marked with the dashed line arrow).

B. Signal Analysis

Each test produced four ECoG signals, one from each of the four actively recording channels. Signals were first pre-processed with a 60-Hz notch filter and then examined in the time domain to observe activities within the somatosensory and motor cortices during heating and upon withdrawal. Fig. 2 shows a typical ECoG signal set of a 25-second recording in each channel during a single thermal plantar test. The radiation was delivered to the left paw in this particular experiment. Peaks lasting two to three seconds consisting of low-frequency, high-amplitude activities, such as that seen occurring at approximately the 21th s in Fig. 2, were observed in all channels for each heat test. This type of activity coincided with the recorded time stamp of paw withdrawal or tail flick, and is thus interpreted to be indicative of a pain response.

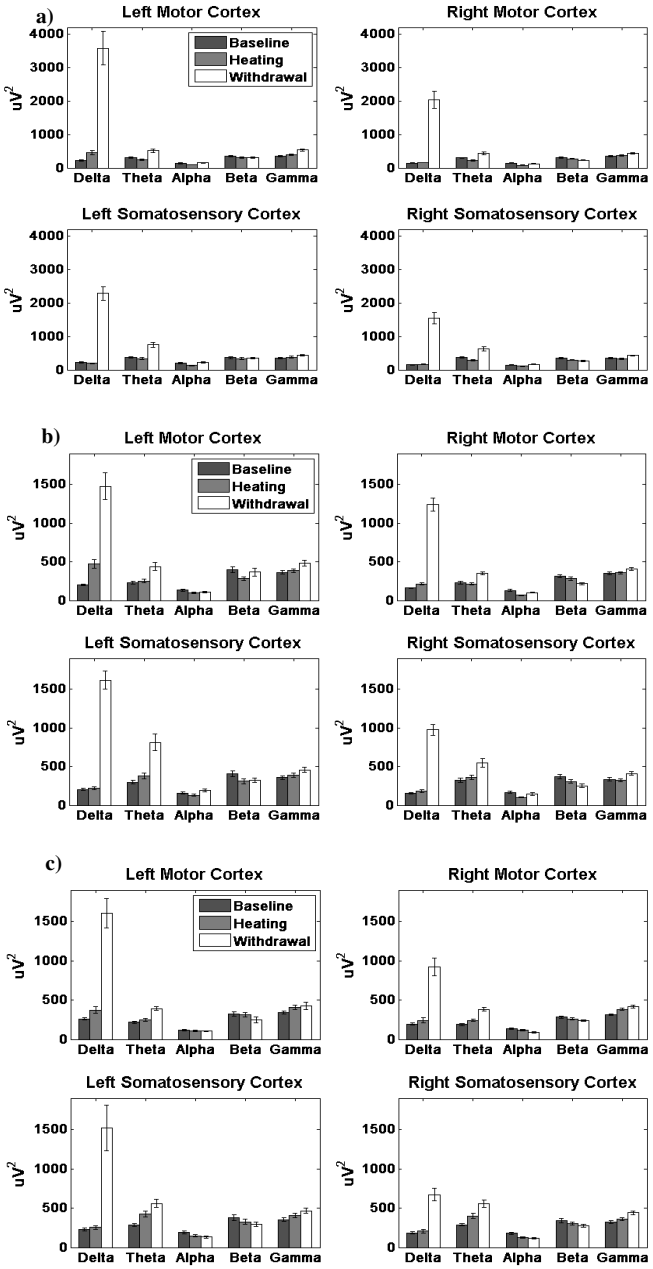

Fig. 3. Average power and standard error of the mean across five brain-wave frequency bands in each recorded area in the brain. The noxious stimuli were applied in the (a) left paw, (b) right paw, and (c) tail of the animal. Four animals were included in each group.

The power spectra of the signals were used to analyze the activities across each brain rhythm during the response to the thermal nociception. In summary, a Fast Fourier Transform (FFT) was performed on three, 2-second segments of each ECoG signal across all channels. The baseline segment, the segment just prior to the withdrawal, and the peak activity segment were served as the control, heating phase, and the pain withdrawal segments, respectively. Power values obtained from FFT were grouped into the delta (0–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), beta (12–30 Hz), and gamma (30–100 Hz) frequency bands. The average power and standard error of the means for each of the five bands were calculated from the data collected from all four animals under the same experimental protocol and shown in Fig. 3.

C. Statistical Analysis

Power spectra data were examined for the four recording channels in each of the five bands to identify areas of significant (p<0.05) changes in the neurophysiological signals for behaviors. Significant changes were tested by student's t-test to compare the mean of the obtained frequency bands in the three different segments. Activities in all frequencies across each channel produced no significant changes between the baseline and heating phase signals in any of the three thermal stimulation areas. However, significant changes in the delta rhythms of the withdrawal-segment signals were found compared to both baseline and heating segments, for all channels in the left and right paws but not in the tail (Table I). Significant differences were observed in low frequency (delta and theta) and high bands (gamma). However, only delta rhythms are shown in this paper since no other frequency bands produced consistent

TABLE I. POWER SPECTRA COMPARISON OF DELTA RHYTHM
M1L: motor cortex left; M1R: motor cortex right; S1HL: somatosensory left; S1HR: somatosensory right. P-value was compared to 0.05. The bold-font numbers demonstrate the significant differences.

Calculated *p-value*	Delta Rhythm			
Channel	*M1L*	*M1R*	*S1HL*	*S1HR*
Baseline vs. Heating				
Left paw	0.29	0.37	0.53	0.80
Right paw	0.14	0.54	0.74	0.53
Tail	0.31	0.49	0.76	0.76
Baseline vs. Withdrawal				
Left paw	**0.04**	**0.03**	**<0.01**	**0.02**
Right paw	**<0.01**	**<0.01**	**<0.01**	**<0.01**
Tail	**0.04**	0.07	0.14	**0.04**
Heating vs. Withdrawal				
Left paw	**0.04**	**0.03**	**<0.01**	**0.03**
Right paw	**<0.01**	**<0.01**	**<0.01**	**<0.01**
Tail	**0.04**	0.09	0.17	0.11

statistical difference upon withdrawal for all cortices in either paw or the tail.

IV. Discussion

This preliminary study compared the effects on brain wave patterns before and during subcutaneous thermal stimuli and demonstrated a distinct rise in the low-frequency activity. This occurred upon the sensation generated by noxious stimulation, possibly producing pain. A new method was introduced in this study involving the use of wireless electrocorticography to develop an experimental model capable of observing the effects of noxious stimuli upon awake, freely-behaving small animals. The wireless system grants an overwhelming convenience in acquiring the neurophysiological signals from both animal and potentially human subjects. In addition, it provides extra validity to the results, since the quantitative data can be further verified through coordinating the behavioral responses of the subjects.

Low-frequency, high-amplitude activities were observed in the time domain for the animal's responses to noxious stimuli. The thermal stimulus upon the plantar regions of both paws produced a significant increase in the delta activities during paw withdrawal. Activities remained constant across all brain rhythms during normal, baseline activity and the heating phase. This may indicate that although some level of nociception might be present, a significant alteration in cortical activity did not occur until the pain specifically reached a magnitude eliciting a purposeful reaction in animal behavior. Although rise in low-frequency activity was also observed in thermal stimulus on the tail, no significance was found to occur in the somatosensory cortex from these trials. Whereas both paws were consistently planted firmly and easily withdrawn upon pain, the tail did not react as uniformly due to the variance in positioning between different rats across multiple trials. This may have contributed to the weaker responses of activities from the thermal stimulus on the tail.

Overall, these findings indicate that ECoG can potentially serve as a potent means for studying nociception in controlled experiments. Future studies will employ a similar model to further explore the effects of thermal as well as mechanical and chemical stimuli in small animals.

V. Conclusion

Thermally induced noxious stimuli produced observable responses in the cortex of small freely-behaving animals with wirelessly recorded ECoG signals. The ECoG signals in the frequency domain indicated a sharp rise of power in the low-frequency bands, particularly the delta rhythm, during the response to a noxious thermal stimulus, verified by behavioral observation. It is thus believed that the observed high-amplitude, low-frequency signals may be associated with the sensation of pain in the rat. The wearable wireless module provides a new experimental method to collect quantitative neurophysiological signals *in vivo* on awake, freely-behaving animals while behaviors can be recorded and correlated with the signals. Additional experiments will be needed to further analyze and affirm the details of the ECoG signals toward specific noxious stimuli.

Acknowledgments

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