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Multi-Channel Recordings of the Motor Activity From the Spinal Cord of Behaving Rats

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Abstract

The objective of this study was to extract voluntary motor signals from the rubrospinal tract in behaving rats and study the correlation between these volitional signals and the elbow movements. Multi-contact silicone substrate surface electrodes were implanted chronically at the cervical C5–C6 and C6–C7 levels of the spinal cord. Recordings were made during face cleaning by the animal as this task involves cyclic movements of the forelimbs and does not require any training. Temporal correlation was performed between the recordings of the proximal pair and the distal pair of contacts within a sliding window to determine whether the signals were sensory or motor. Linear regression technique was used to reconstruct the arm movement from the rectified-integrated version of the neural signals. The preliminary data show that motor signals can be successfully recorded from the cervical region of the spinal cord in behaving rats and reconstruction of elbow movements from the neural signals is feasible using these non-penetrating surface electrodes.

Keywords

rubrospinal tract (RST); brain-computer interface (BCI); lateral corticospinal tract (LCST); spinal cord injury (SCI)

I. Introduction

Injury at the cervical region of the spinal cord results in quadriplegia. Brain Computer Interface, which has demonstrated significant success in the past [1–3], is one way of generating a substitute for the lost command signals in quadriplegic individuals. In this project, as an alternative, we are attempting to record the voluntary motor signals from the descending tracts of the cervical spinal cord.

In humans, the lateral and the medial descending systems send down voluntary control signals to the spinal cord. The medial descending system runs in the ventral funiculus of the spinal cord and mainly controls the axial and proximal limb (girdle) muscles. The lateral system runs in the dorsolateral funiculus of the spinal cord consists of two major tracts: the LCST and RST, which mainly provides the skillful control of the distal limb musculature.

In rats, the normal crossed CST runs in the ventral most portion of the dorsal funiculus white matter [4]. The uncrossed CST is small in the cervical region and cannot be traced below mid-thoracic levels [5]. The RST originates from the magnocellular division of red nucleus [6–7], decussates in the ventral mesencephalic tegmentum, after which the axons descend contralaterally through the dorsolateral funiculus along the entire spinal cord. RST axons have been shown to project ventrolaterally to a specific motoneuron population of intermediate and distal muscles of the limbs [6]. Both the CST and RST have been shown to

be involved in the control of distal, intermediate, and proximal musculature for skilled forelimb movements in rats [8–11].

In this project, we are attempting to record the motor signals from the RST as a means of generating voluntary command signals. This preliminary report demonstrates the feasibility of the approach with recordings from the cervical spinal cord in rats using multi-contact electrodes implanted subdurally. Our future work will involve recordings from the CST also for comparison of results.

II. Experimental set-up

A. Electrodes

Multi-contact silicone substrate electrodes shown in Fig. 1 were prepared in our laboratory. Four 25 μm thick platinum contacts of size 0.5×0.5 mm were welded to multi-strand, 0.0055in. thick, Teflon coated, stainless steel wires (A-M Systems Inc., WA), pressed between two layers of 50 μm thick silicone sheet using a vice, and cured at 150° F for 3 hours. The dimensions of the electrodes with the silicone substrate were 2×2 mm with a thickness of 200 μm . A small square window of 0.2×0.2 mm was opened using a scalpel under microscopic vision to expose the contact surface. The electrode substrate was pre-shaped to conform to the surface of the cord as shown in Fig. 3. All electrode wires were soldered to a micro-connector (H2747-ND, Digi-Key Corporation, MN, USA), that was sutured to the animal's skin on the back.

B. Implantable Length Gauge

Implantation of a saline filled sealed rubber tube anchored to bone or tendon at its ends is a simple and convenient way of monitoring length changes between two points [12–13]. The length gauge shown in Fig. 2, prepared in our laboratory, consisted of a Teflon coated 7-strand stainless steel wires inserted in the silicone rubber tube filled with hypertonic saline, tied at both ends using size 4.0 silk sutures and sealed with silicone.

C. Surgical Procedure

Three Long-Evans male rats, weighing between 300–350 grams were used in this study. The surgical procedures were approved by the Animal Care and Use Committee at Louisiana Tech University. A sterile surgery was performed to implant the spinal cord electrodes and elbow sensor, shown in Fig. 2. Rats were anesthetized with pentobarbital sodium, (Nembutal, 30 mg/kg, IP) and deep anesthesia was maintained by administering additional doses of 0.2 mg/kg (IP) as needed. Dexamethazone (2 mg/kg, IM) was administered at the beginning of the surgical procedure to prevent secretions in the respiratory airways and CNS edema. Dorsal laminectomy was performed at the C5–C6 and C6–C7 levels to expose the spinal cord. The dura was cut using a 26g needle above the dorsal root entry zone at C6–C7 level of the spinal cord. The spinocerebellar tract, which lies just underneath the cord surface above the RST, was severed with a scalpel in the medio-lateral direction. An electrode was implanted by sliding it underneath the dura at C6–C7. Another electrode was implanted at C5–C6 following a similar procedure. Fig. 3 shows the positioning of the electrode contacts on the arachnoid surface. A large Ir reference electrode was also placed on the spinal cord. The electrodes were then covered by the transected dura and fixed in their respective positions using octyl cyanoacrylate tissue adhesive (Nexaband, WPI, Inc, FL).

The upper limb of the animal was shaved and an incision was made on the dorsal side for implanting the length gauge sensor. A fine hole was drilled on the humerus and the radial bone. The sensor was attached to these bones at the holes with sutures. After implantation,

the skin of the animal was closed using absorbable sutures and the wires of the sensor were tunneled on the back of the animal to a micro-connector header (H2761-ND, Digi-Key Corp., MN) for making electrical connections. Antibiotic ointment and analgesic (Nubain, 0.1 mg/kg) were used daily until the wounds healed completely.

D. Recording Procedure

The rats were allowed to move freely in a transparent box during the recording session. Face cleaning behavior with the forelimbs were studied for preliminary studies as they were easily generated without training. Recordings were made from the four contacts of each electrode and the elbow sensor simultaneously using a Grass amplifier (Warwick, RI). The Grass amplifier was interfaced with the computer through a National Instruments data acquisition card (NI-DAQ PCI-6071E) and the signals were acquired into the computer using LabView (National Instruments) data acquisition software. The gain for all channels was 5000; the sampling rate was 40 kHz; and the filtering range was from 100 Hz–6k Hz. All the channels were differentially configured with respect to the reference electrode placed on the surface of the cord.

The animals were terminated after a month of recording time. The implanted section of spinal cord was removed for histology and anatomical verification of the electrode position.

III. Data analysis

Data analysis was performed using MATLAB. The power spectrum of the raw signal was first analyzed for the frequency content to determine if the signals contained significant neural component. The signal from each lateral contact was subtracted from that of the corresponding medial contact. Thus, a total of four channels were produced from the eight contacts in two electrodes. A bandpass filter (100 Hz–4 kHz, FIR) was applied to the signals on the computer to remove some of the noise component. Correlation analysis was performed between the proximal pair and the distal pair of contacts in each electrode within a 2 ms sliding window. A 2 ms duration of the signal from the proximal pair was taken at a time and shifted 500 μ s back and forth and compared to the distal channel to find the point of maximum correlation and the delay at which it occurred. Then the window leaped to the next 2 ms. This process was repeated for the entire duration of the signal. As a result, two matrices were formed: one for the values of maximum correlations and the other for the delays at which these maximum correlations occurred. Correlation value less than 0.7 were rejected. A histogram of the delays was plotted (Fig. 6).

The flow diagram for the reconstruction of the elbow signal from the neural signal is shown in Fig. 5. Principal component analysis was performed on the filtered neural signals to find the principal components. The elbow signal was filtered using a combination of elliptic and butter worth filters; their combined passband being either 0–8 Hz, 4–8 Hz, or 0–4 Hz, respectively. The same filter was then used for the elbow signal and integration of the rectified principal components of the neural signals. Linear regression technique was used to reconstruct the elbow signals from the neural signals. A positive time delay of less than 100 ms in the elbow signal following the neural signal was considered as motor activity. Reconstructions that occurred for a delay greater than 100 ms were discarded.

IV. Results

Electrode contact impedances were measured before (in normal saline) and after implantation at 2 kHz. The electrode impedances were in the range of 10–20k and did not change substantially post-implantation, indicating that the contacts were intact and in place. The recordings show a clean signal with high signal-to-noise ratios. The action potential

amplitudes were as large as 10 times the rms value of the baseline noise, which was in the order of 40–60 μV (Fig. 4).

Fig. 5 shows a histogram of the delay values measured for the maximum correlation points found. Fig. 5A is a histogram of the delay values for a signal recorded from the C5/C6 electrode and Fig. 5B shows the histogram for the delay values for a signal recorded at the C6/C7 level. An area of histogram under positive delay shows that an activity occurs at the proximal contact first and later at the distal contact, thus, exhibiting that the signals recorded is motor in nature. The histogram also shows the presence of sensory activity observed by the peaks for a negative time delay. This suggests that there is some contribution from the sensory fibers at both levels.

It can also be concluded from the histogram that the average velocity with which a motor signal travels between the two contacts (proximal to distal) is less than 10 mm/msec, considering the inter-contact distance in the electrodes is 1.5 mm (1.5 mm divided by 150 μs).

Reconstruction of elbow signals from the neural signals extracted from the RST is shown in Fig. 6 for the frequency ranges of 0–8 Hz, 4–8 Hz, and 0–4 Hz. It can be observed from the plots that the reconstructed trace (dashed line) obtained as a result of linear regression follows the general trend in the filtered elbow signal (solid line). The mean and standard deviation of the correlation values for the 13 episodes tested were 0.70 ± 0.07 for 0–8Hz, 0.76 ± 0.07 for 4–8Hz, and 0.78 ± 0.14 for 0–4 Hz.

V. Conclusion and discussion

The results of this study demonstrate that descending motor signals can be extracted from the cervical spinal cord in behaving rats. The results also suggest that elbow movement can be reconstructed from the neural signals recorded from the cervical cord. Multi-contact silicone substrate electrodes can be successfully used to record the motor signals subdurally from the spinal cord surface. These electrodes are non-penetrating and minimally invasive. Analysis of the spinal cord section and the surrounding tissue after a month's period showed that the electrode were still intact and in place. There was no change in the morphological structure of the spinal cord. The correlation technique, suggested by other groups in the past [14], was successful for separation of motor and sensory activities in this study. Cutting the spinocerebellar fibers must have eliminated a significant amount of sensory activity in the recordings since this ascending tract is located close to the cord surface above the RST fibers. However, the histogram in Fig. 5B shows two peaks suggesting the presence of both motor and sensory activity. Although the sectioning of the spinocerebellar tract was performed at the lower edge of the C6/C7 electrode, there is contribution from the ascending fibers into the recorded signals. Higher correlation between the elbow signal and the neural signals may be achieved if the sensory signals are eliminated completely. This may also improve the reconstruction process of the elbow signals. The sensory contamination should not be a problem in patients with SCI as the ascending fibers would be severed due to the injury. Differential recording helped to eliminate the common-mode signals and emphasize the local activity underneath the contacts from the dorsolateral funiculus.

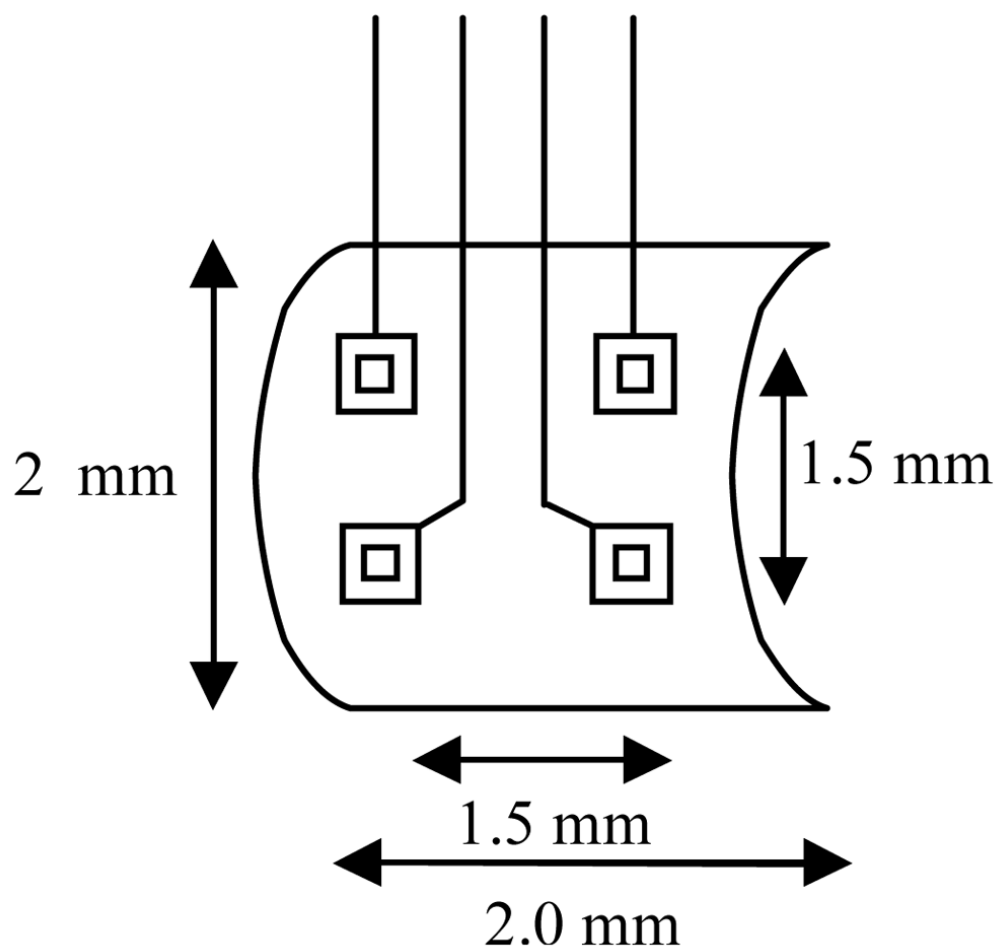
Future work includes increasing the number of contacts and thus the number of neural channels and to apply the same paradigm to CST as well.

Acknowledgments

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**Fig. 1.**

Multi-contact silicone substrate electrodes chronically implanted for subdural recordings of the spinal cord motor activity. The final electrode thickness is about 200 μm and it is pre-shaped to conform to the curvature of the spinal cord surface.

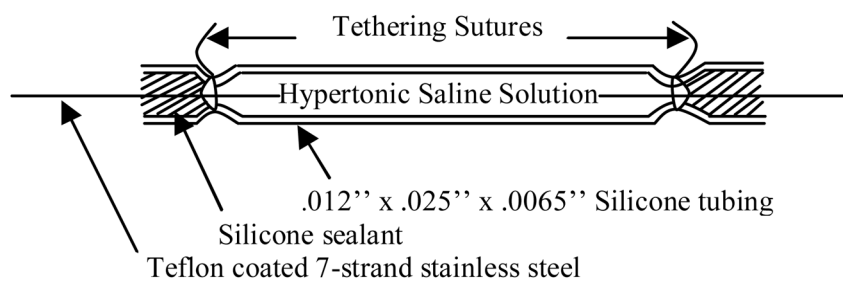


Fig. 2.

Construction of the implantable length gauge. The tethering sutures are used to seal the conductive fluid inside the distensible tubing. Silicone sealant adds reinforcement to the sealed tube.

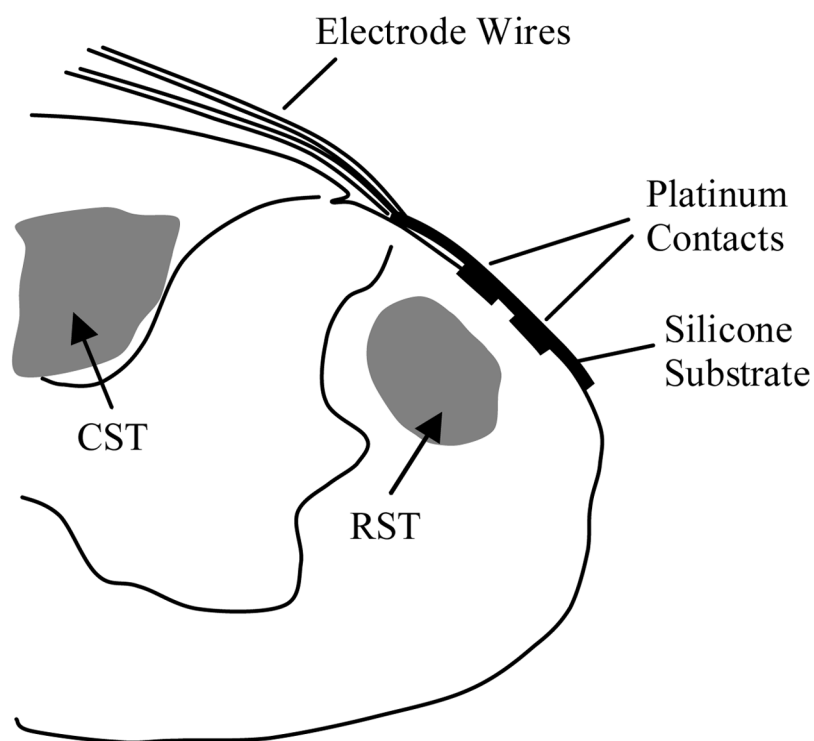


Fig. 3. Positioning of the electrode contacts on the dorsolateral funiculus of the cervical spinal cord in rats. The assumed position of the descending tracts is also shown. One electrode is placed between the spinal roots C5–C6 and another between C6–C7.

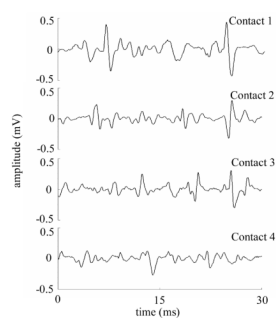


Fig. 4.

A short episode of raw signals recorded from the four contacts of the electrode implanted at C5/C6 border. Contact numbers are 1 through 4 from top to bottom as indicated. Raw amplitudes are shown after dividing the recorded signals by the amplifier gain.

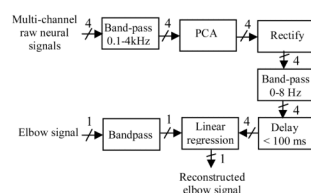
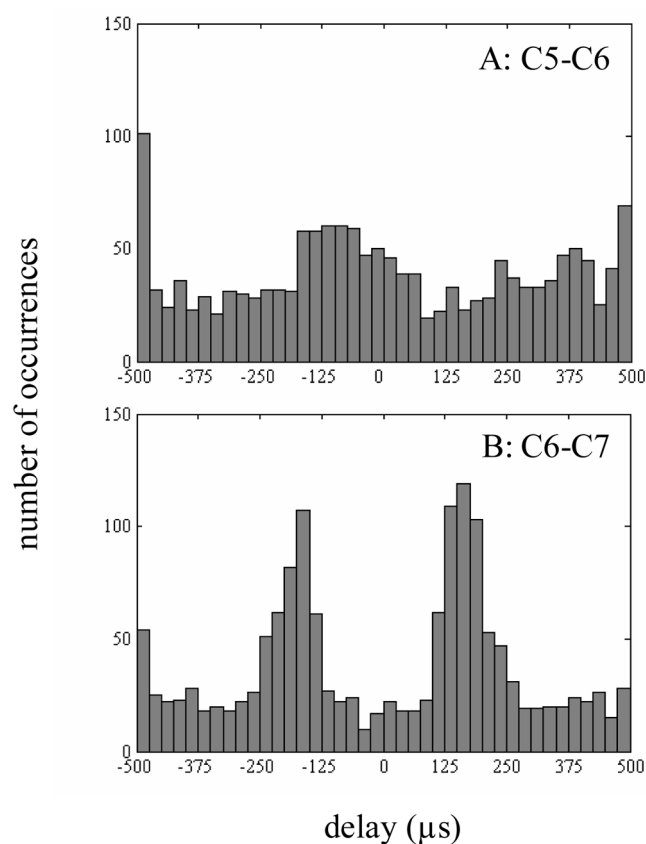


Fig. 5.

The flow diagram for the reconstruction of elbow signal from the neural signals.

**Fig. 6.**

Histogram of the delay values for which the maximum correlations are obtained between the proximal and distal contact pairs for the A: C5/C6 implant and B: C6/C7 implant. Positive delay indicates motor activity. The top figure shows the histogram of the signals from the C5/C6 segment and the bottom from the C6/C7 segment.

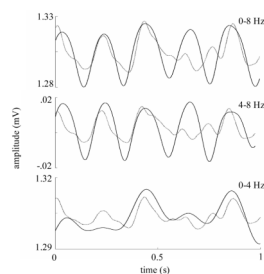


Fig. 7. Reconstruction of the elbow signal using RST signals for the filtering range of 0–8 Hz, 4–8 Hz, and 0–4 Hz for the same episode during face cleaning behavior. The solid trace is the filtered elbow signal and the dashed trace is its reconstructed version from the principal neural components of the RST signals.