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# Collagenase-aided Insertion of Intracortical Microelectrode Arrays: Evaluation of Insertion Force and Chronic Recording Performance

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***Abstract*—Typically intracortical electrodes are required to puncture the intact pia mater during insertion which in the process can lead to brain dimpling and trauma. Furthermore, there is interest in the development of more flexible substrates to reduce relative micromotion after implantation, but such device have difficulty penetrating the pia without buckling. In this paper a strategy for reducing the mechanical integrity of the pia's collagen network by treatment with collagenase is evaluated experimentally. Measurements of the insertion force were carried out with a load cell during computer controlled slow (10μm/sec) electrode insertion into the cortex of rats. It is shown that controlled application of collagenase reduces the peak insertion force experienced by the microwire arrays around 30% on average. In addition, chronic neural recordings (up to 1 month) suggest that there is no appreciable difference in the signal quality as recorded from the collagenase treated and the control sites. The results suggest the technique is useful for reducing insertion forces without compromising neural recording capabilities.**

## I. Introduction

Chronic neural interface technology is important for both basic science and neuroprostheses. Varied solutions are available: bundles of microwires [1, 2], micro-machined tines of silicon [3], and planar multi-site/shank structures [4]. More advanced solutions include embedded microchannels for chemical delivery, on-board signal processing, and micropositioning capability [5-7]. Despite decades of development however, implantable electrodes still do not record neural activity indefinitely. Many sites within an array may never record neural unit activity [8].

Leading hypotheses for failure to record neural activity are encapsulation (scar tissue) and loss of neurons due to the insertion trauma and device presence [9, 10]. The early response (week 1) is thought to be influenced by insertion trauma [9], while the sustained response (>4 weeks) is likely governed by tissue-device interactions. Evidence also suggests the altered microenvironment at the interface may trigger neural death [10]. It is hypothesized that relative micromotion, accentuated by the mechanical impedance mismatch between the brain and device, aggravates the problem. To reduce this effect several groups are developing flexible polymer-based probes [11, 12]. Since the insertion force required to penetrate the brain is on the order of several mN [13, 14], insertion of flexible probes has been challenging [12].

The meninges (*dura* and *pia mater*) are the main structural barriers to intracortical microelectrode insertion. Due to its thickness the dura is usually surgically pierced or excised prior to insertion. This can be done with minimal damage to the brain because the dura is separated by a cushion of cerebral spinal fluid (CSF). In contrast, the much thinner pia layer forms an intimate boundary between blood vessels and the subarachnoid space below the dura making surgical removal nearly impossible [15]. The tips of the electrode array therefore must puncture the pia during the insertion process. This can lead to significant brain dimpling and potential trauma before enough pressure develops to break through. To reduce this effect, some implant systems have adopted a fast or high impact insertion strategy [3]. While this may reduce dimpling, slower insertion speeds might be better to allow blood vessels and brain tissues to accommodate the implant. Furthermore fast insertion requires the electrode to withstand greater insertion forces which is not amenable to flexible structures. An alternative solution may be to reduce the mechanical integrity of the pia.

At a microscopic level, the pia is primarily composed of flattened connective tissue cells and collagen fibrils with varying anatomical thickness [15]. Bundles of collagen are also present in the dura and less so in the arachnoid layer [16]. The application of collagenase has been proposed in the literature [17] to "thin" the pia and therefore may reduce insertion forces and dimpling. It is thought that collagenase acts to breakdown the fibrils by unwinding the collagen triple helix and hydrolyzing the peptide bonds. This action may weaken the extracellular matrix of the pia [18].

In this study, the ability of collagenase-aided microelectrode array insertion to reduce insertion force is quantitatively evaluated. A custom load-cell system measured insertion force during microwire array penetration of rat cortex. The results suggest a consistent and significant reduction in the peak insertion forces without impairing the ability to record neural activity.

## II. Materials and Methods

### A. Surgical Procedures

All animal procedures followed NIH Guidelines for the Care and Use of Animals and approved by the Penn State IACUC committee. Acute experiments were performed on 6 male Sprague-Dawley rats (~350g). Seven additional rats

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(~450g) were implanted chronically for 4-week recording observation.

Subjects were anesthetized with ket/xyl/ace (50:5:1 mg/kg) and monitored via pulse-oximetry. Subjects were placed in a stereotaxic frame and the cranial plates were exposed. For acute experiments, craniotomies were created at 2-4 mm lateral to the midline at two anterior and two posterior sites located 2-4 mm anterior/posterior of bregma, respectively (see Fig. 1). One site each from the anterior and the posterior sites were chosen for treatment with collagenase enzyme. The collagenase mixture was the same as that described in [17] (20 mg/mL collagenase contained in 0.36 mN $CaCl_2$ in 50mM Hepes buffer with equal amount of KY jelly). For control sites, the same mixture minus the collagenase enzyme was applied. Just prior to treatment, the dura was pierced with a 27G hypodermic needle and further opened with micro-scissors to accommodate the electrode array. A small Gelfoam™ sponge soaked in the designated mixture was placed over the implant site for ~15 minutes. Prior to electrode insertion all the sites were thoroughly rinsed with saline. Chronic implantation procedures were essentially identical except that only the two anterior sites were implanted (1 control/ 1 treatment). In these animals, bone screws were placed in the cranial plates and an acrylic headcap was created to hold the electrode connectors.

### *B. Electrode Fabrication and Insertion*

The electrodes were composed of eight 50µm tungsten microwire insulated with polymide (California Fine Wire) arranged in a 2x4 array. The recording tips were blunt and maintained a separation of ~250 µm with islands of dental acrylic (similar to [1]). The microwires were connected to a small PCB interface board which was soldered to a miniature connector. A stainless steel ground wire was tied to one of the bone screws to serve as ground for neural recording.

The force experienced by the electrode array during insertion was monitored by attaching the array to a load-cell coupled to an in-line amplifier (Honeywell Sensotec model MBL 25gms; 0.1% FS linearity; 0.03% FS repeatability). The system was calibrated to produce an output of 5V for a force of 245.25mN (25gms). Despite working in the lower 5% portion of the dynamic range, the sensor was tested with calibrated weights and found to be linear and accurate down to a load of 0.049mN (5mg). The analog output of the in-line amplifier was simultaneously monitored on a digital multimeter and acquired and stored on a computer for subsequent analysis.

The entire assembly consisting of the load-cell and the microwires was manually lowered toward the craniotomies using a 3-axes micromanipulator. Once a deflection was registered with the digital multimeter, the electrodes were retracted by approximately 50µm to ensure that the tips were close but not touching the brain. For insertion, a custom-built computer controlled stepper-motor setup was used to drive an oil hydraulic micromanipulator (Narishige MWO-3). This action directed the microwire array at a constant speed of ~10µm/sec into the cortex (similar to [2]). The final depth of insertion for acute studies was 2mm to ensure complete penetration of the electrodes. The final depth for the chronic studies was restricted to 1mm to enable recording from cortical layer V of primary motor cortex.

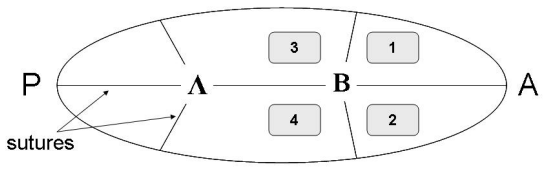


**Fig. 1: Top view schematic of skull showing craniotomy sites. A=anterior; P=posterior; B=bregma. Chronically implanted subjects only received implants at sites 1 and 2.**

### *C. Neural Recording and Data Analysis*

A commercial multi-channel acquisition system (Tucker-Davis Technologies) was used to collect simultaneous neural recordings from the electrodes of chronically implanted animals. Signals were digitally band-pass filtered 300-5kHz, down-sampled at 12kHz and streamed to disk for offline analysis. Recordings were carried out in regular sessions with the animal either awake or lightly sedated with isoflurane. During each session, a 5-minute block of recordings was obtained from each of the implanted arrays. The electrode impedances were also measured at 1kHz with a Bak™ electrode impedance meter.

Initial screening of data was made with OpenScope (Tucker-Davis) with subsequent analysis handled in Matlab (Mathworks, MA). Signals were pre-processed to eliminate periods of prevalent mastication artifact (>300µV). Neural spikes were threshold-isolated and extracted as waveform segments (2-ms in duration) exceeding 3 standard deviations of the raw signal.

## III. RESULTS

### *A. Insertion Forces*

*Acute Experiments.* Acute experiments were conducted in 6 rats to examine the effects of inter-electrode spacing, anatomical location, and collagenase application on insertion force. It was found that the insertion forces were consistently higher for the posterior sites (sites 3 and 4 of Fig. 1) as compared to the anterior sites regardless of the number of electrodes in the array or their spacing. It is therefore important for the control site to be located at the same anterior/posterior location as the test site. Partly due to the small sample size, the effect of inter-electrode spacing on insertion force was less conclusive and not considered in this paper (all data presented from arrays with ~250um spacing).

Multiple peaks in the insertion force vs. depth curves were commonly observed; especially for the anterior insertion sites (see Fig. 2A). It was assumed that the initial peak was caused by penetration of the array through the pia, while the second peak was most likely caused by drag forces from surrounding tissue. Since this study was concerned with the effect of collagenase treatment on the pia layer, the magnitude of the first peak (typically the largest) was taken as the peak insertion force. Comparing within subjects, the

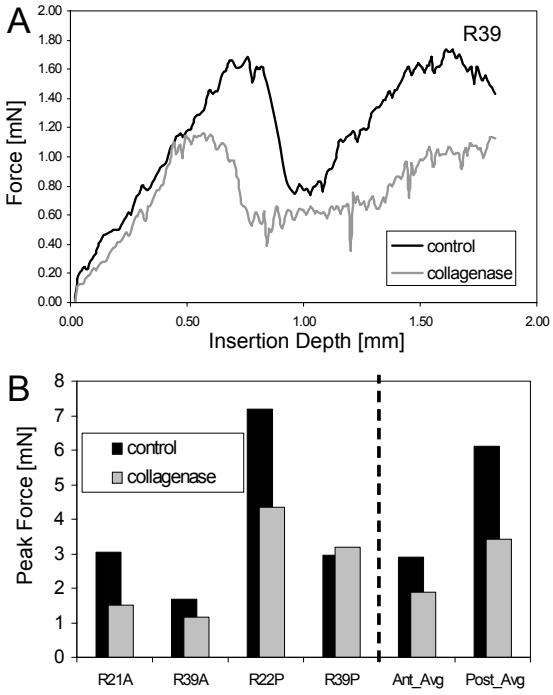


**Fig 2. A: Representative force-distance curve for 2x4 array insertion in an acute animal. B: Summary of peak insertion forces for acute experiments. "A" and "P" indicate anterior and posterior sites, respectively. Right of vertical dashed line are the averages for all anterior and posterior sites in the study (N=3 for each).**

collagenase-aided site on average exhibited 28% lower peak insertion forces vs. un-treated site (Fig. 2B). In addition, the dimpling (taken as penetration distance at peak penetration force) appeared to be reduced.

*Chronic Experiments.* Except for R47 (data not shown), none of the animals showed a clear peak in the force-distance curve for the 1mm insertion depth (Fig. 3A). However comparing within subjects the forces measured were lower for the collagenase-treated site throughout the insertion run compared with the contra-lateral control site. Since peaks did not always occur, two measures were extracted from the force-distance curves for comparison purposes: max insertion force and slope of best linear fit through the force curve. Similar to acute results, the average slope of the force curve and the average max force was reduced by 36% and 32%, respectively for the collagenase treated sites in the same animal (Fig. 3B). The overall mean and standard deviation of the max insertion force for control and collagenase were 3.65 ± 0.93 mN and 2.45 ± 0.56 mN, respectively ($p<0.04$, unpaired 2-tailed t-test); slopes were 4.22 ± 1.27 N/m and 2.57 ± 0.22 N/m, respectively ($p<0.05$, unpaired 2-tailed t-test).

### *B. Neural Recording Performance*

A brief summary of recording performance results is shown in Fig. 4. In general, a majority of electrodes in both collagenase and non-collagenase implant sites recorded neural activity over the 4 week duration. A slightly greater proportion of electrodes implanted in the collagenase treated sites recorded spikes greater than 100μV p-p. Immediately following surgery the percentage of electrodes with neural spikes >100μV p-p was found to be 82% for collagenase sites as opposed to 69% for control sites. Over the duration of recordings the percentage of electrodes that met this criterion was 56% for collagenase treated sites vs. 45% for controls. Thus it is clear that the collagenase treatment does not seem to affect long term recording performance and may in fact enhance it.

## IV. Discussion and Conclusion

The penetration forces experienced by standard micro-wire implants were quantified in this study with and without application of collagenase to the pia surface prior to insertion. Insertion forces were consistently lower for collagenase treated sites (~30% lower on average; within subject comparison). The results are significant considering the relatively crude control over the micro-wire electrode arrays (hand-fabricated) and the inherent variability in surgical technique from implant to implant. This reduction in force may allow insertion of more flexible probes or those with a smaller cross-sectional area (lower buckling force).

The range of pia insertion forces observed here are in agreement with those reported in the literature [13, 14]. A surprising discovery was the strong influence of anatomy. In particular the posterior insertion sites consistently produced higher force readings. The reason for this may be due to

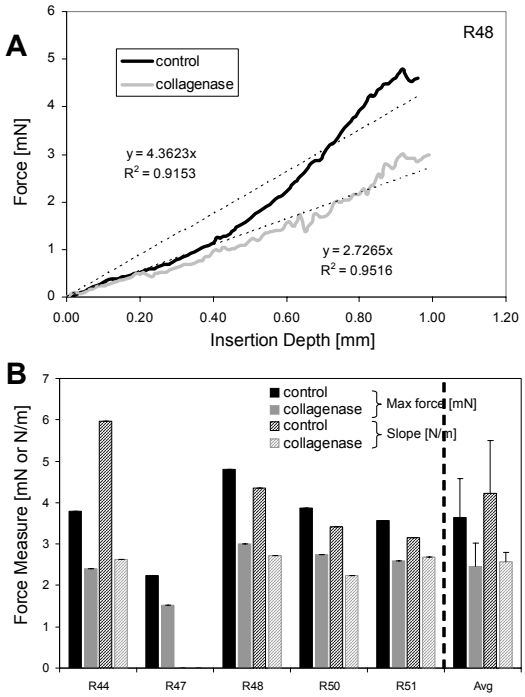


**Fig 3. A: Representative force-distance curve for 2x4 array insertion during chronic implant. B: Summary of max insertion forces and slope for chronic implants. Only anterior sites were implanted. Right of dashed line: average and standard deviation for all subjects.**

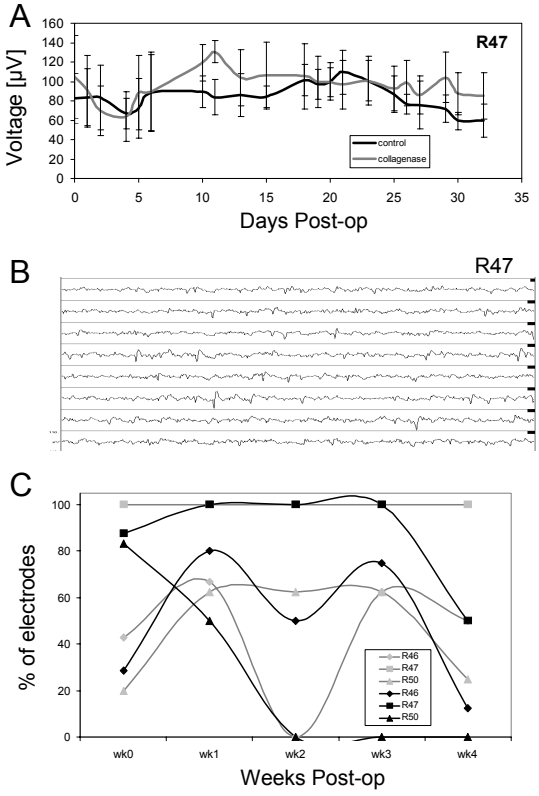


**Fig 4 A: Magnitude (p-p) of mean spike waveform over time in one subject (R47). B: Sample of recordings on final day from collagenase treated site in same animal. C: % of electrodes which recorded neural spike >100µV p-p over duration of implant. Solid black lines: controls; Dashed gray lines: collagenase treated sites.**

variations in pia thickness [15].

The fact that peaks were not observed during chronic implant insertions suggests the electrode array did not penetrate the pia by the end of electrode advancement. This was further supported by visual inspection through the surgical microscope. The reason for this apparent discrepancy with the acute results is not clear. One variable may be differences in age among the groups of animals. Also since the electrodes were only advanced 1mm, direct comparison with the acute results is difficult. The authors are confident the pia was likely pierced by end of surgery because neural recordings were consistently obtained immediately following surgery.

The electrophysiological recordings for the treated and the untreated sites were comparable. In fact, on most the recording days the collagenase treated sites showed better signal-to-noise ratios and recorded higher spike amplitudes. This may be attributed to reduced dimpling and the accompanying damage during the initial insertion process.

Future studies will carry out histological analysis to compare any tissue differences that may have resulted from the initial treatment. The study could be expanded to include a variety of flexible and stiff neural probes, as well as the inclusion of other enzymes like elastase, dispase and their cocktails.


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