

# DEVELOPMENT OF AN ANATOMICALLY CONFORMAL PARYLENE NEURAL PROBE ARRAY FOR MULTI-REGION HIPPOCAMPAL RECORDINGS

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## ABSTRACT

We report the first Parylene C neural probe array that is anatomically matched to multiple regions of the hippocampus and successfully demonstrate recordings from targeted regions. No prior polymer probe array has achieved recordings at the depth of the hippocampus (5.5 mm probe length) and at such a high electrode density (64 electrodes). In addition, bare probes were implanted without the use of any insertion shuttles thereby enabling, for the first time, acquisition of dense neural information from deep brain targets of interest while restricting acute tissue disruption to the as-fabricated probe cross-section. Here, we present design and fabrication of the Parylene neural array, its electrical packaging scheme, and acute, *in vivo* recordings from pyramidal cells of both the cornu ammonis (CA) 1 and CA3 regions, essential players in the one-way hippocampal circuit that encodes memory.

## INTRODUCTION

Parylene C, a USP class VI polymer recognized for its biocompatibility, is an attractive MEMS material for implantable devices and especially neural probes [1]. Soft, flexible substrates can mitigate the immune response caused by micromotion between brain tissue and probes constructed from rigid materials such as metal and silicon, thereby improving the quality of chronic recordings [2-4]. However, polymer neural probes do not have sufficient stiffness required for implantation and require bulky insertion shuttles or overcoats which greatly increase acute tissue trauma and have effectively limited their application to superficial recordings in the cortex (< 3 mm deep) [2, 5, 6]. We achieved minimally invasive, deep brain implantation of bare probes into the hippocampus by employing a biodegradable brace that temporarily shortened the effective shank length, thereby achieving the required transient increase in stiffness while maintaining the as-fabricated probe cross-section [7].

The hippocampus plays a role in the formation of long-term memories via a one-way neuronal circuit that is formed from the dentate gyrus, CA1, and CA3 [8]. Simultaneous recordings from neurons in these different layers of the hippocampus can provide a mechanistic understanding of how memory is encoded. This is not only important for basic neuroscience research, but has implications on neural prostheses for memory. Such a prosthetic could repair lesions in the neuronal circuit by recording input from one region, using external circuitry to reproduce the function of the damaged area, and then stimulate the appropriate effectors, downstream from the site of injury. Hippocampal prostheses have the potential to restore function in diseases such as Alzheimer's, in which hippocampal damage interrupts memory formation.

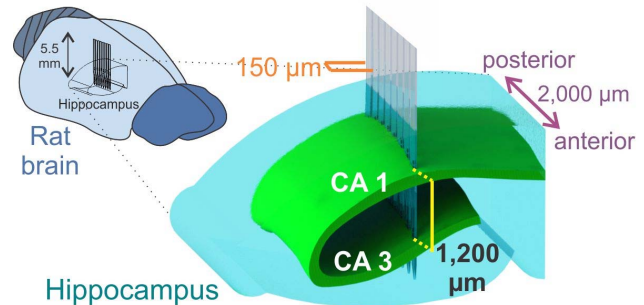


Figure 1: Diagram of Parylene neural probe array, with eight probes designed to match the variegated laminar anatomy of the hippocampus as it changes in depth anteriorly to posteriorly. Probes were 5.5 mm long, 28  $\mu\text{m}$  thick, and 110-150  $\mu\text{m}$  wide. Two groups of four platinum electrodes (30  $\mu\text{m}$  exposed diameter) target the CA1 and CA3 hippocampal layers.

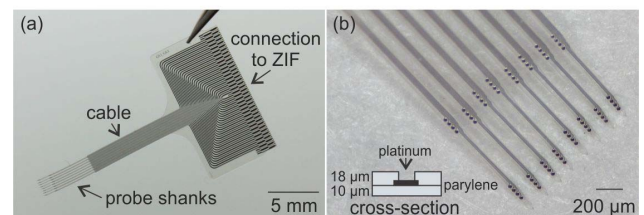


Figure 2: (a) Photograph of fabricated array and (b) close-up of electrode groups with inset drawing of cross-section through recording site.

## DESIGN

The electrode layout of the probe array was anatomically matched to acquire simultaneous recordings from the CA1 and CA3 layers and to span the 2,000  $\mu\text{m}$  anterior-posterior distance along a rat's hippocampus (Figure 1) [9] when fully implanted. These microprobe arrays, consisting of a Parylene-metal-Parylene sandwich, contained eight probe shanks that were 5.5 mm long with 250  $\mu\text{m}$  center-to-center spacing. Probe shanks were 28  $\mu\text{m}$  thick, 110-150  $\mu\text{m}$  wide, and were patterned with two four-electrode groups having 30  $\mu\text{m}$  diameter exposed platinum (Pt) areas (Figure 1 and Figure 2).

Individual shanks were collected into a flat, flexible Parylene ribbon cable with an enlarged contact pad region sized to mate with a 71 pin zero insertion force (ZIF) connector mounted to printed circuit board (PCB) 1. Two PCBs were used to minimize the load on the rat's head. PCB 1 served as an adapter containing an SSB6 receptacle and was designed to be permanently dental cemented to the rat's cranium. PCB 2 connected to PCB 1 via an SSB6 plug and facilitated connection to two 32-channel Omnetics plugs required by the Plexon electrophysiological recording system (Figure 3). PCB 2 was designed to be removable: it was connected to PCB 1

during recording experiments and disconnected during rest periods.

To successfully insert the probe array into deep brain targets, the flexible polymer probe must be reinforced to increase stiffness. Our strategy entails exposing approximately half of the length of the probes during the initial stage of insertion by embedding the base of the shanks in a biodegradable brace. This enables probe penetration into brain tissue without buckling and without an increase in cross-sectional area, thereby minimizing acute tissue damage caused during implantation.

## FABRICATION AND IMPLANTATION

A 10  $\mu\text{m}$  thick layer of Parylene C was deposited onto a carrier silicon wafer. Image reversal photoresist AZ5214 was patterned to define electrode, electrode trace, and contact pad locations. 2,000  $\text{\AA}$  of Pt was deposited via sputtering and excess metal was lifted off in an acetone bath heated to 50  $^{\circ}\text{C}$ . An 18  $\mu\text{m}$  Parylene insulation layer was deposited followed by lithography to define an etch mask. Then the openings for the electrode sites and contact pads, and the outline around each array were etched away by  $\text{O}_2$  plasma-based switched chemistry etching in a deep reactive ion etcher. Arrays were lifted off from the silicon carrier wafer by wetting with water and gently peeling off devices with tweezers.

Post-processing of arrays included cyclic voltammetry (CV) cleaning, electrochemical impedance spectroscopy (EIS) measurements, encapsulating the arrays in a biodegradable brace, and electrical packaging. CV of devices in  $\text{N}_2$  purged  $\text{H}_2\text{SO}_4$  from -0.2 to 1.2 V was used to clean the electrode surfaces from any residual scum that may have marred the surface during fabrication and handling of devices. EIS measurements were used to confirm proper function of electrodes at frequencies of 1 kHz and to identify faulty traces.

After electrochemical testing, arrays were sandwiched between two layers of polydimethylsiloxane (PDMS) while resting atop an acrylic backing. Molten polyethylene glycol (PEG), heated to 50 $^{\circ}\text{C}$ , was injected into the cavity made by the PDMS mold (Figure 4) [7]. PEG served as the biodegradable base. Once the PEG cooled and hardened, the braced array supported by the acrylic backing was removed from the PDMS mold, and the Parylene cable with contact pads was inserted into the ZIF connector of PCB 1 (Figure 3). PCBs 1 and 2 were prepared through reflow soldering of the surface mount connectors onto each PCB on a hotplate.

A fully fabricated array, with electrode groups targeting the CA1 and CA3 hippocampal layer, was electrically packaged with PCBs 1 and 2 and implanted into an anesthetized, male Sprague-Dawley rat as described above, at an insertion speed of 10  $\mu\text{m}/\text{s}$  (Figure 5). All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) as well as the Department of Animal Resources of the University of Southern California (USC).

A 2 x 4 cranial window was removed above the rat's dorsal hippocampus along with three screw holes for two anchor screws and a single screw that served as a ground electrode. The dura was carefully removed from the

implantation site with forceps prior to array placement. During insertion, the array was slowly advanced at increments of 0.1 mm after the probe reached 2 mm, the expected depth of the CA1, to allow for electrical recordings. Once the first group of electrodes began recording spikes from the more superficial, CA1 layer, the array was advanced until the second group of electrodes reached the CA1, at which point the first group of electrodes recorded from the deeper CA3 layer. To confirm proper placement of the array, recordings were evaluated for the presence of complex spikes which are defined as a burst of 2-6 spikes of decreasing amplitudes at  $\leq 6$  ms durations [10]. Complex spikes are characteristic of pyramidal cells in the hippocampus.

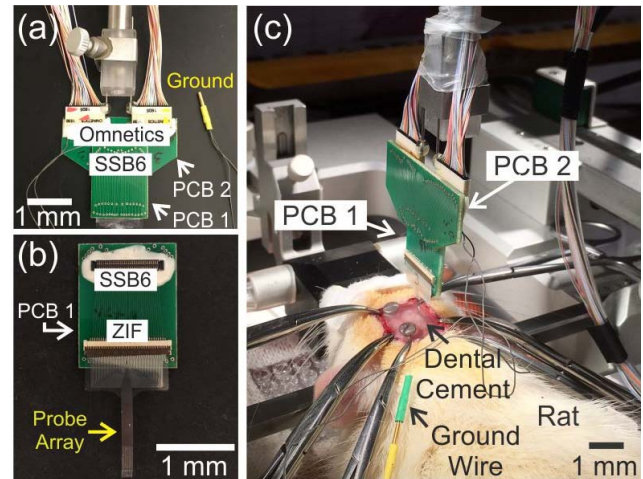


Figure 3: Electrical packaging of neural probe array showing (a) probe array connected via two PCBs to the electrophysiological recording system. (b) Close-up of PCB 1 with 71 pin zero insertion force (ZIF) connector and an SSB6 PCB-PCB connector. (c) PCB 2 contained the SSB6 mate, enabling connections between PCBs 1 and 2 and connection to the Plexon recording system via Omnetics plugs. The fully assembled device implanted in rat hippocampus.

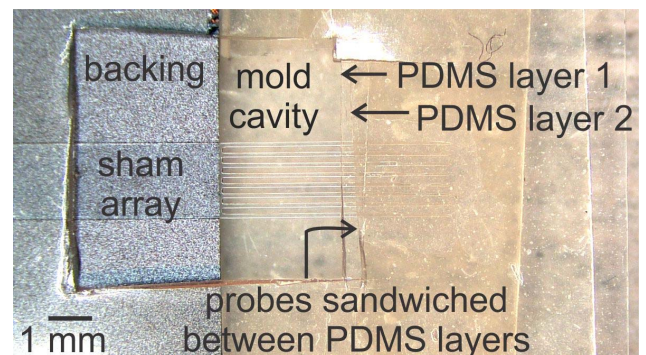
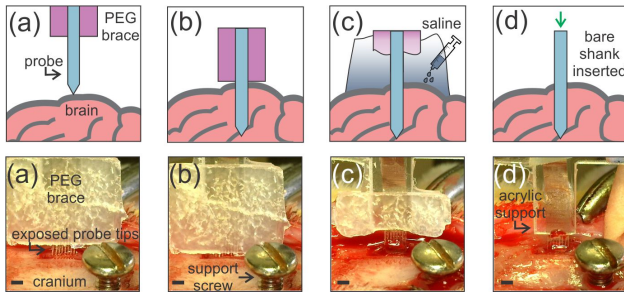


Figure 4: PDMS mold used to create the PEG brace that supported the array during insertion. The array was laid across an acrylic backing and its probe tips supported by layer 1 of the PDMS mold. Layer 2 is aligned on top of the array, and completes the formation of the mold cavity which is filled with molten PEG to brace the top length of the probe shanks.



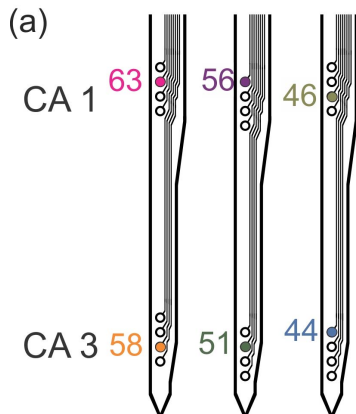


**Figure 5: Insertion of array supported by dissolvable PEG brace into rat hippocampus.** Exposed probes were inserted into brain, (a) the PEG brace was incrementally dissolved in saline, (b & c) and the remainder of the probe length was inserted to desired depth (d). Probes were advanced with pauses for electrical recording to ensure proper placement. Bare probes supported by PEG brace inserted without buckling. Scale bar = 1 mm.

## RESULTS

Arrays were successfully fabricated and packaged. CV cleaning prior to EIS measurements resulted in 51 (out of 64) working traces, with an average impedance of  $248 \pm 125$  k $\Omega$  at the physiologically relevant frequency of 1 kHz for action potential measurements. A packaged array was attached to the arm of a stereotaxic stage and implanted into rat hippocampus without buckling. Temporary pauses allowed time for brace dissolution using saline rinses and intraoperative recordings to confirm placement in the hippocampus. The detection of complex spikes from hippocampal pyramidal cells in the CA1 and CA3 layers in adjacent probes, with their characteristic sequential spikes of decreasing amplitudes indicated accurate placement of the array in the hippocampus and confirmed probe functionality (Figure 6). Analysis of adjacent channels 63, 56, and 46 confirmed recording of complex spikes from the CA1, while neighboring channels 58, 51, and 44 recorded from the CA3.

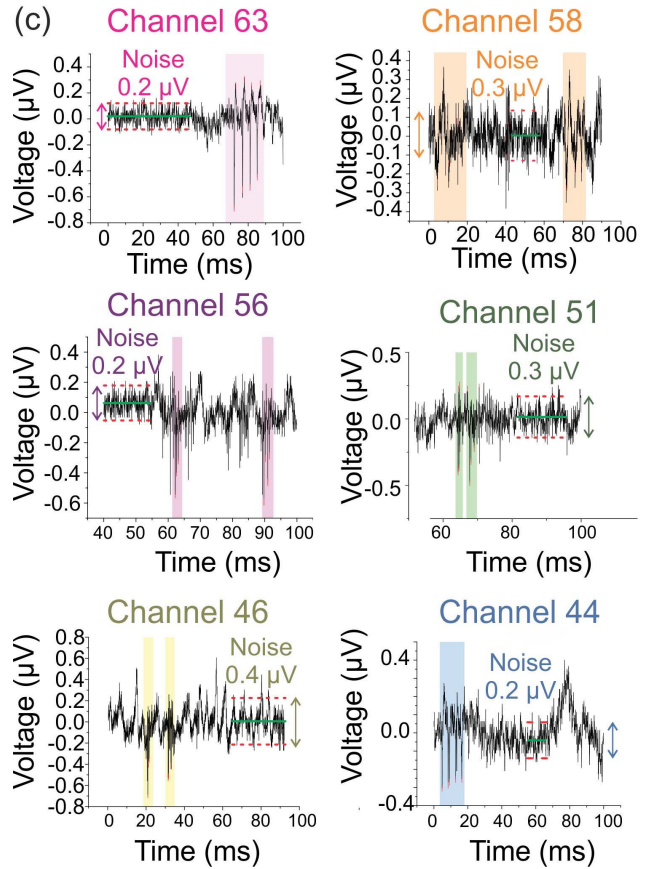
Complex spikes are specific to pyramidal neurons in the hippocampus, however they are infrequently observed in the cortex. The presence of simultaneous complex spikes recorded from adjacent electrodes lend credibility to the assumption that the complex spikes witnessed during recording originate from pyramidal cells, and not from cells in the cortex that can disguise themselves as pyramidal cells by also giving off complex spikes.



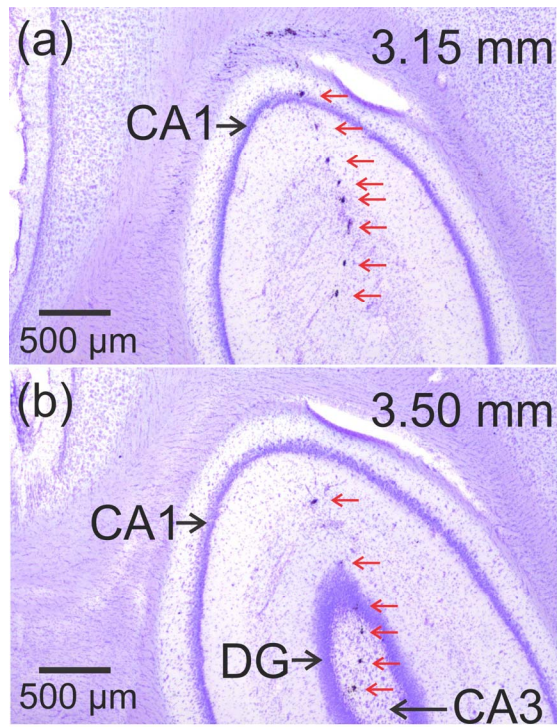
(b)

Channel 63		Channel 56		Channel 46	
Time (ms)	Voltage ( $\mu$ V)	Time (ms)	Voltage ( $\mu$ V)	Time (ms)	Voltage ( $\mu$ V)
72.0	-0.69	62.2	-0.54	21.0	-0.69
76.8	-0.54	62.6	-0.40	21.3	-0.40
81.1	-0.53	63.1	-0.32	21.6	-0.35
85.2	-0.45	89.8	-0.59	31.5	-0.53
		90.3	-0.30	31.8	-0.51
		91.0	-0.46	32.9	-0.42
		91.4	-0.29	33.3	-0.45

Channel 58		Channel 51		Channel 44	
Time (ms)	Voltage ( $\mu$ V)	Time (ms)	Voltage ( $\mu$ V)	Time (ms)	Voltage ( $\mu$ V)
4.5	-0.29	64.6	-0.37	4.8	-0.30
10.3	-0.27	64.9	-0.22	8.4	-0.29
15.2	-0.26	67.7	-0.48	9.1	-0.27
71.5	-0.35	68.1	-0.32	12.8	-0.26
76.1	-0.27	69.0	-0.22		
79.5	-0.24				



**Figure 6: In vivo hippocampal recordings.** (a) Schematic showing active neural probe recording sites. Top group of four electrodes targeted CA1 and the bottom group targeted CA3. (b) Tabulated time and voltage stamps for complex spike peaks confirming characteristic peak spacing and amplitudes. (c) Recordings showing complex spikes (burst of 2-6 spikes of decreasing amplitudes at  $\sim 6$  ms durations) and average noise (2 standard deviations indicated by dotted red lines; mean by solid green line). Recordings were acquired at a depth of 4.15 mm for channels 63, 56, 46, and 44, and from a depth of 3.9 mm for channels 58 and 51.



**Figure 7:** Nissl stained transverse slices of array sacrificed one month after the acquisition of acute recordings, showing histological confirmation of placement. Red arrows point to probe cross-sections. (a) Transverse slice at a depth of 3.15 mm from bregma, showing cross-sections of all eight probes and the dense neural layer of the CA1. (b) Transverse slice (depth 3.50 mm), with the cross-sections of six probes readily visible, and the most posterior three probes of the array entering the CA3 layer of the hippocampus.

Post-surgery, the rat chewed off the microwire used as a grounding electrode, making repeat recordings from this rat impossible. Histological evaluation of Nissl stained hippocampal slices from this rat sacrificed one month after implantation revealed that each of the eight shanks in the array crossed through the CA1 and CA3 layers of the hippocampus (Figure 7). The three most posterior probes were placed in the CA3 region and correspond to recordings.

## CONCLUSION

A Parylene-based deep brain neural probe array was designed, fabricated, and demonstrated in rat hippocampal recordings. Unlike previously reported polymer probes, these probes were advanced to deep regions of the brain without the use of bulky overcoats or insertion shuttles. Instead, a novel insertion method based on beam mechanics was used to temporarily stiffen the probes for insertion and minimize tissue damage. Packaged arrays were slowly implanted into rat brain with pauses to dissolve the biodegradable brace reinforcing the probe array and to take recordings to verify the desired placement within the hippocampus. Electrophysiological recordings exhibiting the characteristic complex spikes associated with hippocampal neurons were observed. Subsequent histological analysis confirmed proper placement of a subset of probes in agreement with the

electrophysiological recordings obtained. Chronic recording studies to test electrode signal to noise ratio and array lifetime are underway.

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