A Library of Polymer-based Microelectrode Array Designs for Recording from the Brain of Different Animal Models*

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***Abstract*—Large-scale network recording technology is critical in linking neural activity to behavior. Stable, long-term recordings collected from behaving animals are the foundation for understanding neural dynamics and the plasticity of neural circuits. Penetrating microelectrode arrays (MEAs) can obtain high-resolution neural activity from different brain regions. However, ensuring the longevity of implantable devices and the consistency of neural signals over time remains one big challenge. A potential solution is to use flexible, polymer-based MEAs to minimize the foreign body response and prolong the lifetime of neural interfacing devices. Rodents and nonhuman primates (NHP) are commonly used animal models in neuroscience and neuroengineering studies. Specially designed MEAs that capture morphological features of different animal brains and various brain structures are powerful tools to simultaneously obtain neural activities from multiple brain regions. In this work, we develop a set of prototype designs of polymer MEAs that cover cortical, sub-cortical, and multiple brain regions of rodents and NHP.**

I. Introduction

One main objective of neuroscience and neuroengineering studies is to understand the dynamics of neural networks involved in sensory, motor, and high-order cognitive functions. Stable long-term recordings collected from anatomically and functionally connected brain regions of behaving animals are the foundation for understanding such dynamics and are crucial in investigating brain functions, especially neural plasticity involved in learning and memory[1][2]. Synaptic plasticity, which occurs at timescales ranging from milliseconds to months is believed to be one complementary mechanism underlying optimal learning and memory encoding[3][4]. To fully comprehend neuronal rules in encoding complex behaviors, chronic recording of unitary activities of neuron populations from multiple brain regions consists of a well-defined neural circuit are needed. Over decades, breakthrough insights were gained from network recordings of behaving animals. With spike trains recorded simultaneously from hippocampal CA3 and CA1 regions of rats performing a delayed-nonmatch-to-sample memory task, a computational model which captured the nonlinearity of the hippocampal circuit was developed [5]. Similarly, with neuronal activity recorded from six cortical regions of monkeys reporting the color or motion of stimuli, the information flow during flexible sensory-motor decisions were investigated [6].

As the essential tool to obtain *in vivo* neural activities from the brain of behaving animals, the development of implantable microelectrode arrays (MEAs) significantly advanced the quantity and quality of neuronal recordings [7-12]. An ideal neural recording device should simultaneously sample from a statistically representative subset of neurons in multiple brain regions of interest. Numerous animal models have been used in neuroscience studies. The brain of different species varies greatly in size and anatomy. Nonhuman primates have relatively large brains rich of sulci while rodent brains are much smaller and have distinct morphology [13][14]. Individual brain structures also have diverse dimensions, cell distribution, neuron density, and complexity. High-density MEAs with unique geometry and electrode layouts are needed for assorted research goals. In addition, the recording should have high spatial and temporal resolution and be stable and consistent throughout long term. While microwires with find tips enabled the recording of cellular-scale activities, their limited number of effective recording points remains a hindrance for high channel counts and unit yield. Dense samplings of neurons from behaving animals' brains were achieved with high-density silicon probes, but the long-term function of these devices and the stability of neural signals recorded with these stiff implants are largely confined due to tissue response to the implant [15].

Encapsulation of recording electrodes by activated astrocytes and microglia that electrically and mechanically

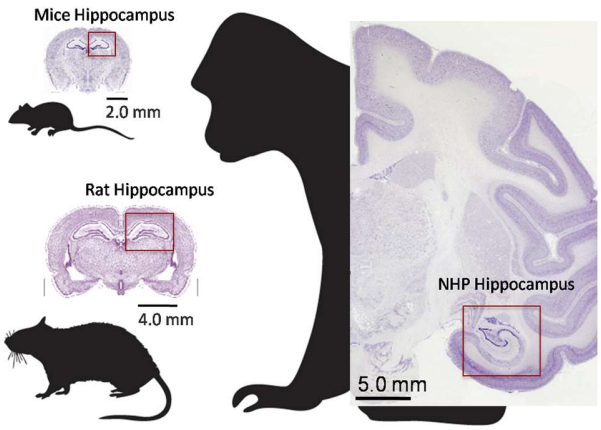

Figure 1. A diagram shows the size variation between mouse, rat and NHP. Histology of coronal brain slices shows obvious distinctions between their brains and critical brain structures such as the hippocampus.

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isolate the electrodes from surrounding neurons has long been hypothesized as the major reason for signal loss over time [16]. Many studies indicated that MEAs built with flexible, compliant materials can reduce inflammation and foreign body response and prolong the lifetimes of implanted devices [17]. Parylene and polyimide are both commonly used structural and coating materials in FDA-approved implants. MEAs fabricated with these flexible polymers can achieve a 100x reduction of stiffness (Young's modulus ~10^2 GPa for metals and silicon compared to ~10^0 GPa for polymers) [18]. Although there remains a gap in the softness of brain tissue, a significant improvement in recording longevity was observed by multiple groups [17][19]. Micro-fabrication techniques empowered the development and fabrication of polymer MEAs with almost any geometry. With specially arranged electrodes, multiple brain regions can be accessed simultaneously. To further promote the development of polymer MEAs, we developed a library of polymer MEA designs covering various cortical, sub-cortical, and multi-regions of the brains of mice, rats, and nonhuman primates (NHP).

II. Methods

A. Design Strategy

Anatomical features of different species' brains and the morphology of brain structures are deterministic factors in MEA designs. The initial MEA configurations (D_0) are determined based on anatomical considerations and surgical feasibility. Geometric parameters such as the number of shanks (*N*s), shank length (*L*s), spacing between shanks (*S*s) are estimated according to measurements gathered from the brain atlas and histological data of different species. By combining measurements of dimensions of brain structures and references to parameters of commercially available MEAs, the arrangement of electrodes, the number of electrodes per shank (*N*e), inter-electrode spacing (*S*e), and diameter of recording electrodes (*De*) of each MEA array is determined.

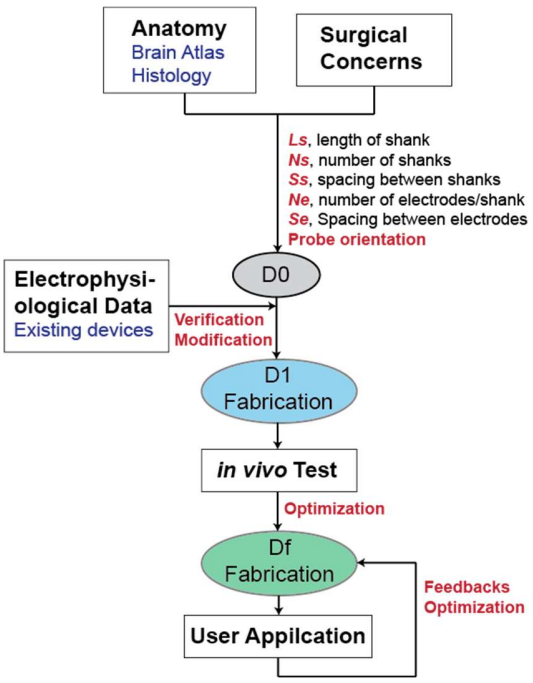

Figure 2. The flow chat of the systematic design strategy of the library of polymer MEAs.

Further verification of the initial designs for rodents is conducted with existing recording devices. *in vivo* electrophysiological recordings with microwires and silicon probes are used to verify the layout of electrodes. Characteristic electrophysiological patterns of specific brain regions and cell types, such as complex spikes generated by hippocampal pyramidal cells, are used to estimate spacing between different hippocampal sub-regions. According to feedback provided by *in vivo* recordings, the arrangement of electrodes is modified and optimized. The first design (D_1) for the fabrication of MEAs for mice and rats is generated. The initial design of MEAs for NHP is reviewed and evaluated by a group of experts in the field of NHP studies. With their feedback, D_1 for fabrication of MEAs for NHP are determined.

After the first modification round, MEAs are fabricated following the D_1 design. Performance of these MEAs is tested in living animals. Geometric and layout parameters will be iteratively optimized and finalized (D_f) based on the *in vivo* results.

B. MEA Fabrication

Parylene-based MEAs are fabricated with a standard microfabrication technique. The 10μm thick Parylene and the 10μm thick insulation are deposited using chemical vapor deposition. Platinum (Pt) traces, contact pads, and electrodes are fabricated with photolithography and metal evaporation. An adhesion promoter, A174 silane, is coated between the insulation and metal layer to strengthen the bond between the two materials. Reactive ion etch (RIE) is conducted to remove insulation above electrodes and contact pads. To further improve adhesion between the Parylene C layers and prevent delamination, Parylene-MEAs were sandwiched between ceramic alumina plates and annealed for 48 hours under vacuum at 200 °C.

III. Results

An initial library is developed, including 12 MEA designs for three animal models and a generic MEA design for rodents.

A. The Generic MEA Design for Rodents

The generic MEA has 64 channels arranged on four Parylene shanks. With *Ls*=5 mm and *Ss* (center to center)=500 μm distance (center to center), the MEAs can cover multiple cortical layers of mice and rats and access deep sub-cortical structures like the hippocampus with its sufficient length. To ease the insertion of the flexible MEA, shanks are tapered in width from 180 μm to 80 μm. On individual shanks, 16 Pt electrodes (*Se*=120μm) with 30μm diameter are placed at the tip and evenly distributed along both edges of the Parylene shank.

B. MEAs for Rat

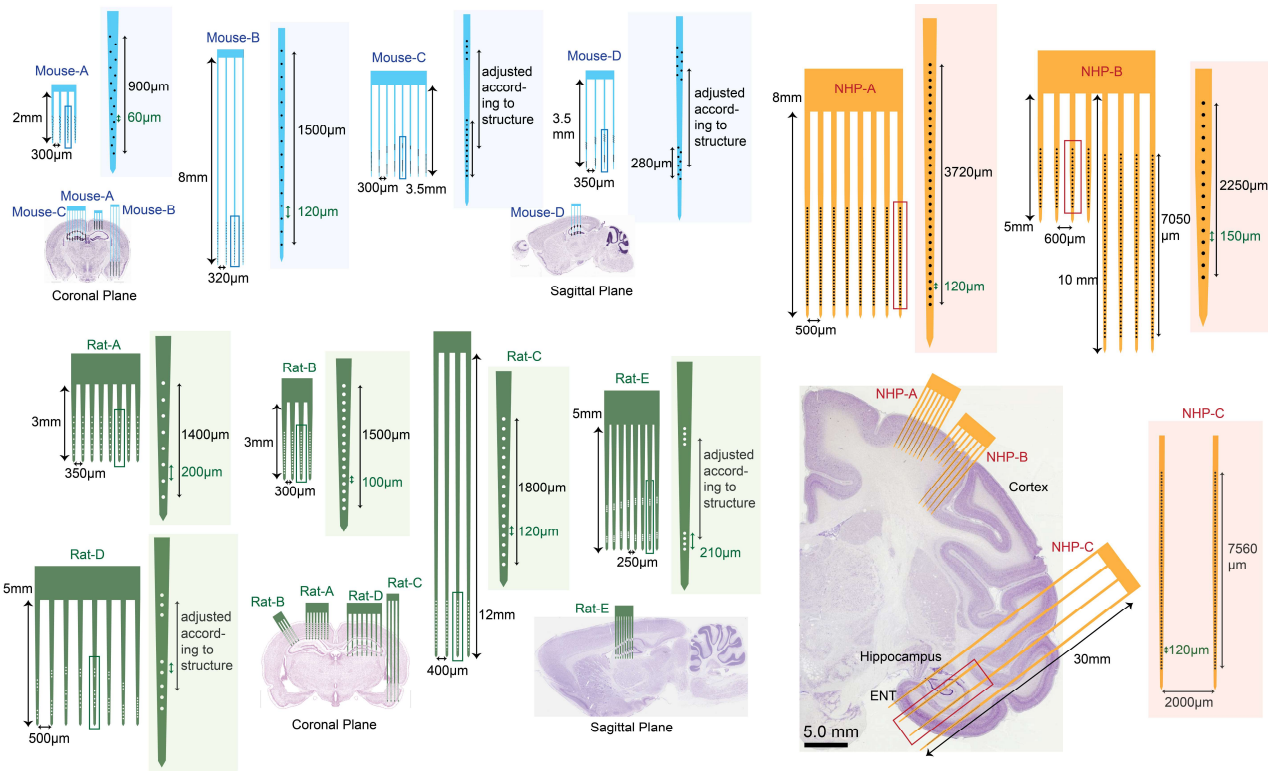

Figure 3. A schematic representation of the initial MEA designs targeting cortical and sub-cortical regions in mouse (blue), rat (green), and NHP (orange).

Table 1. Geometric and layout parameters of mouse, rat , and NHP MEAs

Species	Channel Number	*Ns*	*Ls* (mm)	*Ss* (μm)	*Se* (μm)	Coverage(HxWμm)	Applications
Rat	64	8	3.0	350	200	1400*2450	Individual and multiple cortical regions
	64	4	3.0	300	100	1500*900	Local cortical circuits
	64	4	12.0	400	120	1800*1200	Deep cortical regions, entorhinal cortex
	64	8	5.0	522	Conformal	~2000*3500	Individual hippocampal slice
	64	8	5.0	250	70, conformal	~1500*1750	CA1 and CA3 sub-regions across multiple hippocampal slices
Mouse	64	4	2.0	300	60	900*900	Dorsal cortex
	64	4	8.0	320	100	1500*960	Ventral cortex, entorhinal cortex
	64	8	3.5	300	Conformal	~1500*2100	Individual hippocampal slice
	64	4	3.5	350	40, conformal	~1500*1050	Multiple hippocampal laminae
NHP	256	8	8.0	500	120	3720*3500	Multiple layers of the cortex
	256	8	5.0;10.0	600	150	2250*1800;7050*1800	Sulcus & gyrus
	256	4	30.0	2000	120	7560*6000	Hippocampus, entorhinal cortex
Generic	64	4	5.0	500	120	1800*1500	Rodent brain

Five MEAs, each with 64 electrodes (*De*=15/30 μm) are designed to interface with multiple cortical and sub-cortical regions of the rat brain. **Rat-A:** The MEA features eight 3 mm long shanks and is designed for broader areas of the dorsal cortex. Eight evenly distributed electrodes (*Se*=200 μm) span 1400 μm vertically to cover multiple cortical layers. With *Ss*=350 μm, the MEA can span more than 2400 μm of the cortex. **Rat-B:** This cortical array has four 3 mm long shanks (*Ss*=300 μm), each with 16 electrodes (*Se*=100 μm). This denser design is intended to collect concentrated neural activities from local cortical circuits. **Rat-C:** This long array targets ventral cortical regions such as the entorhinal cortex. The MEA has four shanks (*Ls*=12 mm, *Ss*=400 μm) and 16 electrodes (*Se*=120 μm) at the tips. **Rat-D:** This hippocampal MEA has eight shanks (*Ls*=5 mm, *Ss*=500 μm) spanning the entire hippocampal transverse lamina. Each shank has eight electrodes arranged to match the curvature of hippocampal cell body layers, enabling simultaneous recording of the tri-synaptic hippocampal circuit. **Rat-E:** The 5 mm long MEA records from multiple hippocampal laminar (*Ns*=8, *Ss*=250 μm). There are two recording groups, each with 4 electrodes (*Se*=70μm), targeting CA1 and CA3 sub-regions. Spacing between two recording groups is fine-adjusted according to the anatomy of hippocampal cell body layers.

C. MEAs for Mouse

Four 64-channel MEA designs are developed for the mouse. **Mouse-A:** is an MEA with four 2 mm long shanks that target superficial cortical regions. Each shank has 16 electrodes (*Se*=60 μm) placed along the edges to sample 900 μm of the cortex. With *Ss*=300 μm, this MEA spans 900 μm of the cortical region. **Mouse-B:** this longer MEA targets deep areas such as the entorhinal cortex via four shanks (*Ls*=8 mm, *Ss*=320 μm) with 16 linearly distributed electrodes (*Se*=100

μm). **Mouse-C:** an MEA for recording from a hippocampal lamina is designed with eight 3.5 mm long shanks (*Ss*=300 μm) to span the width of the hippocampus. The locations of 8 electrodes on each shank are adjusted to conform to the curvature of cell body layers. **Mouse-D:** this MEA can assess multiple hippocampal laminar using four 3.5 mm long shanks (*Ss*=350 μm) to span the dorsal hippocampus. On each shank, 16 electrodes are divided into two densely packed recording groups (8 electrodes, *Se*=40 μm).

D. MEAs for NHP

Three high-density 256-channel NHP MEAs are designed. **NHP-A:** is an MEA with eight 8 mm long shanks (*Ss*=500 μm) for cortical recordings. On each shank, 32 evenly distributed electrodes (*Se*=120 μm) are placed at the tip of the shank. To access the sulci and gyri of the NHP brain, MEAs with different shank lengths are designed. **NHP-B:** an 8-shank MEA with four 10 mm long shanks for the sulcus and four 5 mm shanks for the gyrus (*Ss*=600 μm; *Se*=150 μm) are designed in which 64 electrodes (*Ne*=16) are placed on shorter shanks, and 192 electrodes (*Ne*=48) are placed on longer shanks. **NHP-C:** a long MEA for simultaneous recording from multiple hippocampal layers and the entorhinal cortex is designed. This MEA has four 30 mm long shanks (*Ss*=2000 μm) and 64 electrodes (*Se*=120 μm).

E. in vivo Recording with the Generic Rodent MEA

The generic rodent MEA is implanted into the brain of rats under both acute and chronic preparations. Two MEAs are implanted to a depth of 2.45 mm and 3.10 mm. Unitary activities from the somatosensory cortex are recorded during the implantation. Additionally, six generic MEAs are chronically implanted in the hippocampal region, and up to 44 units were recorded from a single animal. The MEAs captured typical hippocampal neural activities, such as theta rhythm and complex spikes in anesthetized and free-moving animals.

IV. Future Works

In this work, the initial design of a comprehensive library of MEAs that target various cortical, sub-cortical, and multiple sub-regions of rodent and NHP brains is developed. The initial design will be subject to expert review and feedback, leading to modification and refinement of the MEAs. The first batch of functional MEAs will then be fabricated. Following the implantation of these MEAs, the design of MEAs will be optimized based on electrophysiological data, which may require additional iterative modifications.

It is challenging to simultaneously get access to certain brain regions and sub-regions in neural networks with complex geometry and connections. We are also exploring innovative solutions such as developing a 3D, polymer-based MEA matrix and corresponding implantation method to effectively interface with complex brain structures and obtain large-scale recordings from neural circuits.

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References

[1] D. L. Alkon *et al.*, “Learning and memory. FESN Study Group,” *Brain Res.Brain Res.Rev.*, 1991.

[2] H. de J. G. Antunes and P. G. Pinheiro, “Linking knowledge management, organizational learning and memory,” *J. Innov. Knowl.*, 2020, doi: 10.1016/j.jik.2019.04.002.

[3] R. S. Zucker and W. G. Regehr, “Short-term synaptic plasticity,” *Annual Review of Physiology*. 2002, doi: 10.1146/annurev.physiol.64.092501.114547.

[4] T. V. P. Bliss and T. Lømo, “Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path,” *J. Physiol.*, 1973, doi: 10.1113/jphysiol.1973.sp010273.

[5] D. Song, R. H. M. Chan, V. Z. Marmarelis, R. E. Hampson, S. a. Deadwyler, and T. W. Berger, “Nonlinear dynamic modeling of spike train transformations for hippocampal-cortical prostheses,” *IEEE Trans. Biomed. Eng.*, vol. 54, no. 6, pp. 1053–1066, 2007, doi: 10.1109/TBME.2007.891948.

[6] M. Siegel, T. J. Buschman, and E. K. Miller, “Cortical information flow during flexible sensorimotor decisions,” *Science (80-.).*, 2015, doi: 10.1126/science.aab0551.

[7] D. R. Kipke, R. J. Vetter, J. C. Williams, and J. F. Hetke, “Silicon-substrate intracortical microelectrode arrays for long-term recording of neuronal spike activity in cerebral cortex,” *IEEE Trans. Neural Syst. Rehabil. Eng.*, vol. 11, no. 2, pp. 151–155, 2003, doi: 10.1109/TNSRE.2003.814443.

[8] R. E. Hampson, C. J. Heyser, and S. A. Deadwyler, “Hippocampal Cell Firing Correlates of Delayed-Match-to-Sample Performance in the Rat,” *Behav. Neurosci.*, vol. 107, no. 5, pp. 715–739, 1993, doi: 10.1037/0735-7044.107.5.715.

[9] D. K. Rangel Guerrero, J. G. Donnett, J. Csicsvari, and K. A. Kovács, “Tetrode recording from the hippocampus of behaving mice coupled with four-point-irradiation closed-loop optogenetics: A technique to study the contribution of hippocampal SWR events to learning,” *eNeuro*, vol. 5, no. 4, pp. 1–15, 2018, doi: 10.1523/ENEURO.0087-18.2018.

[10] A. Berényi *et al.*, “Large-scale, high-density (up to 512 channels) recording of local circuits in behaving animals,” *J. Neurophysiol.*, vol. 111, no. 5, pp. 1132–1149, Mar. 2014, doi: 10.1152/jn.00785.2013.

[11] M. Vandecasteele *et al.*, “Large-scale Recording of Neurons by Movable Silicon Probes in Behaving Rodents,” no. March, pp. 1–6, 2012, doi: 10.3791/3568.

[12] N. A. Steinmetz *et al.*, “Neuropixels 2.0: A miniaturized high-density probe for stable, long-term brain recordings,” *bioRxiv*, 2020, doi: 10.1101/2020.10.27.358291.

[13] T. A. Paxinos G, Huang XF, Petrides M, “The Rhesus Monkey Brain in Stereotaxic Coordinates,” *Academic Press*, 2009. .

[14] L. W. Swanson, “Brain maps 4.0—Structure of the rat brain: An open access atlas with global nervous system nomenclature ontology and flatmaps,” *J. Comp. Neurol.*, 2018, doi: 10.1002/cne.24381.

[15] V. S. Polikov, P. a. Tresco, and W. M. Reichert, “Response of brain tissue to chronically implanted neural electrodes,” *J. Neurosci. Methods*, vol. 148, no. 1, pp. 1–18, 2005, doi: 10.1016/j.jneumeth.2005.08.015.

[16] J. N. Turner *et al.*, “Cerebral astrocyte response to micromachined silicon implants,” *Exp. Neurol.*, 1999, doi: 10.1006/exnr.1998.6983.

[17] L. Luan *et al.*, “Ultraflexible nanoelectronic probes form reliable , glial scar – free neural integration,” no. February, pp. 1–10, 2017, doi: 10.1126/sciadv.1601966.

[18] A. Weltman, J. Yoo, and E. Meng, “Flexible, penetrating brain probes enabled by advances in polymer microfabrication,” *Micromachines*, vol. 7, no. 10, 2016, doi: 10.3390/mi7100180.

[19] J. E. Chung *et al.*, “High-Density, Long-Lasting, and Multi-region Electrophysiological Recordings Using Polymer Electrode Arrays,” *Neuron*, vol. 101, no. 1, pp. 21-31.e5, 2019, doi: 10.1016/j.neuron.2018.11.002.