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Custom-designed high-density conformal planar multielectrode arrays for brain slice electrophysiology

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Abstract

Multielectrode arrays have enabled electrophysiological experiments exploring spatio-temporal dynamics previously unattainable with single electrode recordings. The finite number of electrodes in planar MEAs (pMEAs), however, imposes a trade-off between the spatial resolution and the recording area. This limitation was circumvented in this paper through the custom design of experiment-specific tissue-conformal high-density pMEAs (cMEAs). Four configurations were presented as examples of cMEAs designed for specific stimulation and recording experiments in acute hippocampal slices. These cMEAs conformed in designs to the slice cytoarchitecture whereas their high-density provided high spatial resolution for selective stimulation of afferent pathways and current source density (CSD) analysis. The cMEAs have 50 or 60 μm center-to-center inter-electrode distances and were manufactured on glass substrates by photolithographically defining ITO leads, insulating them with silicon nitride and SU-8 2000 epoxy-based photoresist and coating the etched electrode tips with gold or platinum. The ability of these cMEAs to stimulate and record electrophysiological activity was demonstrated by recording monosynaptic, disynaptic, and trisynaptic field potentials. The conformal designs also facilitated the selection of the optimal electrode locations for stimulation of specific afferent pathways (Schaffer collaterals; medial versus lateral perforant path) and recording the corresponding responses. In addition, the high-density of the arrays enabled CSD analysis of laminar profiles obtained through sequential stimulation along the CA1 pyramidal tree.

Keywords: Planar; Conformal; High-density; Multielectrode array; Current source density analysis; Hippocampal slice

1. Introduction

The capability of simultaneously recording electrical activity at multiple sites in vitro has enabled investigations of neuronal network dynamics previously not possible with single electrode recordings (Droge et al., 1986; Singer, 2000; Soussou et al., in press; Warland et al., 1997). Planar multielectrode arrays (pMEAs) present one currently available technology to record from multiple neurons simultaneously in vitro (Duport et al., 1999; Egert et al., 1998; Gross and Schwalm, 1994; Jahnsen et al., 1999; Jimbo and Robinson, 2000; Novak and Wheeler, 1988; Oka et al., 1999; Stoppini et al., 1997). The distribution of pMEA electrodes captures the spatio-temporal dynamics of neuronal activity, while their transparent conductive leads permit microscopic visualization of the relative position of the tissue with respect to electrodes. Planar MEA electrodes can be used for both recording and stimulation, thereby providing self-contained sterile systems with no need for external electrodes (Gross et al., 1993; Novak and Wheeler, 1988).

Low cost of photolithographic fabrication coupled with advances in signal acquisition hardware and fast computers with large data storage, has led several groups to independently develop their own pMEAs. These investigators developed thin-film pMEAs in a variety of configurations to monitor extracellular electrophysiological activity in acute and cultured slices from different brain areas: retina (Grumet et al., 2000; Meister et al., 1994), spinal cord (Borkholder et al.,

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1997), and hippocampus (Boppart et al., 1992; Egert et al., 1998; Heuschkel et al., 2002; Novak and Wheeler, 1988; Oka et al., 1999; Thiebaud et al., 1999). In addition, several commercial planar multielectrode recording systems have recently become available such as MEA60 from Multi Channel Systems, Reutlingen, Germany and MED64 from Panasonic, CA, USA.

The advantages offered by pMEAs over traditional extracellular pulled-glass or sharp-wire electrodes depend on the number of electrodes, which is limited by current technological constraints, such as electrode and lead overcrowding, cross-talk, connector design, and data acquisition. These limitations create a trade-off between spatial sampling resolution and coverage area, preventing stimulation and recording from every location of a tissue preparation. Most of these currently available pMEAs have electrodes distributed in square matrix configurations with relatively large inter-electrode spacing (greater or equal to 100 μm). Since neural cytoarchitecture changes dramatically in the spatial domain in a non-symmetrical fashion, these low-density geometrically regular electrode arrangements do not provide the necessary resolution for selectively stimulating afferent pathways or flexibility in recording from small subregions. Furthermore, the low electrode density does not permit adequate current source density (CSD) analysis (Freeman and Nicholson, 1975; Nicholson and Freeman, 1975; Nicholson and Llinas, 1975; Wheeler and Novak, 1986). Therefore, there is a need to create tissue-specific high-density cMEAs that conform to the cytoarchitecture of the nervous tissue of interest. This paper resolves this compromise by describing custom pMEAs (cMEAs) that have a high-density of electrodes in tissue-conformal configurations for specific experimental applications.

This study describes methodology for designing, fabricating and using such conformal high-density cMEAs, and presents four examples of such pMEAs suited for CSD analysis, three of which are conformally mapped to hippocampal slice cytoarchitecture. The conformality refers to electrode distributions that correspond to the organization of intrinsic hippocampal circuitry. Stimulating electrodes are thus concentrated under afferent fibers or presynaptic cells, while recording electrodes are arranged under postsynaptic dendrites and somas. These layouts were designed for in vitro stimulation and recording from different hippocampal subregions (Fig. 1): cMEA#1 is a 3×20 rectangular array created for CSD analysis, and is well suited for electrophysiological investigations of the pyramidal and granular cells of the hippocampus, since these cells are densely packed into columns of parallel dendrites; cMEA#2 was designed to stimulate Schaffer Collateral (SchC) afferents to CA1 and records their responses; cMEA#3 design was intended for the stimulation of perforant path (PP) fibers in order to excite the dentate gyrus (DG) and the CA3 area; cMEA#4 was designed to stimulate PP and record DG, CA3 and CA1 responses in order to trigger trisynaptic responses.

Conformal topographical mapping and high electrode density enabled fine control and easy optimization of stimulation and recording sites. Large signal-to-noise ratios (>10:1) and high spatial density of electrodes has enabled CSD analysis of responses recorded from all four designs. This analysis disentangles field potentials to accurately map sources and sinks of synaptic currents. CSD was combined with sequential stimulations through a column of electrodes to generate a laminar profile of CA1, and to demonstrate independence of spatially distinct inputs. Selective stimulation of afferent fibers was hence optimized with ease even with adjacent pathways. These experiments with acute rat hippocampal slices established that conformal high-density MEAs could be custom-designed for slice preparations to ideally suit experiments requiring selective stimulation of afferent pathways and CSD analysis.

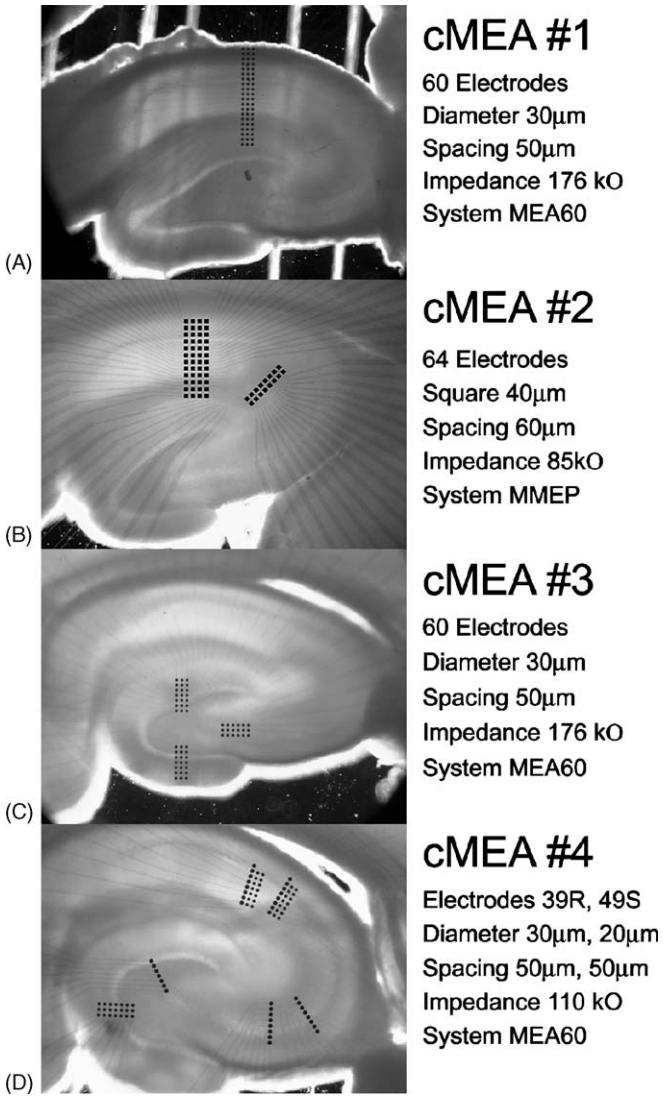

Fig. 1. Conformal probes: (A) cMEA#1 is a 3×20 rectangular array of electrodes. (B) cMEA#2 has a 2×8 sub-array to stimulate Schaffer Collateral (SchC) fibers and a 4×12 sub-array to record output responses from CA1 pyramidal cells. (C) cMEA#3 has two 3×7 sub-arrays to stimulate perforant pathways (PP) and record in dentate gyrus (DG) and one 3×6 sub-array to record CA3 output. (D) cMEA#4 has one stimulation sub-array consisting of seven triplet electrodes aligned to PP, and two other sub-arrays of seven pairs of electrodes for stimulating SchC. Electrodes in pairs or triplets act in unison, as they are connected to each other and lead to one contact pad. cMEA#4 also has four linear sub-arrays of seven or eight electrodes to record from DG, CA3 and CA1.