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·Review·

Use of multi-electrode array recordings in studies of network synaptic plasticity in both time and space

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Abstract: Simultaneous multisite recording using multi-electrode arrays (MEAs) in cultured and acutely-dissociated brain slices and other tissues is an emerging technique in the field of network electrophysiology. Over the past 40 years, great efforts have been made by both scientists and commercial concerns, to advance this technique. The MEA technique has been widely applied to many regions of the brain, retina, heart and smooth muscle in various studies at the network level. The present review starts from the development of MEA techniques and their uses in brain preparations, and then specifically concentrates on the use of MEA recordings in studies of synaptic plasticity at the network level in both the temporal and spatial domains. Because the MEA technique helps bridge the gap between single-cell recordings and behavioral assays, its wide application will undoubtedly shed light on the mechanisms underlying brain functions and dysfunctions at the network level that remained largely unknown due to the technical difficulties before it matured.

Keywords: multi-electrode arrays; acute hippocampal slices; spatial neural plasticity; temporal neural plasticity; network electrophysiology

1 Introduction

The human brain is comprised of ~10^{11} neurons, each of which connects to at least 500 others, leading to the ~50 trillion neuronal connections (synaptic contacts) that are responsible for various brain functions, such as sensory perception, motor action, memory and cognition[1]. This means that any brain function cannot be fully executed without the integrity of the whole system or sub-systems. For example, if the synaptic networks (or neuronal circuits) in one brain region are disrupted by external/internal environmental factors (trauma, injury or inflammation), functional disorders would occur, such as neuropathic pain, epilepsy, Parkinson disease and Alzheimer disease. In fact, the brain has been well studied at the single-neuron level over the past decades; however, less is known at the network level due to long-term difficulties in the development of new techniques for such investigations[2,3]. Thus, understanding how synaptic transmission and modulation occur at the network level in both physiological and pathophysiological states is critically important for unraveling the

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mechanisms of both brain functions and dysfunctions. On the other hand, pharmaceutical research and development of drugs for the treatment of brain diseases require novel, simple techniques that allow reliable large-scale screening of targeted lead compounds in experimentally stable brain models both *in vitro* and *in vivo*. Moreover, the possible toxic effects of drugs also need to be fully evaluated *in vitro* before their wide application in animal behavioral tests, due to ethical issues. Collectively, these problems cannot be simply solved by traditional electrophysiology using single or double microelectrodes. This has motivated the development of multisite recordings using multi-electrode arrays (MEAs) in the brain of behaving animals[3] as well as in brain slices *in vitro*[2] in the past several decades. This review starts with a brief introduction to the development of MEA recording techniques, followed by a description of their use in studies of synaptic plasticity in both time and space at the network level. Other fields in which MEA techniques are used are beyond the scope of this review.

2 Historical issues

The first design for MEA recordings was introduced by Thomas *et al.*[4] who applied a 2 × 15 array of 30 microelectrodes (7 μm^2 in size and 100 μm apart) to cultured embryonic chick heart cells recording. In 1977, Gross and colleagues used another set of MEAs with an array of 36 microelectrodes (100 or 200 μm apart) to record action potentials from dissociated snail ganglia[5,6]. Combining intracellular recordings with extracellular recordings by MEAs (2 × 16 array of 32 microelectrodes, 8 × 10 μm in size and 250 μm apart), Pine[7] successfully correlated the intracellular and extracellular events by simultaneous recordings from dissociated rat superior cervical ganglion neurons, making the MEA recording technique a useful electrophysiological tool to study network physiology and pharmacology. However, before the beginning of the 21st century, high quality and efficiency in the use of MEA techniques were not widely successful despite the efforts made in cultured preparations ranging from invertebrates to vertebrates[8-14].

One central problem hindering the advance of the MEA technique was the materials used to fabricate the microelectrodes and the insulation layer embedded within the MEA substrate. Ideally, all materials used in MEA fabrication should have: (1) good biocompatibility that should not produce any toxic effect and have adhesive properties in biological preparations; (2) good electrical properties for microelectrodes that can maintain a high signal-to-noise ratio; (3) good transparency for observation of the acute or cultured tissue under an inverted microscope; and (4) low cost. On the other hand, micro-fabrication technology is also required in the process of MEA fabrication. This includes photolithography, metal deposition techniques, wet chemical etching, and other back-end technologies. In 1998, Egert *et al.*[15] reported a novel planar MEA of 60 microelectrodes that was successfully used to record from organotypic cultures of hippocampal slices living for up to 4 weeks on the MEA. Both spontaneous action potentials and electrically-evoked local field potentials (LFPs) were recorded in the CA1 region after stimulation of the Schaffer collaterals in CA3[15]. The basic design of the MEA60 consists of gold strip conductors with titanium nitride (TiN) electrodes on a glass substrate. The insulating layer is composed of silicon nitride (Si_3N_4) (Table 1 and Fig. 1A). The MEA60 is the primary product of the MEA system commercially provided today by Multi Channel Systems (Reutlingen, Germany). The next year, Oka *et al.*[16] reported another new planar multi-electrode dish (MED) probe with 64 microelectrodes that was successfully used to record from acutely-dissociated hippocampal slices. Stable field excitatory postsynaptic potentials (fEPSPs) that were blocked by 6,7-dinitroquinoxaline-2,3-dione, an antagonist of non-NMDA glutamate receptors, were evoked in the CA1 region after applying electrical stimulation to the Schaffer collateral fibers. In that experiment, long-term potentiation (LTP), a type of neural plasticity that shows enhanced synaptic efficacy in response to high frequency stimulation, was evoked following conditioning stimulaton (4 pulses, 100 Hz burst repeated ten times at 200 ms interval) and lasted for 60 min. The basic design of the MED64 consists of transparent indium-tin-oxide (ITO, 100 nm) conducting leads with electrodes made

of gold (50 nm thick) and nickel (500 nm thick) that are electroplated with platinum black. The insulating layer is composed of polyimide that replaces silicon (Si_3N_4), a material with semiconductor properties (Table 1 and Fig. 1B). This glass-substrated MED64 is the primary product of the MED system commercially provided by Alpha MED Scientific Inc. (Osaka, Japan). These two commercially-available MEA systems make possible the wide use of the technique on various biological preparations, including acutely-dissociated brain slices (e.g., hippocampus, cortex, hypothalamus, substantia nigra, amygdala, superior colliculus, and brain stem), cultured slices (e.g., hippocampus, spinal cord, suprachiasmatic nucleus, cerebellum, and septo-hippocampal co-culture), retina, myocardial (ventricular and atrial slices) and smooth muscle from mammals[2,17-22]. Meanwhile, applications of MEAs in drug discovery have also been expanded[2,20,23].

Planar MEAs have been widely used to record from multiple kinds of preparations ranging from cultured neurons and organotypic brain slices to acutely-dissociated brain slices. However, the latter becomes more useful in studies on synaptic plasticity due to the following advantages: (1) synaptic structures within local brain regions remain relatively intact; (2) tissue modification or aberrant connections caused by culture are avoided; (3) recordings can be made on acute slices from animal models of brain diseases; (4) MEA recordings can be combined with both behavioral and molecular/cellular assays; and (5) the procedures are time-saving. However, the use of planar MEAs in acutely-dissociated brain slices may yield less stable recordings due to a layer of dead cells at the surface of the slice caused by cutting, leading to a low signal-to-noise ratio. The so-called "dead cell layer" indicates the border of the slice (~50 μm in depth) in which few cells remain active after injury and edema caused by the cutting procedure. This forms an electrically passive layer producing a shunt between the planar MEA and active cells inside the slice, resulting in small signal amplitudes. Thus, reducing the distance between the recording electrodes and active cells is a good way to obtain high-amplitude signals. In consequence, Thiebaud *et al.* introduced a 60-channel MEA with 3-dimensional (3D) tip-shaped protruding microelectrodes[24]. Heuschkel *et al.*[25] later applied this 3D MEA to acute hippocampal slices and obtained better

Table 1. Planar multi-electrode array recording setups

Standard setup	Multi-channel recording system[1]	
	MEA System	MED System
Manufacturer	Multi Channel Systems (Germany)	Alpha MED Scientific Inc. (Japan)
Recording electrode	MEA (microelectrode arrays)	MED Probe (multi-electrode dish)
Classic pattern	8 × 8 or 6 × 10 arrays of 60, 120, and 240 channels	8 × 8 arrays of 64 channels
Electrodes	titanium nitride	platinum black + gold + nickel
Conducting layer	gold leads	indium-tin oxide leads
Insulating layer	polyimide (or silicon nitride)	polyacrylamide (or polyimide)
Amplifier	MEA 1060 series	MED64 Amplifier
		MED Head Amplifier (MED-A64HE1)
		MED Main Amplifier (MED-A64MD1)
Connector	USB-MEA60 Systems	MED Connector (MED-C03)
Software	MC _Rack	MED64 Mobius (old version: Conductor and Performer)
Electrical stimulation	StimMEAs (four pairs of large stimulation electrodes made of titanium nitride)	Can be applied through each of the 64 recording electrodes

[1]Details available at http://www.multichannelsystems.com/ and http://www.med64.com/.

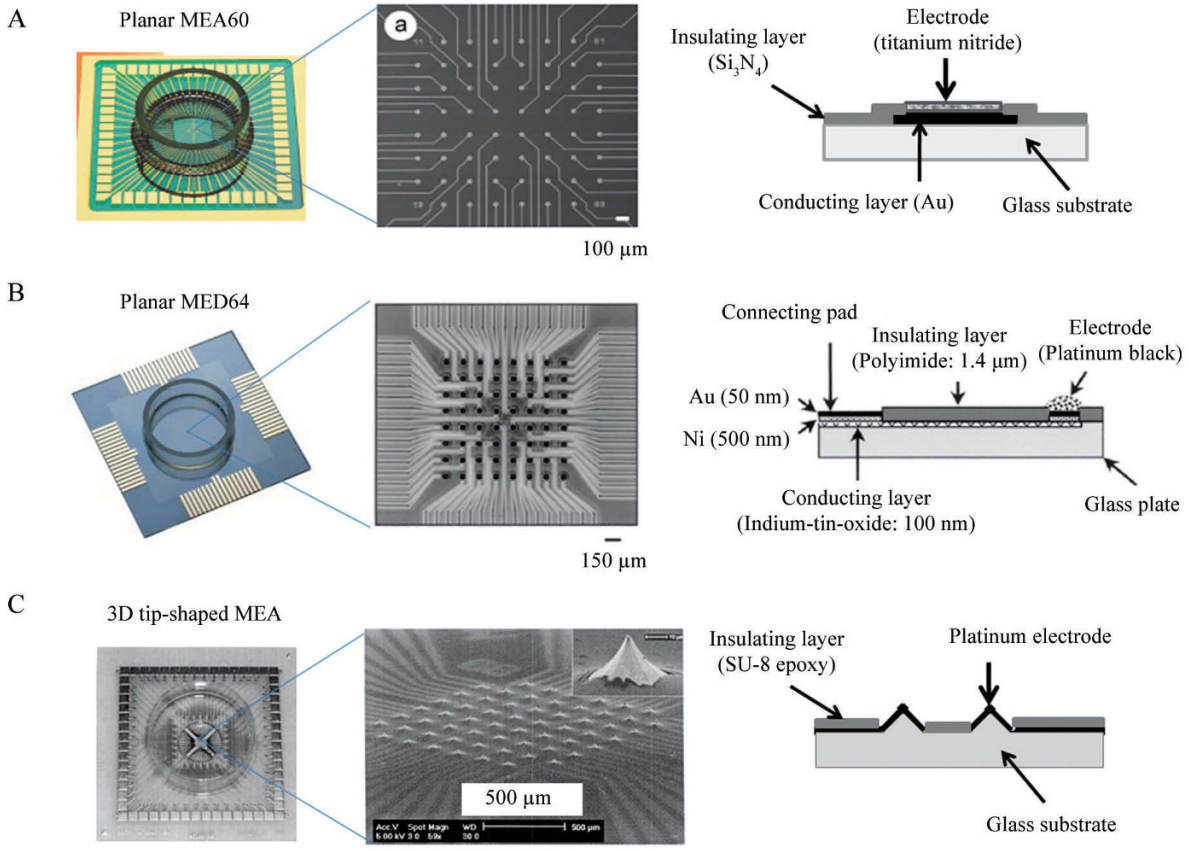

Fig. 1. Three types of standard multi-microelectrode arrays (MEAs). A: The planar MEA60 biochip. Left, an overview of the MEA60 chamber; middle, design of MEA60 showing that the recording field is composed of 60 microelectrodes connected to strip conductors; right, cross-sectional scheme of the basic design of the MEA60 composed of a gold conducting layer (Au, black) with titanium nitride electrode (dotted) placed on a glass substrate (light grey). The insulating layer is silicon nitride (Si_3N_4, dark grey). Modified from Egert *et al.*, Brain Res Brain Res Protoc, 1998[15] with permission from Elsevier. B: The planar multi-electrode dish 64 (MED64) biochip. Left, overview; middle, design of MED64 showing that the recording field is composed of 64 microelectrodes connected to strip conductors; right, cross-sectional scheme of the basic design of the MED64 composed of indium-tin-oxide strip conductors (crossed) with electrodes consisting of platinum black + gold (Au) + nickel (Ni) plated on a glass substrate (light grey). The insulating layer is polyimide (dark grey). Modified from Oka *et al.*, J Neurosci Methods 1999[16] with permission from Elsevier. C: The 3-dimensional (3-D) tip-protruding MEA biochip. Left, overview; middle, design of 3D MEA photographed under a scanning electron microscope showing that the recording field is composed of 60 tip-protruding microelectrodes connected to strip conductors; right, cross-sectional scheme of the basic design of the 3D MEA composed of platinum electrodes (black) on a glass substrate (light grey). The insulating layer is SU-8 epoxy (dark grey). Modified from Heuschkel *et al.*, J Neurosci Methods 2002[25] with permission from Elsevier. Scale bars: A middle, 100 µm; B middle, 150 µm; C middle, 500 µm.

recordings with a higher signal-to-noise ratio compared with planar MEA60 recordings. The 3D tip-protruding MEAs consist of a 1.4 × 1.4 mm^2 recording area on a glass substrate, 60 platinum electrodes in 8 × 8 arrays (without 4-corner electrodes), and an SU-8 epoxy insulating layer (Fig. 1C). The microelectrode surface size is 40 × 40 μm^2 and they are spaced 200 µm apart in the 3D MEA configuration. The glass tips are 60 µm long so they can penetrate the "dead cell layer" and make contact with active cells. Well-fabricated 3D MEAs have each electrode surface area between 2809 and 3600 μm^2, 1.75–2.25 times larger than the single planar MEA, leading to a decrease in resistance (2.14 times smaller) and an increase in capacitance (2.08 times larger) than the planar MEA60, and

allowing good contact between each electrode and active cells. The advantages of the 3D tip-shaped MEA are: (1) tissue slice penetration that enables reduction of the distance between electrodes and active neurons; (2) increase in geometrical surface that reduces electrode impedance, thus enhancing the signal-to-noise ratio; and (3) increase in geometrical surface that produces a higher electrical field around the electrode during stimulation due to the ability to store large electrical charge and to safely deliver a higher injected charge (for details see Heuschkel *et al.*, chapter 4 in *Advances in Network Electrophysiology Using Multi-electrode Arrays*[2]).

3 Experimental setups

The details of the two standardized planar MEA recording setups, the MEA System (8 × 8 arrays of 60 microelectrodes) and the MED System (8 × 8 arrays of 64 microelectrodes) are shown in Table 1. Their primary designs have been reported by Egert *et al.*[15] and Oka *et al.*[16].

4 Use of MEA recordings in studies of synaptic plasticity in space

4.1 Examples of spatial hippocampal plasticity induced by peripheral nociception The term "spatial plasticity" was first used by Ramón y Cajal, who proposed the connective foundation of neural memory, and provided an 'explanation of the adaptation and professional skill of the functional proficiency due to exercise (physical education, operations of speech, writing, piano playing, mastery of fencing, *etc.*) … by the creation of new cellular appendices … capable of improving the adjustment and the extension of contacts, and even of organizing completely new relations between previously independent neurons'[26]. However, so far, little electrophysiological evidence has been provided, although several morphological descriptions exist in the literature. More recently, researchers have argued that the spatial properties of synaptic transmission and connection, like the well-documented temporal synaptic plasticity like LTP and long-term depression (LTD), may also have great potential for furthering our understanding of the complex phenomena of neuroplasticity that occur under normal and pathological conditions[27,28]. In this respect, our lab investigated the possible existence of spatial plasticity in the hippocampal formation (HF) induced by persistent peripheral nociceptive stimuli in a rodent model of bee venom (BV)-evoked persistent inflammatory pain[29]. Using the newly-established MED64 system (Alpha MED Scientific Inc.) in acutely-prepared hippocampal slices (Fig. 2), we stably recorded simultaneous responses across both dentate gyrus (DG) and CA1 regions after electrical stimulation of the perforant pathway (PP) (Fig. 3)[31]. Because the PP fibers project mainly to the molecular layer of the DG and molecular and radiatum layers of CA1 where the dendritic trees of granular neurons and apical dendrites of pyramidal

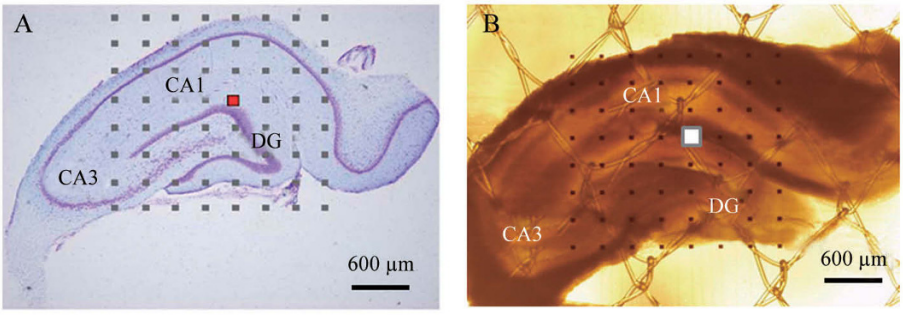

Fig. 2. Positioning of a standard (8 × 8) MED64 biochip on the hippocampal formation. A: Positioning of the biochip on a Nissl-stained slice. Red square indicates the site of electrical stimulation at the perforant path (PP) formed by entorhinal-hippocampal projection fibers; the remaining 63 black squares are recording electrodes across the dentate gyrus (DG), CA3, and CA1. B: One photomicrograph taken by a CCD camera under an inverted microscope (Olympus X71, Japan) showing the actual position of the MED64 probe on the slice. White square indicates site of electrical stimulation at PP fibers. Scale bars, 600 μm.

neurons are localized (Fig. 2), it was expected that the first PP-evoked depolarization should occur within these areas. Moreover, because the LFPs are believed to be produced by the inward flow of cations across voltage-gated ion channels, negative-going waveforms, namely current sinks (see below), could represent local membrane depolarization[2,30]. As expected, the negative-going LFPs evoked by PP stimulation at the site (asterisk, electrode #29, Fig. 3A) were mainly confined to the dendritic distribution areas of both granular and pyramidal neurons in the normal or saline control state[31]. The spread of the PP-evoked depolarization was clearly shown by 2-dimensional current source density (2D-CSD) imaging (Fig. 4A)[31]. The current sink (blue color) started to occur 5 ms after PP stimulation, and then protruded toward the DG granular cell layer previously occupied by current source (yellow color) from 11 ms. At about 17 ms, the sink was localized within the DG granular cell layer and disappeared from the dendritic compartment of both granular and pyramidal cells. The current source, transformed from positive-going waveforms, is believed to be produced by passive current outflow within the circuitry related to membrane depolarization, and thus may represent compound potentials including postsynaptic membrane depolarization and overriding action potentials. As shown in Fig. 3A and 4A, the current source appeared simultaneously in the granular cell layer and hilus of the DG with current sink, and spread slowly toward the track of mossy fibers projecting to CA3. To summarize, the

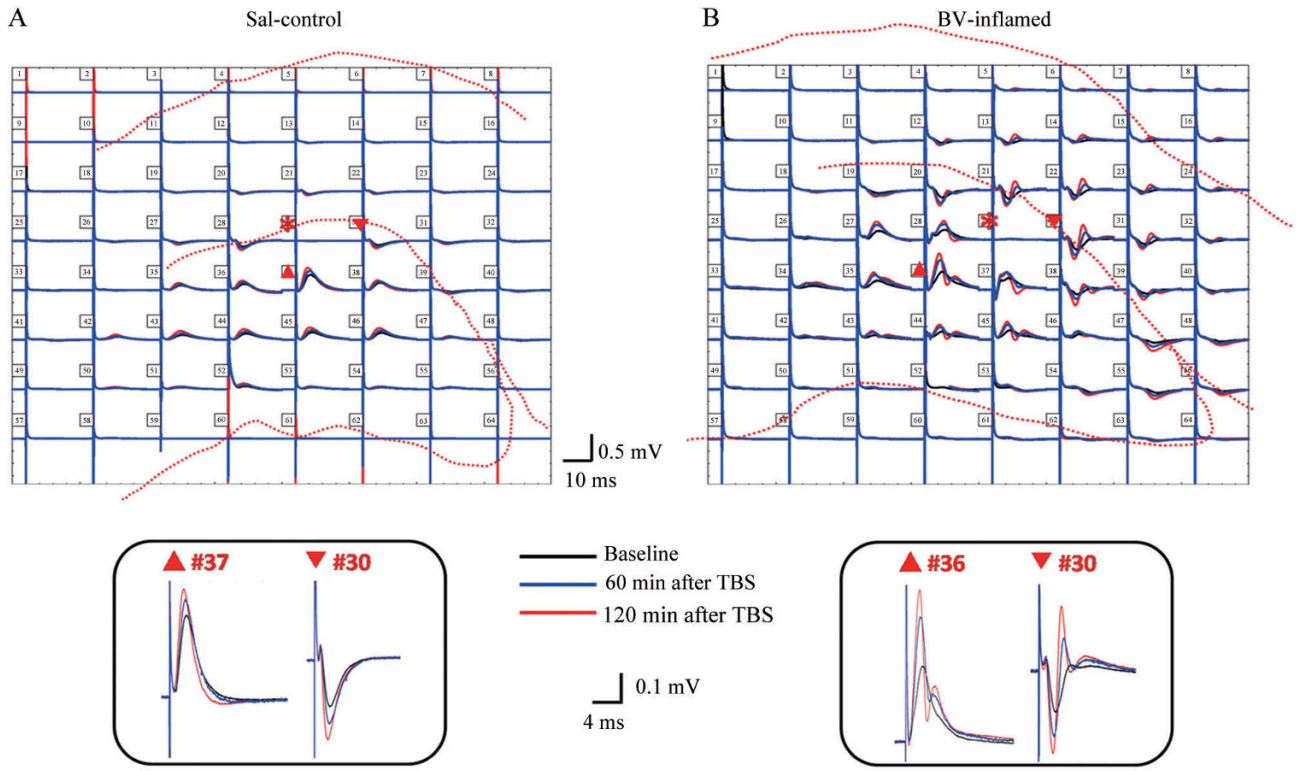

Fig. 3. Two typical examples of MED64 probe recordings from the hippocampal slice of rats receiving hindpaw injection of saline (Sal, A) or bee venom (BV, B). Upper panels: raw traces from 63 recording electrodes across the dentate gyrus and CA1 in response to perforant path (PP) test stimulation before (baseline), 60 min and 120 min after theta burst stimulation (TBS). Dashed lines indicate the anatomical contours of hippocampal formation. Lower panels: Examples of field potentials from the electrodes indicated in upper panels by upward and downward arrowheads. In a slice from a saline control rat (A), the amplitudes of the field potentials in both areas were potentiated for a prolonged period after TBS conditioning of the PP fibers (asterisk in upper panel). In a slice from a BV-inflamed rat (B), the shape of the field potentials was deformed or split after TBS conditioning. The amplitudes of the field potentials in both areas were appreciably potentiated for a prolonged period after TBS conditioning of the PP fibers (asterisk in upper panel). Modified from Zhao *et al*., Mol Pain, 2009[31] with permission.

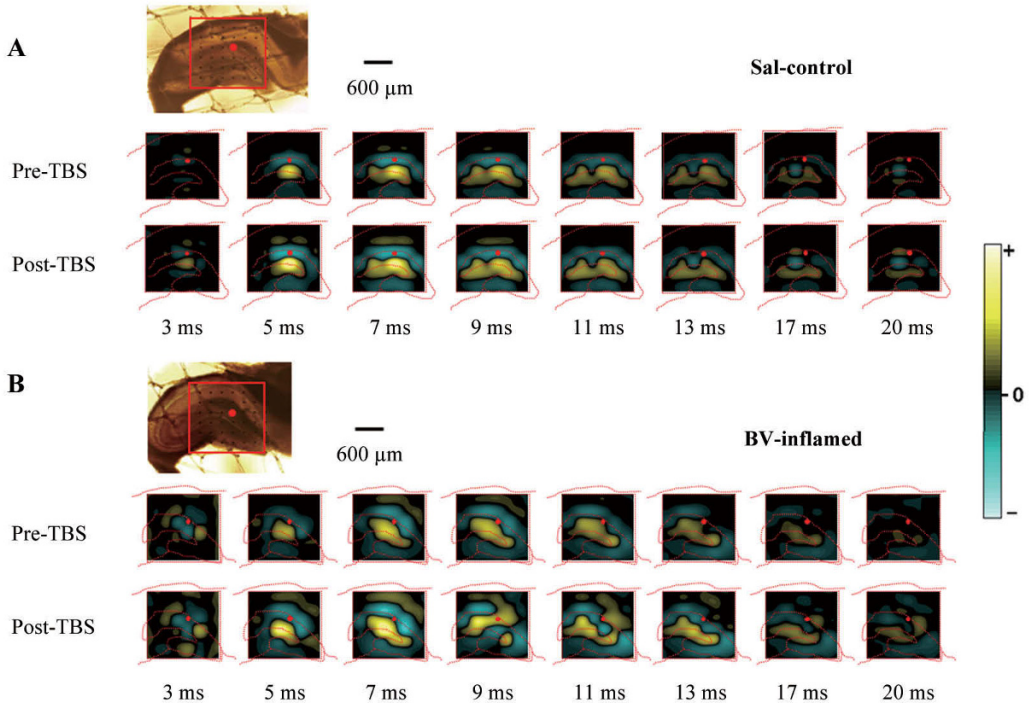

Fig. 4. Two-dimensional current source density (2D-CSD) imaging of 8 × 8 array (inset, inter-electrode spacing, 300 μm; scale bar, 600 μm) recordings in the hippocampal formation. Examples of 2D-CSD imaging of the network response across the dentate gyrus (DG) and CA1 to theta burst stimulation (TBS) of the perforant path (PP) in a hippocampal slice from a rat receiving saline injection (Sal-control, A) or bee venom injection (BV-inflamed, B). Recordings were made from the remaining 63 sites in the slice in response to PP electrical stimulation (red dots). Each image represents the instantaneous 2D-CSD plot computed at selected time points. Negative current sinks are depicted in blue and positive current sources are shown in yellow (see color scale). The positions of the DG and CA1 are marked by red, dashed lines. Pre-TBS, baseline; Post-TBS, 120 min after TBS conditioning. Note the conversion from a single current source-sink dipole to biphasic events after TBS in the BV-inflamed group. Adapted from Zhao *et al.*, Mol Pain, 2009[31] with permission.

prominent field potentials recorded in control conditions could be characterized as a current source-sink dipole occurring mainly from 5 to 17 ms after PP stimulation.

However, what are the pharmacological characteristics of these LFPs? Are they mediated by excitatory glutamate transmitters and receptors? To determine whether they are fEPSPs, the PP-evoked electrical responses were tested pharmacologically. Perfusion of the slices with the selective fast Na^+ channel antagonist TTX (0.5 or 1 μmol/L) or a high Mg^{2+}-low Ca^{2+} solution ($CaCl_2$, 0.25 mmol/L; $MgSO_4$, 4.0 mmol/L) resulted in a dramatic and partially reversible decrease in the amplitude of LFPs within both DG and CA1 (see Fig. 4 of Zhao *et al.*[31]), suggesting that the multisite synaptic responses recorded under our conditions are activity-dependent, relying on both action potential propagation and Ca^{2+}-associated transmitter release from nerve terminals following electrical stimulation of PP fibers. Then AP5 (an NMDA glutamate receptor antagonist, 50 or 100 μmol/L) and CNQX (a non-NMDA glutamate receptor antagonist, 10 μmol/L) were separately added to the recording system to verify the postsynaptic receptor types mediating this synaptic response. CNQX produced a much larger reduction in the amplitude of LFPs than AP5, although the latter at 100 μmol/L also significantly reduced the whole network response (see Fig. 5 of Zhao *et al.*[31]). These results indicate that the local potentials recorded in DG and CA1 are fEPSPs principally mediated by ionotropic glutamate receptors, with non-NMDA receptors comprising the main component and NMDA receptors partially involved[31]. Moreover, these data suggest that the current

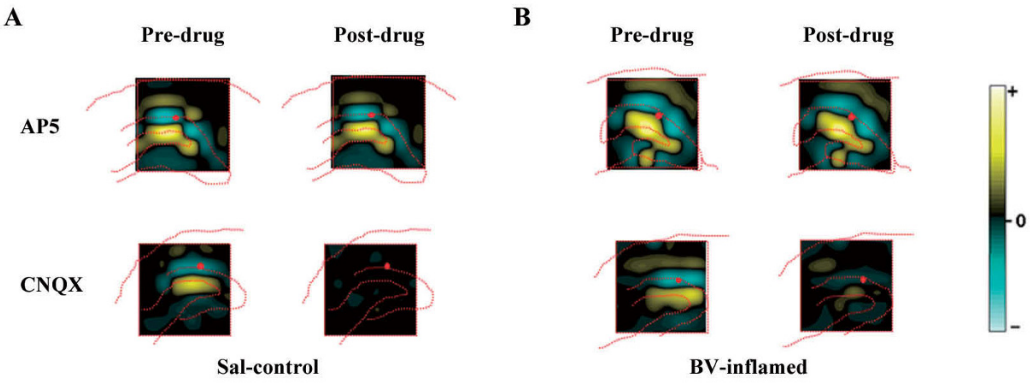

Fig. 5. Effects of bath application of AP5 (100 μmol/L) or CNQX (10 μmol/L) on the spatial expansion of a synaptic network. Two-dimensional current source density imaging of changes in the spatial distribution of positive current sources and negative current sinks across the dentate gyrus (DG) and CA1 before (Pre-drug) and after (Post-drug) AP5 (upper) or CNQX (lower) infusion in saline control (A) and BV-inflamed (B) groups. Bath infusion of CNQX, but not AP5, robustly decreased the intensity of current signals around the DG and CA1. For other details see legend of Fig. 4. Adapted from Zhao *et al.*, Mol Pain, 2009[31] with permission.

source is also formed by the postsynaptic events mediated by ionic glutamate receptors, confirming the existence of two direct entorhinal (EC)–hippocampal pathways: one is the EC–CA1 synaptic pathway, and the other is the EC–DG–CA3 pathway.

To determine whether synaptic connections and functions change in the HF after persistent peripheral nociception, we next assessed the spatial dimensions of synaptic connections and transmission. First, we found that spatial plasticity occurs at three levels: (1) enlargement of synaptic connection size at the network level that may be caused by recruitment of new pre-synaptic inputs or an increase in post-synaptic components such as the growth of new dendritic spines; (2) increased synaptic efficacy at the cellular level that may involve both increased pre-synaptic transmitter release and elevated post-synaptic responsiveness (increased synthesis of receptors and channels, *etc.*); and (3) changed fEPSP shape at the local circuit level that may result from disinhibition of inhibitory interneuron-mediated tonic suppression[31]. Specifically, persistent nociception in the periphery resulted in an increase in the number of fEPSPs across the hippocampal network (see Fig. 6 of Zhao *et al.*[31]), a left-ward shift of input-output curves of synaptic transmission (see Fig. 8 of Zhao *et al.*[31]), and dramatic changes in the structure or form of fEPSPs evoked by theta burst stimulation (TBS) in the pain state (Fig. 3B and Fig. 4B). These results are interesting because the MEA recordings on acute hippocampal slices at least disclose three types of spatial plasticity at the cellular, local micro-circuit, and network levels, providing insights into the mechanisms underlying brain dysfunction caused by chronic pain and other stressors.

To probe the potential receptor and signaling mechanisms underlying pain-related spatial plasticity at the network and cellular levels, we next evaluated possible roles of non-NMDA receptors, NMDA receptors, group I metabotropic glutamate receptors (mGluRs) and the mitogen-activated protein kinase (MAPK) family members, extracellular signal-regulated kinase (ERK), p38 MAPK and c-Jun N-terminal kinase (JNK), using pharmacological receptor antagonists or kinase inhibitors. Our results showed that the non-NMDA receptor might play a more important role than the NMDA receptor, suggesting a mechanistic difference between nociception-induced spatial plasticity and classical forms of learning and memory (Fig. 5)[31]. In addition, mGluR1 and p38 MAPK appear to be involved in the tonic inhibition of EC–DG and EC–CA1 synaptic enhancement, while ERK may mediate persistent pain-associated spatial extension[32,33]. Moreover, the enhancing effects on the spatial organization of network synaptic con-

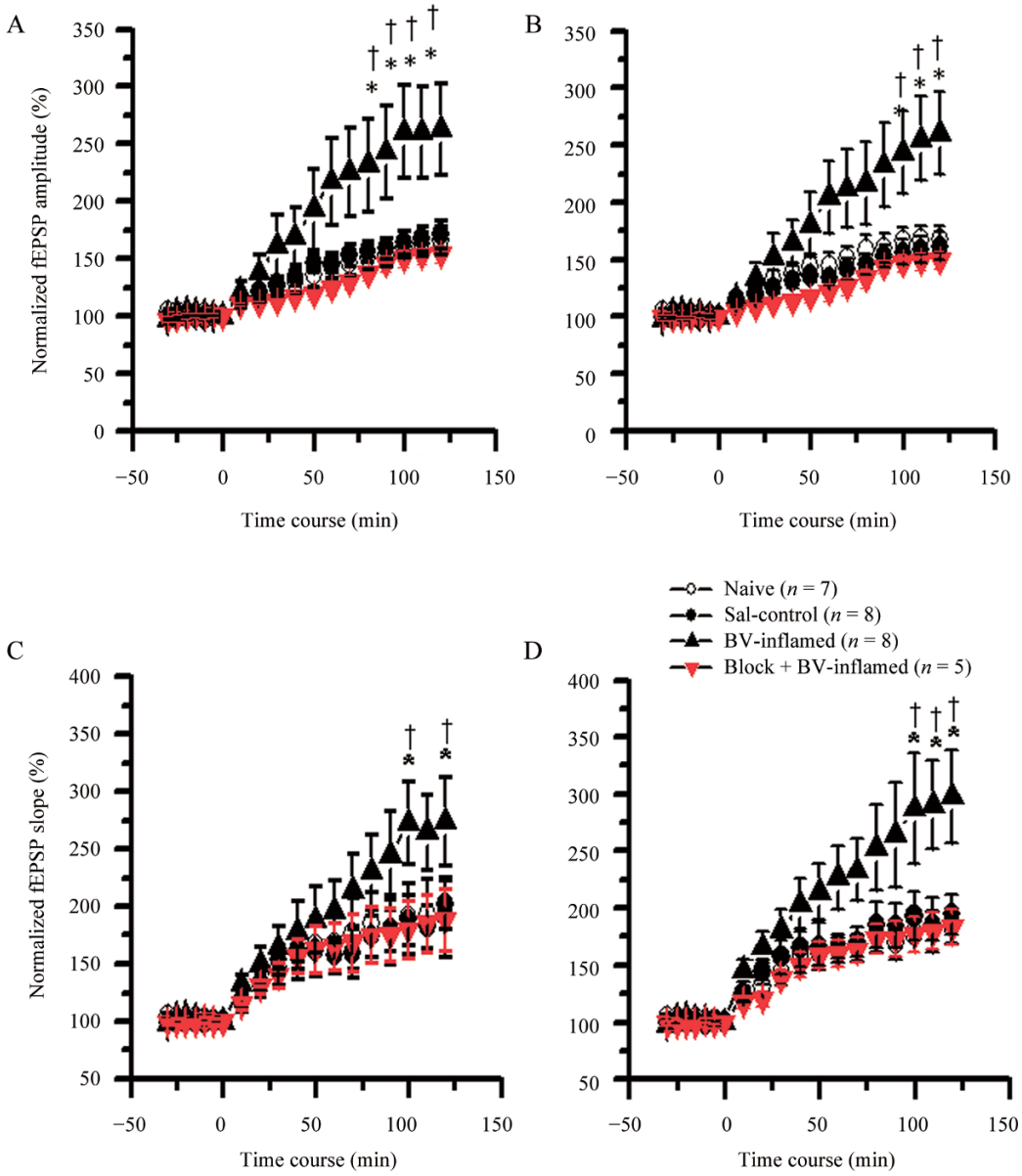

Fig. 6. Comparison of long-term potentiation (LTP) of field excitatory postsynaptic potentials (fEPSPs) in the hippocampal formation induced by perforant path theta burst stimulation (TBS) conditioning between groups of rats in naïve, saline (Sal-control), bee venom (BV)-inflamed and peripheral impulse blockade states. The amplitude (A, B) and slope (C, D) of both the dentate gyrus (A, C) and CA1 (B, D) fEPSP were normalized as percentages of the pre-TBS baseline and plotted as a function of time. Enhancement of network LTP by BV-induced persistent nociception was reversed by local pre-blockade of nerve impulses from the injury site. The number of slices used to plot the graph is indicated in parentheses. *P <0.05 *versus* naïve control; †P <0.05 *versus* saline control. Error bars, ± SEM. Adapted from Zhao *et al.*, Mol Pain, 2009[31] with permission.

nections observed in the HF have been shown to depend upon the painful state in the periphery, because peripheral blockade of ongoing impulses originating from the injury site was effective in preventing the spatial changes in the hippocampus. Taken together, these spatial characteristics of synaptic plasticity, as well as their pharmacological profiles, are far beyond the scope of classical electrophysiological recording techniques (such as *in vivo* electro-

physiology and *in vitro* patch clamp recording), and further highlight the superiority of multisite recording using MEA techniques.

4.2 2D-CSD imaging analysis As noted above, another unique advantage of MEA recording is that the multisite synaptic responses can be used for 2D-CSD imaging analysis to dynamically visualize the spatial and temporal electrical activity by generating movies of current movements at millisecond resolution[34]. In our previous study, we also used this analysis method by bilinear interpolation at each point of the 64 electrodes and transformed the MED64 data to spatially-visualized imaging of network responses in the HF. By comparing the 2D-CSD images captured at 3, 5, 7, 9, 11, 13, 17, and 20 ms, dynamic spatial changes in the configuration of current sources and sinks were monitored in parallel with the original waveforms (Fig. 4A)[31]. More importantly, a novel type of spatial plasticity at the local circuit level was revealed by the 2D-CSD analysis, that is, the deformed fEPSP shape or structure (reflected by altered spatial distribution of the source-sink dipole) after TBS conditioning that could only be visualized in the persistent peripheral pain state but not in the saline control or naïve state[31]. Time-lapse changes in 2D-CSD imaging showed a disrupted pattern of source-sink arrangement with a prominent change at 9–11 ms after PP test stimulation (Fig. 4B), implying a functional rearrangement of synaptic connections due to micro-circuit damage in the state of persistent nociception. In addition, the 2D-CSD image analysis can be used to map spatial patterns of synaptic connections and functions not only in hippocampal slices but also in cerebral cortex slices such as anterior cingulate cortex (ACC) and primary somatosensory cortex[21,22,31].

5 Use of MEA recordings in studies of synaptic plasticity in time

5.1 Examples of LTP recording using MEA techniques Because of its rapid induction, long duration and predominance in hippocampal pathways, LTP remains the most widely-used paradigm to study the forms of synaptic plasticity *in vitro* that underlie certain kinds of information processing and memory storage[35-37]. However, most previous data on LTP induction and expression in the HF were from glass microelectrode recordings that mainly focused on one specific pathway within a single slice. The network properties of hippocampal LTP cannot be evaluated with traditional electrophysiology, and the recording period of LTP maintenance is also limited (at most 6 h). These defects typically preclude any chance of testing hypotheses regarding neuronal interactions in spatially extended circuits over a long period. In this context, the MEA technique is a unique tool to investigate, at the macroscopic level, the network LTP phenomenon within a local neuronal circuit for a relatively longer duration. As mentioned above, the pioneering work of Oka *et al.*[16] attempted for the first time to use the MED64 system to record LTP at Schaffer collateral-CA1 synapses in acutely-prepared hippocampal slices. Subsequently, Shimono *et al.*[38] made chronic recordings of LTP in hippocampal slices cultured on MED64 probes and demonstrated that LTP in this condition can last for up to 48 h. Next, using the same MED64 system, our group addressed the LTP induction property in different neuronal circuits of the primary somatosensory cortex and found that the thalamocortical circuit was much more plastic than the intracortical circuit[22]. We also explored the effects of different TBS protocols on the induction rate of LTP in brain slices containing the ACC[21]. A probable layer-related difference in the network properties of synaptic activities in the ACC was also examined. Our results showed that long-train TBS conditioning more readily induced sustained cingulate LTP than short-train TBS, and this difference was independent of the layer in which post-TBS responses were recorded[21]. Except for the somatosensory cortex and the ACC, we also elucidated the effects of peripheral nociception on hippocampal LTP and found that both the probability of induction and magnitude of LTP were significantly enhanced by BV-triggered persistent nociceptive input (Fig. 6). This augmented LTP is dependent on the peripherally painful state and activation of mGluR5, ERK and JNK, but with negative modulation by mGluR1 and p38 MAPK in the EC–DG and EC–CA1 pathways[32,33]. Overall, using MEAs to record LTP has the following advantages: (1) it can reveal the spatial proper-

ties of LTP distribution in different microcircuits or layers within a single slice; (2) it provides a much longer period of LTP recording than conventional electrophysiological techniques; and (3) it maps LTP induction within a wider scope and reveals its alteration under pathological conditions.

5.2 Examples of LTD recording using MEAs Besides LTP, a sustained decrease in synaptic efficacy, namely LTD, is thought to contribute equally to information storage and memory acquisition in the brain[36,39-41]. Albeit with a large number of previous studies on LTD induction in many brain regions, little information is available regarding LTD recordings using MEAs in brain slices. Therefore, after dissecting out the influence of persistent nociception on hippocampal LTP, we next investigated its impact on LTD induction. In normal slices, low frequency stimulation produced an enduring synaptic depression of hippocampal synaptic responses lasting for 1 h. Interestingly, while the induction rate of LTP was apparently elevated in slices from animals experiencing inflammatory pain, the LTD induction probability was significantly lower than that in the control group (unpublished data). However, this is not the case of neuropathic pain. In hippocampal slices from animals with neuropathic pain, LTD was much more likely to occur while LTP was largely abolished (unpublished data). In summary, both LTP and LTD can be reliably recorded with the MEA system in acute or cultured brain slices for a prolonged time and across a wide spatial map.

6 Technical advantages and disadvantages of the MEA technique

6.1 Advantages The MEA technique is a unique and well-established tool for investigating the electrophysiological properties of living brain slices or cultured neuronal networks at the macroscopic level, linking single cell testing and behavioral studies[2,20]. Compared to traditional electrophysiology, the MEA technique is superior in that it enables: (1) gathering large amounts of spatial information on the internal dynamics of networks with multisite recordings[42,43]; (2) long-term analysis of the spatiotemporal distribution of network-level electrical activity[20,44]; (3) multisite stimulation and recording within one slice[20,42]; (4) evaluation of network physiological properties and the pharmacological effects of compounds[16,22,45,46]; (5) stable recording that is less sensitive to factors such as mechanical vibration[42,43]; and (6) a tremendous variety of research applications involving acute brain slices[10,16,45,47], organotypic slice cultures[38,44,46,48] and dissociated cell cultures[7,17]. It is well-suited for studying synaptic plasticity[21,31-33], single unit activity[49,50], rhythmic activity[17,45,51], and pharmacological drug testing[23,46,52]. Furthermore, powerful analytical softwares (e.g. Conductor and Mobius) make it possible to perform a broad set of analyses for different kinds of application.

6.2 Disadvantages One major disadvantage of MEA recording is that only extracellular events (spikes and LFPs) can be recorded. The signal-to-noise ratio is greatly affected by the quality of MEA probes and is far below that of intracellular or patch-clamp recording. However, 3D tip-shaped MEAs and combinations of MEA recording with Ca^{2+} imaging, patch-clamp recording, and optogenetic techniques, can resolve these problems. Another disadvantage of MEA recording is the problem of spatial resolution. Although CSD analysis provides better localization of synaptic activation sites, the inter-electrode distance is usually too large to give an accurate estimation of events within micro-circuits. However, the spatial resolution can be greatly improved by using 'high-density' conformal MEAs that have proven effective in improving the spatial mapping of electrical activity within the hippocampus (see Soussou *et al.*, chapter 4 in *Advances in Network Electrophysiology Using Multi-electrode Arrays*[2]). On the other hand, whether the source current is produced by a passive current loop or by active post-synaptic inhibitory currents cannot be derived directly by calculation. One must correlate the CSD data with the morphological data and single-cell electrophysiology before any conclusion is drawn.

7 Perspectives

Besides the fundamental work on the basic processes of brain functions and dysfunctions, the MEA technique has also been used as a test-bed for neuroprosthetic de-

vices, since it allows direct stimulation of specific brain regions and multisite recordings of spatiotemporal dynamics. For example, a group led by Theodore W. Berger at the University of Southern California has been working on 'a hippocampal cognitive prosthesis' for many years. Recently, they demonstrated that implantation of 'a biomimetic multi-input/multi-output nonlinear model' in the damaged hippocampus, which provides the capability for predicting spatio-temporal spike train outputs of CA1 based on the inputs recorded in CA3 (pre-synaptic input to CA1), successfully restored the functions of the damaged hippocampus in animal's delayed nonmatch-to-sample behavioral test[53]. Before doing this test in living animals, they designed and built a 'high-density' conformal MEA for simultaneous recordings of spatiotemporal activities along the DG–CA3–CA1 trisynaptic circuit in the hippocampal slice[2]. This successful translation of *in vitro* MEA data to pre-clinical use in behaving animal models of memory loss strongly supports the use of this technique as a rapid testing tool for the development of biomimetic neuroprosthetic devices for the treatment of brain diseases. Moreover, this success in translational research highlights the importance of network electrophysiology that enables simultaneous acquisition and analysis of both input and output electrical activities in both spatial and temporal dimensions with the unique MEA technique.

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