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A new planar multielectrode array for extracellular recording: application to hippocampal acute slice

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Abstract

The present paper describes a new planar multielectrode array (the MED probe) and its electronics (the MED system) which perform electrophysiological studies on acute hippocampal slices. The MED probe has 64 planar microelectrodes, is covered with a non-toxic, uniform insulation layer, and is further coated with polyethylenimine and serum. The MED probe is shown to be appropriate for both stimulation and recording. In particular, multi-channel recordings of field EPSPs obtained by stimulating with a pair of planar microelectrodes were established for rat hippocampal acute slices. The recordings were stable for 6 h. Finally a spatial distribution of long-term potentiation was studied using the MED system.

Keywords: Multi-channel; Acute slice; fEPSP; LTP

1. Introduction

In order to answer the question of how the brain functions, it is useful to record electrical responses of brain neural circuits to external stimuli. Since the circuit is very complicated, data should be recorded simultaneously from various points of the circuit for certain periods of time, which is necessary for the circuit to change. Among various studies pursuing multi-site recording from brain circuits, planar microelectrode arrays have been shown to be appropriate for recording responses of dissociated cultured neurons obtained from fetal and neonatal brains of rats (Pine, 1980; Gross et al., 1982). It is recognized that the networks of dissociated cultured neurons might respond differently from the neural organization of intact brains. Application of planar microelectrode arrays to acute brain tissue was reported by Novak and Wheeler (1988), in which they showed that their array can both stimulate and record from multiple sites on acute hippocampal slice preparations. Although their work definitely showed that planar microelectrode arrays should be useful for studies of brain tissue, the stimulation by their array electrodes seemed somewhat ineffective, and their recordings were limited from 2 to 4 h.

Our approach to achieve both effective stimulation and longer periods of recording time is based on two key issues: a larger size area of each planar microelectrode and the coating on the surface of the array. We have developed an array of 64 planar microelectrodes, each having a size of 50×50 μm, arranged in an 8 by 8 pattern with 150 μm of separation (the MED probe). The material of the insulating layer of the MED probe, which directly contacts the brain tissue, was carefully chosen from various materials available. The coating material and method of preparation for the surface of the MED probe were also studied. These technologies, together with electronics designed exclusively for the MED probe (namely, the MED system), enabled multi-channel recordings of field EPSPs induced by stimulating adult rat hippocampal slices with a pair of array electrodes for more than 6 h. Performance of the MED probe and the MED system was shown by an experiment in which long-term potentiation was induced and recorded in the CA1 region of rat hippocampal slice.

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2. Materials and methods

2.1. Planar microelectrode array (the MED probe)

Fig. 1A and B show the entire view and the cross-sectional view of the MED probe, respectively. First, indium–tin oxide was evaporated to form a transparent conductive layer having a thickness of 100 nm on a square glass plate ($50 \times 50 \times 1.1$ mm, Pyrex 7059) and photo-etched by the standard procedure to create 64 microelectrodes, lead wires, and correspondent connecting pads at the edge of the glass plate. The overall size of each microelectrode was 60×60 μm, and the 64 microelectrodes were arranged in an 8×8 array with an interpolar distance of 150 μm. Secondly, the microelectrodes and the connecting pads were non-electrically plated with nickel (500 nm in thickness) and gold (50 nm in thickness). Negative photosensitive polyimide (Toray: semicofine SP-910) was spin-coated to form an insulation layer having a thickness of 1.4 μm and photo-etched by the standard procedure to expose the microelectrodes and the terminals. The size of the square hole centered on each was 50×50 μm, which is the effective conductive area of the microelectrode. The insulation layer could be constructed from various materials, however, we have chosen the above-mentioned material because of its non-toxicity and transparency. Thirdly, a glass ring (I.D. = 22 mm, O.D. = 26 mm, height = 0 mm) was attached at the center of the glass plate by a silicon adhesive (Shin-Etsu Kagaku: KE 42) to form a culture chamber. Finally, 64 microelectrodes were electroplated with platinum black, using a platinum chloride solution containing 1% $H_2PtCl_6/6H_2O$, 0.01% $Pb(CH_3COO)_2/3H_2O$ and 0.0025% HCl (Gesteland et al., 1959). Plating was monitored optically under a microscope and usually took about 60 s to achieve sufficient plating. The impedance of each of the 64 microelectrodes of the MED probe was measured with an LCR meter (Hewlett Packard: 4263A) at 1 kHz, 50 mV and at 50 Hz, 50 mV signal. Typical microelectrode impedance was 6.21 ± 0.25 kΩ at 1 kHz and 41.4 ± 4.45 kΩ at 50 Hz.

The MED probe was composed of transparent materials (glass, ITO, polyimide) except for the sites of the microelectrodes and terminals, thereby allowing microscopic observation of the tissue. The light transmittance of the MED probe was over 80% (i.e. < 0.1 of absorbance) at a wavelength between 400 and 900 nm.

2.2. Measuring apparatus

2.2.1. Multi-channel extracellular recording system (the MED system)

Fig. 2 shows a diagram of the multi-channel, extracellular recording system (the MED system). The MED system consists of three units: the MED unit, the measuring unit, and the controlling unit. Electrical stimulation patterns are both designed and delivered by custom software and sent to the isolator via the data acquisition board. The stimulation signals are then directed by the 8/64 switch-box to be applied to appropriate pairs of microelectrodes on the MED probe through the MED connector. Electrical potentials captured by the MED probe are sent to the 8/64 switch-box through the MED connector. Eight selected microelectrode potentials then amplified by a factor of 10 by built-in, 8-channel, differential head amplifiers. The potentials are further amplified and filtered as necessary by the 8-channel main amplifier and are digitized by the 12-bit resolution data acquisition board operating at a 20 kHz sampling rate. The digitized data are displayed on the monitor screen and stored onto the hard disk

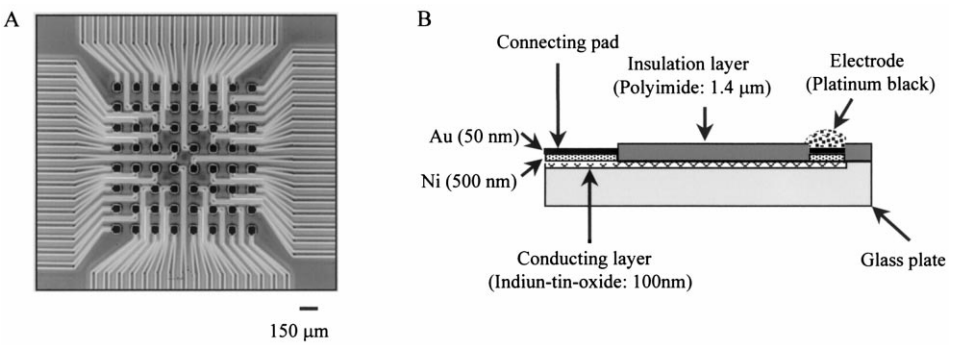


Fig. 1. Structure of the MED (Multi-Electrode Dish) probe. (A) Sixty-four planar microelectrodes arranged in an 8×8 array with an interpolar distance of 150 μm. The size of each microelectrode surface is 50×50 μm. (B) Cross-sectional view of the MED probe. Indium–tin oxide was evaporated to form an insulating layer, etched by the standard procedure to create each microelectrode, wiring and connecting pad. The microelectrodes and the connecting pads were covered with nickel (500 nm in thickness) and gold (50 nm in thickness). The entire surface was then coated with negative photosensitive polyimide resin having a thickness of 1.4 μm, and photoetched to expose the electrodes and the connecting pads. Finally, the microelectrode was electroplated with platinum black.

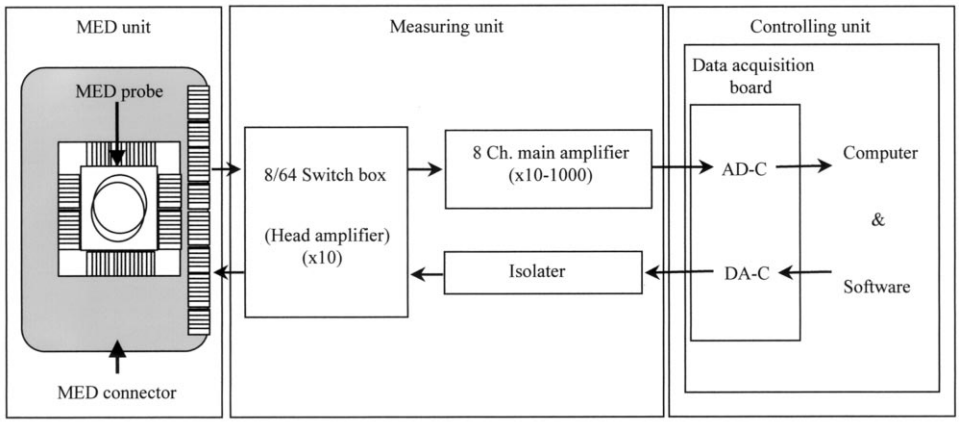


Fig. 2. Block diagram of the MED system. The MED system consists of three units: the MED unit, the measuring unit and the controlling unit. The MED unit comprises the MED probe and the MED connector, which is designed for exclusive use with the MED probe. The half-split MED connector enables easy handling of the MED probe when slices, neurons, and medium are placed in it. The six-layered printed circuit board of the MED connector allows for well-shielded experimental conditions because of its ground plane layers (1st, 3rd, 4th, and 6th) surrounding the signal layers (2nd and 5th). The measuring unit contains the 8/64 switch-box (Panasonic: SH-MED8), the 8-channel main amplifier (Panasonic: SU-MED8), and an isolator (BAK Electronics Inc.: model BSI-2). The controlling unit contains a data acquisition board (National Instruments: AT-MIO-16E-1), a personal computer running Windows 95, and custom data acquisition software (Panasonic: SAFC-80F). The software was designed to collect, display and store 8 channels of electrophysiological data recorded by the MED probe with 12-bit resolution and a maximum sampling rate of 20 kHz for each channel. Experimental parameters such as stimulating pattern, sampling rate, recording time, and the number of repetitions are set by the software. Data is stored in real-time on a hard disk drive and may be replayed and exported in several different formats for data analysis.

2.2.2. Coating of the MED probe

The surface of the MED probe was treated with 0.1% polyethylenimine in 0.15 M borate buffer, pH 8.4, for 8 h at room temperature. The probe surface was rinsed three times with sterile distilled water. The probe (chamber) was then filled with DMEM/F-12 mixed medium, containing 10% fetal bovine serum (Gibco: 16050-122) and 10% horse serum (Gibco: 16141-020), for at least 1 h at 37°C. DMEM/F-12 mixed medium is a 1:1 mixture of Dulbecco's Modified Eagle's Medium and Ham's F-12 (GIBCO: D/F-12 medium, Lot No. 1006274), supplemented with insulin (5 mg/ml, Sigma; P8783), hydrocortisone (20 nM, Sigma, H0888), putrescine (100 mM, Sigma, P5780), and selenium (20 nM, Wako).

2.2.3. Preparation of hippocampal slices

The general procedures for the preparation of the slices are similar to those described previously (Alger et al., 1984; Edwards et al., 1989). A 3-week-old male Sprague–Dawley rat was sacrificed by decapitation after anesthesia using 2-bromo-2-chloro-1,1,1-trifluoroethane (Fluothane: Takeda Chemical), and the whole brain was removed carefully. The brain was immediately soaked in ice-cold, oxygenated preparation buffer of the following composition (in mM): 124 NaCl, 26 $NaHCO_3$, 10 glucose, 3 KCl, 1.25 NaH_2PO_4, 2 $CaCl_2$, 2 $MgCl_2$, for approximately 1 min. Appropriate portions of the brain were trimmed and placed on the ice-cold stage of a vibrating tissue slicer (Dosaka: DTK-1500). The stage was immediately filled with both oxygenated and frozen preparation buffers. The thickness of each tissue slice was 300 μm. Each slice was gently taken off the blade by a painting brush, trimmed, and immediately soaked in the oxygenated preparation buffer for 1 h at room temperature. Then a slice was placed on the center of the MED probe, which was previously coated with polyethylenimine and filled with the DMEM/F-12 mixed medium containing 10% fetal bovine serum and 10% horse serum. The slice was positioned to cover the 8×8 array except for the 8 microelectrodes in the top row, which were used as reference electrodes for recording. After positioning the slice, the MED probe was immediately placed in a box filled with 95% O_2 and 5% CO_2 and allowed to recover at 37°C for 1 h.

2.2.4. Electrophysiological recording

During electrophysiological recording, the MED probe and the MED connector were placed in a small CO_2 incubator (Asahi Lifescience: model 4020) at 34°C. After recovery of the slice on the MED probe, the medium was replaced with oxygenated recording buffer of the following composition (in mM): 124 NaCl, 26 $NaHCO_3$, 10 glucose, 3 KCl, 1.25 NaH_2PO_4, 2 $CaCl_2$, 1 $MgCl_2$. The recording buffer was continuously replaced with pre-incubated (at 34°C) and oxygenated, fresh recording buffer, at the rate of 2 ml/min.

A single pair of planar microelectrodes of the 64 available was used for stimulating. Bipolar constant current pulses (10–50 μA, 0.1 ms) were produced by the data acquisition software through the isolator. The

stimulating pair of microelectrodes was selected by the 8/64 switch-box to stimulate Schaffer collateral axons in the CA3 region. Eight pairs of planar microelectrodes were used for differential recording. Each pair consisted of a recording electrode in the CA1 region and a reference electrode in the top row of the array, which was not covered by the tissue slice. Evoked field potentials at eight sites in the CA1 region were recorded simultaneously with the MED system at a 20 kHz sampling rate.

3. Results

3.1. Effect of the coating

The number of slices, which adhered to the MED probe was compared with different coating procedures. The hippocampal slice reproducibly adhered to the coated MED probe (8 adhered/8 tested), but never adhered to the non-coated MED probe (0/5). The MED probe coated only with polyethylenimine showed intermediate adhesion to slices (2/5). Coating effectiveness was also investigated by impedance measurement at 1 kHz between the microelectrode and the medium. When the slice was placed on the non-coated MED probe, the impedance was 11.9 ± 3.5 kΩ. When the slice was placed on the coated MED probe, the impedance was 37.9 ± 13.1 kΩ. Since the microelectrode itself only has an impedance of 6.21 ± 0.25 kΩ (Materials and Methods), the much greater impedance of the coated MED probe suggests sufficient adhesion of the tissue slice to the probe.

3.2. Recording of field excitatory post synaptic potential (fEPSP)

When Schaffer collateral axons in the CA3 region were stimulated by a pair of planar microelectrodes, field potentials were recorded at all eight planar microelectrodes positioned in the CA1 region. The positions of the microelectrodes for stimulation and recording are shown in Fig. 3A. In order to confirm that the recorded field potentials were field excitatory post synaptic potentials (fEPSPs), we conducted paired-pulse facilitation and inhibition using 6,7-dinitroquinoxaline-2,3-dione (DNQX), an AMPA receptor antagonist. Fig. 3B shows paired-pulse facilitation in

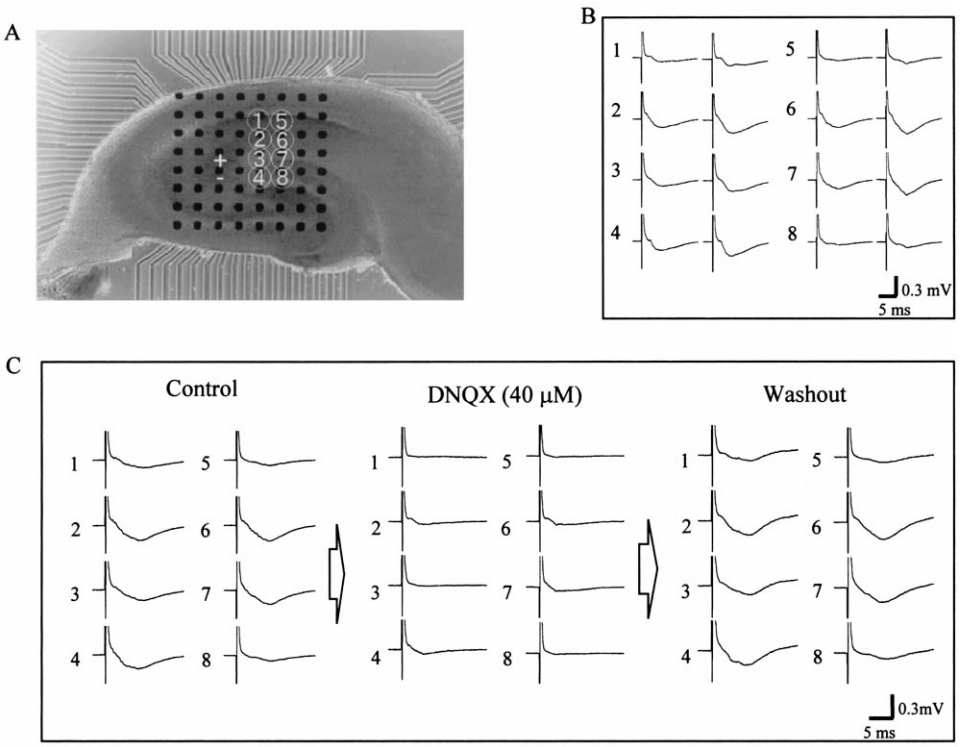


Fig. 3. Recording of field excitatory post synaptic potential (fEPSP). Schaffer collateral axons in region CA3 were stimulated by a pair of microelectrodes (+ and −) with biphasic constant current pulses (amplitude, ±20 μA, duration, 100 μs), and the evoked potentials were recorded at the stratum radiatum in CA1. Each waveform is an average of five consecutive recordings. (A) Phase-contrast microphotograph of the rat hippocampal slice as positioned on the MED probe. (B) Paired-pulse facilitation of evoked response. Two biphasic pulses were applied at a 40-ms interval. (C) Effect of DNQX (40 μM) on evoked responses. Control evoked responses were recorded for 5 min (left panel), then DNQX was applied to the chamber (for a final concentration of 40 μM). Evoked responses were recorded 10 min after DNQX application (middle panel). The slice was rinsed with recording buffer for 10 min and evoked responses were recorded (right panel).

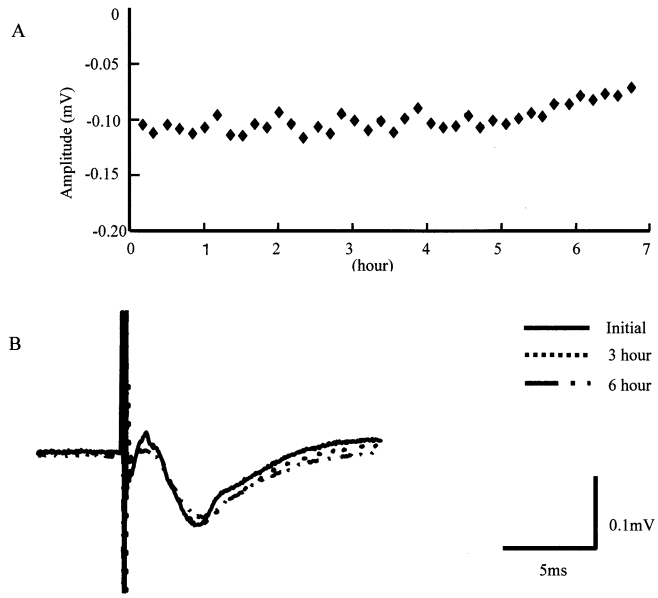


Fig. 4. Stability of fEPSP. (A) Measurements of fEPSP amplitude with time. Schaffer collateral axons in region CA3 were stimulated by a pair of microelectrodes with biphasic constant current pulses (amplitude, ±18 μA, duration, 100 μs), and fEPSPs were recorded at the stratum radiatum in CA1 for 7 h. The slice was perfused at 2 ml/min with oxygenated recording buffer at 34°C. (B) Comparison of raw waveforms recorded at channel 7 at 0, 3, and 6 h.

which two biphasic pulses were applied with a 40 ms interval. The second pulse produced larger field potentials at all eight sites. Fig. 3C shows that the evoked field potentials were totally blocked in the presence of 40 μM DNQX and subsequently recovered by its being washed out. These results confirm that the recorded field potentials using the MED probe together with the MED system were typical fEPSPs recorded at the CA1 region as a result of the stimulation of Schaffer collateral axons.

3.3. Stability of fEPSP

Stability of the fEPSPs recorded with the MED system was tested using the same positioning of the acute hippocampal slice on the MED probe. The fEPSPs were recorded every 30 s for 7 h, and peak amplitudes of all sweeps for each microelectrode were measured. Fig. 4 shows the typical change in amplitude of the fEPSP and the waveforms of fEPSPs recorded at 0, 3, and 6 h from channel 7. The peak amplitudes varied with channels (from −0.038 to −0.178 mV), however, standard deviations of the peak amplitudes were less than 0.01 for all eight channels (0.006–0.010). These results demonstrated that the recording of fEPSPs with the MED probe was stable for more than 6 h.

3.4. Observation of long-term potentiation in hippocampus CA1 region

Finally, long-term potentiation (LTP) was induced and recorded using the same experimental parameters and positioning of the slice on the MED probe (Fig. 5A). Control fEPSPs were recorded for 10 min before the conditioning stimulation. LTP was then induced by theta burst conditioning stimulation (Larson et al., 1986), which is four-pulse, 100 Hz bursts repeated ten times at 200 ms intervals (Fig. 5B). After the conditioning bursts, fEPSPs were recorded from eight sites in the CA1 every 20 s for 60 min. Potentiation occurred at all eight sites in the CA1 region and lasted for at least 60 min (Fig. 5D). Fig. 5C shows the waveforms of 8 fEPSPs at 5 min before and 60 min after the conditioning stimulation. Amplitudes after LTP were largest at channels 3 and 7, which were located at the distal area of apical dendrites of CA1 pyramidal neurons, mainly innervated by Schaffer collateral axons. LTP was reproducibly induced and recorded in more than ten slices that we have tested.

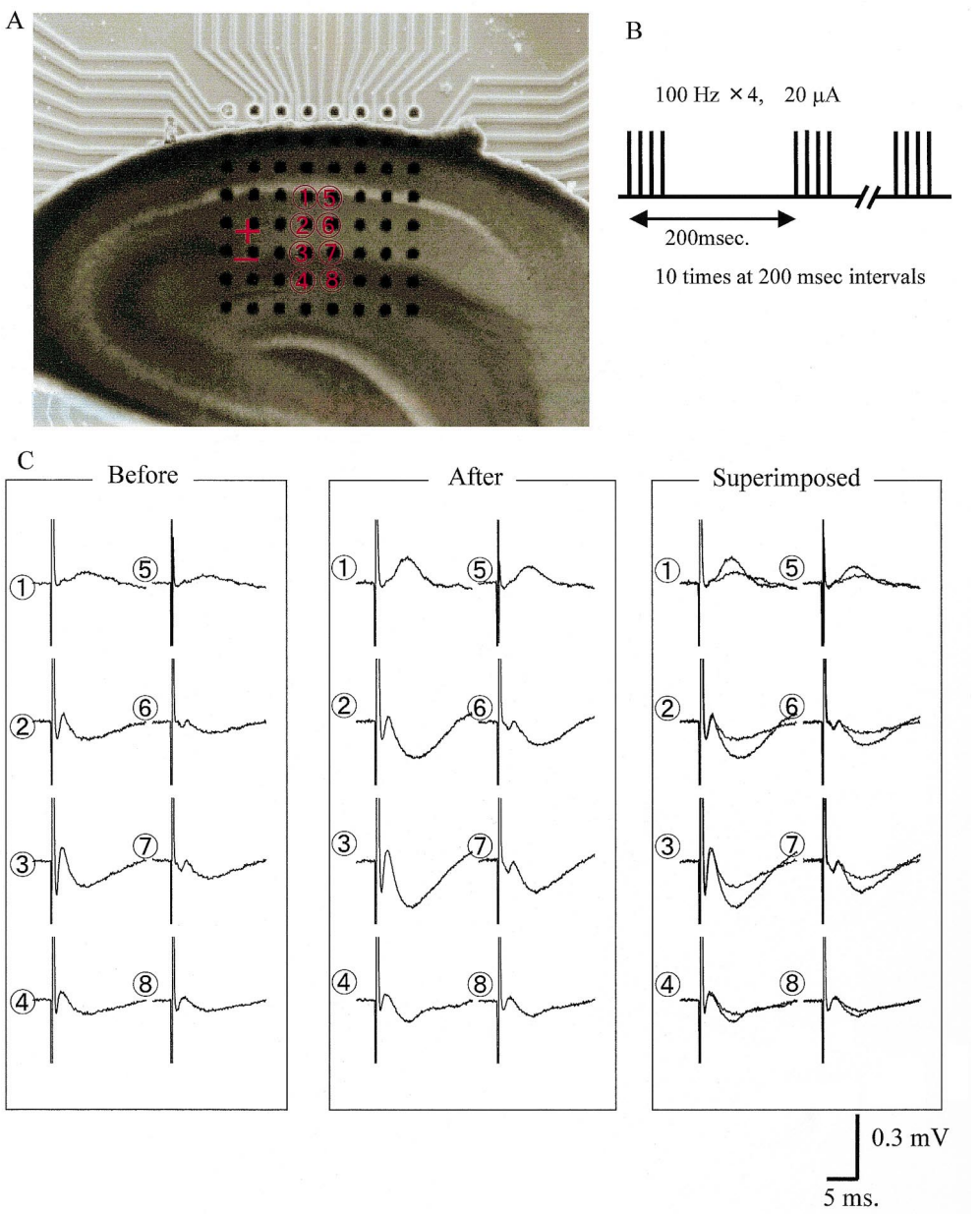
4. Discussion

We present a new planar multielectrode array (MED probe) which is used to perform electrophysiological studies on acute hippocampal slices. Long-term stable recordings of fEPSPs induced by stimulating with a pair of array microelectrodes were achieved because of two key features of the MED probe: a larger size area of each microelectrode and the method of surface coating.

The size of each microelectrode of the MED probe is 50 × 50 μm, which is quite larger than the 8 × 10–30 × 30 μm microelectrodes previously studied (Pine 1980; Gross et al., 1982; Novak and Wheeler, 1988). The most significant advantage of the larger size microelectrode is its lower impedance. With platinum black electroplating, the impedance of the microelectrode is only 6.21 ± 0.25 kΩ at 1 kHz. This low impedance microelectrode enables both reliable stimulation and higher levels of signal to noise ratio recordings.

The coating of the MED probe by polyethylenimine and serum established sufficient adhesion of the slice to the probe surface. Sufficient adhesion between the slice and the probe surface results in higher shunt resistance between the microelectrode and the medium (Gross, 1979). This explains the increase of total impedance when the slice was placed on the coated MED surface (from 11.9 ± 3.5 to 37.9 ± 13.1 kΩ). The combination of a low impedance microelectrode and a high shunt resistance results in effective electrical stimulation with little artifact (Fig. 4B).

Fig. 5.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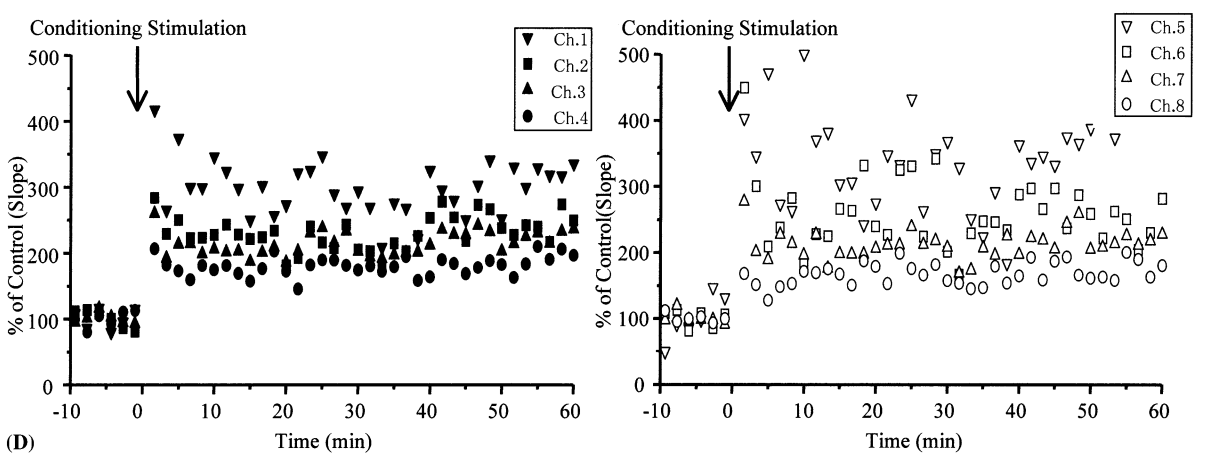
Conditioning Stimulation
Ch.1
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Time (min)
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Conditioning Stimulation
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Ch.7
Ch.8
% of Control(Slope)
Time (min)

Fig. 5.

One of the disadvantages of the planar microelectrode array is that unlike glass microelectrodes one can not individually move planar microelectrodes toward a source signal to find the best site for recording. The result is that voltage potential at the planar microelectrode is often much smaller than that of the source signal. Both planar and glass microelectrodes demonstrate further voltage drop as impedance increases, thereby resulting in a deteriorated signal to noise ratio. However, since the impedance of the MED probe at the 50 Hz principle frequency of EPSPs is only 41.4 ± 4.45 kΩ (or less than one tenth that of glass microelectrodes), we recorded EPSPs with satisfactory signal to noise ratios (Fig. 4B).

Sufficient adhesion of the slice to the MED probe surface also enabled enough perfusion by the recording buffer (2 ml/min) to keep the slice healthy for more than 6 h of EPSP recording. The adhesion was strong enough that no additional effort was necessary to hold the slice (e.g. covering net, which sometimes causes mechanical damage to the tissue).

Finally, we tested the performance of the MED probe and the MED system by inducing and recording LTP. The LTP was reproducibly induced and recorded in more than ten slices that we have tested. Average and standard deviation of the initial slope of fEPSPs before ($n = 6$) and after conditioning ($n = 25$, between 20 and 60 min after conditioning) were calculated. Slopes after LTP were larger at channels 3 (-60.4 ± 4.7 mV/s) and 7 (-29.5 ± 2.7 mV/s) which were located at the distal area of apical dendrites of the CA1 pyramidal neurons mainly innervated by Schaffer collateral axons (Fig. 5A). However, the percentage changes of slopes were larger at proximal channels 2 and 6 (230 and 256%, respectively) than the distal channels 3 and 7 (217 and 213%, respectively). The greater LTP induced at the proximal site is probably due to larger depolarization caused by current flow from both local and distal synapses during conditioning stimulation. On the other hand, depolarization at the distal site is only produced by the local synapses. Similar results were reported by Kolta et al. by measuring two sites using conventional techniques (Kolta et al., 1995).

In addition to acute slice, the MED probe seems to be appropriate for dissociated cultured neurons. Honma et al. reported an application using dissociated neurons from the rat suprachiasmatic nucleus cultured on the MED probe, in which they continuously recorded circadian oscillation of spike frequencies for more than a few months (Honma et al., 1998). While the present MED system allows only eight-channel simultaneous sampling, a 64-channel recording system is being developed in our laboratory, which will allow recording of neuronal activity from all 64 microelectrodes of the MED probe.

In conclusion, the present method of multi-channel extracellular recording may represent an early attempt for long-term, multi-site studies of brain networks in vitro. Simultaneous recording of many sites with the MED system enables the detection of 'network level' spatiotemporal patterns. Compared to conventional electrophysiology, the richness and extent of network level patterns make it particularly appropriate for studying psychoactive drugs and for defining the types of computations carried out by various brain networks.

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Fig. 5. Multi-point recording of LTP. Schaffer collateral axons in region CA3 were stimulated by a pair of microelectrodes (+ and −) with biphasic constant current pulses (amplitude, ± 20 μA, duration, 100 μs), and fEPSPs were recorded at the stratum radiatum in CA1. The slice was perfused at 2 ml/min with oxygenated recording buffer at 34°C. (A) Phase-contrast microphotograph of the rat hippocampal slice as positioned on the MED probe. (B) Conditioning stimulation is four-pulse, 100 Hz bursts repeated ten times at 200-ms intervals. (C) Field EPSPs recorded 5 min before and 60 min after the conditioning stimulation, respectively (left and middle panel). Superimposed waveforms are shown in the right panel. Each waveform is an average of five consecutive recordings. (D) Measurements of initial slope before and after conditioning stimulation.