Cortical signal recording using an economical microelectrode fabricated on printed circuit board

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Abstract: - This work presents a simple, flexible and economical microwire array electrode for extracellular cortical recordings. The proposed procedure is relatively simple, even for a novice worker to implement in-house. These main steps include design and sculpturing PCB, straightening microwires, connecting PCB pattern, arraying and soldering microwires and packaging the microelectrode. A practiced researcher can assemble the microelectrode in about two hours and implant it in approximately three. The mass of this assembled microelectrode is 1.96g. The cost of the materials in the entire array is less than US$1.5, and the array is suitable for implantation in the cortex of rats for neurophysiology studies. In this study, electrochemical impedance spectroscopy is also applied to measure the impedance and the phase between the electrode and the electrolyte, and then to obtain an equivalent circuit. The improved microwire array electrode is adopted to record the extracellular cortical signal of cerebrum. The microwire array electrode can be fabricated and used for multi-site, multiple single-unit recording experiments.

Key-Words: - Microelectrode, Printed circuit board, Flexible flat cable, Extracellular, Electrochemical impedance spectroscopy

1 Introduction

Numerous neural probes have been developed, including bundles of microwires, microwires embedded in neurotrophic assemblies, polymer substrate probes and several silicon-substrate probes. Various microwire array electrodes have been utilized in multi-site, multiple single-unit recording experiments. For example, Chapin *et al.*, (1999) constructed 24 microwires to use in rats and Wessberg *et al.*, (2000) designed 96 microwires to apply in monkeys can be feasibly used to record neural signal in conscious animals [1-2]. Many multi-electrode arrays can be bought from commercial vendors to suit various experimental designs. They involve various cost, time, materials, tools, and technique of microelectrode manufacture, offering flexibility of the experimental design. Developing in-house fabrication methods remains very worthwhile especially for those research teams that are limited by funds.

This work describes a simple, flexible and economical microwire array electrode for extracellular cortical recording. The fabrication of the microwire array electrode includes connecting PCB pattern, arraying and soldering microwires and packaging the microelectrode. A six-channel microwire array electrode for multi-site is described to elucidate the fabrication procedure. Electrochemical impedance spectroscopy is used to measure the impedance and the phase between the electrode and the electrolyte, and then to obtain an equivalent circuit. Finally, the proposed microelectrode is applied successfully to record extracellular cortical signals at the right S1HL of the primary somatosensory cortex (SI) upon mechanical stimulation using a brush.

2 Materials and methods

A six-channel microwire array electrode for a multi-site is described to illustrate the fabrication procedure. Implementing a microelectrode requires about two hours.

2.1 Materials

In the presented approach, the microwire array electrode is composed of Teflon insulated tungsten microwires, a printed circuit board (PCB) pattern, a flexible flat cable (FFC) and connector and viscose of epoxy A+B that is proposed herein. Fabricating a microelectrode requires under US$1.5 in materials.

2.1.1 Microwire

In this study, the microwire array electrode consists of 50um-diam Teflon-insulated Tungsten wires (A-M Systems, Carlsberg, WA; #795500). Tungsten wire offers the highest strength and stiffness of all A-M system wires. Teflon-insulated tungsten wires are typically 25 feet (7.5 m) long per spool. They are cut every 20 mm into microwire probes. Every spool costs $60. Therefore, the average cost of each section is $0.157. This material is chosen because it is cheap, hard and, above all, easily processed by an individual.

2.1.2 Printed circuit board

The printed circuit board is double-sided, 1ounce of copper and FR-4 epoxy glass with a thickness 0.064". The market cost of an area of 100 square cm of glass-epoxy double-sided photo-resist board. Therefore a PCB with an area of around 1 square centimeter costs under US$0.012. Such a PCB after design and sculpturing is regarded as the base of the microelectrode, and is called the PCB pattern. The PCB is used because it is light and thin, universal, inexpensive and easy to obtain. In particular, the PCB pattern has a flat surface and a convenient workbench can be used to fabricate the microwire array electrode. The user can conveniently and rapidly revise it to various experiments.

2.1.3 Flexible flat cable

The flexible flat cable is B-type (with conductor exposed on one side), with a 1.0 mm pitch, a total length of 100 mm and conductor pins. A connector, mounted to one or both ends of the FFC, is generally employed with a set of electrical receptacles or sockets that are designed to receive terminal posts or contact pads on the printed circuit board. The FFC connector has a 1.0 mm pitch and ten conductor pins. The price of one set is US$ 0.358; each set includes both FFC and connector. The advantages of selecting this FFC and connector include precision, recyclability and flexibility. In particular, plugging in and pulling out can be repeated easily. These advantages reduce injury to the rat during the experiment.

2.2 Methods

The PCB pattern of eight-channel microelectrodes is used to implement the fabricative process. A six-channel microwire array electrode is fabricated to cooperate with the bio-signal capture and recording system and is applied to measure the cortical signal. These main steps of a microelectrode fabrication include design connecting PCB pattern, arraying and soldering microwires and packaging the microelectrode. Before an electrode fabrication can work, the layout and the sculpturing of the PCB must be completed, and the microwires straightened. The procedures and techniques of operation are introduced separately below.

2.2.1 PCB design and sculpturing

In the experiment, a two-sided PCB is used. The PCB for each microelectrode is 11.0 mm long, 9.0 mm wide, and approximately 1.6 mm thick. A specially designed PCB is developed to bond the microwire array to the electrode. The PCB after finish procedure of design and sculpture becomes PCB pattern. The front of the PCB pattern contains nine elliptical pieces of copper which have an area of 2.2 × 1.2 mm^2 and one rectangular piece of copper which has an area of 0.7 × 4.7 mm^2. The vertical separation between the ellipses is 1.75 mm and the horizontal separation is 3.0 mm, as displayed in the picture on the left in Fig.1. The tops of the individual microwires are cleaned and soldered onto an elliptic piece of copper. The back of the PCB pattern contains ten finger connectors, which match correspond to a high-density ten-pin single row FFC connector matrix, presented in the right-hand image in Fig.1, for use in chronic experiments. The inter-finger connector separation is 1.0 mm for a total width of about 9.0 mm. The routing connections of are from the penetrating pads to the finger connectors.

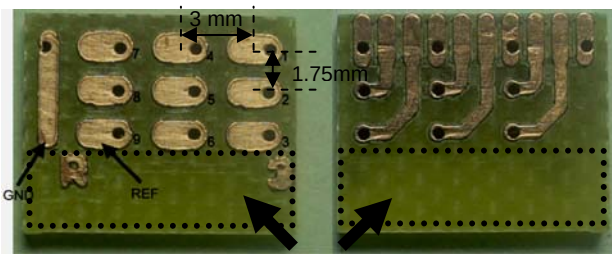

Fig.1 PCB pattern of eight-channel microelectrodes. The serial numbers of nine elliptical pieces of copper run from the top-right to the bottom-left. The left-hand image is the front of the PCB pattern. The right-hand image is the back of the PCB pattern.

The PCB can be made by a board manufacturer or by the experimenter, and its dimensions depend on the practical experiment. The PCB pattern provides a flat surface to supply as the fabrication plate, facilitating the fabrication of the microwire array electrode. Especially while arranging

microwires into a desirable configuration and soldering microwires to the corresponding copper. In order to increase the precision and convenience while arranging the spacing of the electrode tips and separation of the inter-electrode, the microwire slots can be processed on the under blank of PCB pattern by PCB sculpture machine, laser cutting machine or board manufacturer.

2.2.2 Straightening the microwires

Microwires cut from the spool are curved using nickel-plated steel wire scissors (5 1/2 inches); thus, they are unsuited to the fabricating electrode arrays. The curved microwires are straightened by a pair of nickel-plated hemostats (5 1/2 inches, Kelly hemostat, straight). Spooled microwires were cut into 10 cm segments. A hemostat is clipped to each end of the curved microwires; the upper end is fixed while the lower end is gently allowed to spin until the microwires are straightened. The straightened microwires are cut into 2.0 cm segments, and around 2 mm of insulation is removed from one end by briefly holding it over a small flame.

2.2.3 Connecting PCB pattern

The first operation is the linking of the FFC connector to the PCB pattern. A soldering iron is used to weld each pin of the connector to a pin on the bottom layer of the PCB, as shown in Fig.2A. Then, the two-sided adhesive tape is cut into lengths of 5mm and widths of 2mm. The tape is stuck on the back of the connector which has already been welded onto the PCB. The two-sided adhesive tape is used to fix the PCB to the work bench. After it has been positioned, the PCB is not moved during the subsequent steps. This entire process takes approximately ten minutes.

2.2.4 Arraying and soldering microwires

The two-sided adhesive tape is again cut to lengths of 3mm and widths of 1mm. It is stuck onto the layer below the top layer of the PCB pattern: this blank space has no copper. The tape is then used to fasten lightly the microwires to the PCB pattern. Six cut microwires are placed on the adhesive tape sequentially. A dissection microscope is applied to help adjust the relative positions of the two tips. When all of the microwires are properly positioned, a small drop of epoxy glue is spread on a site close to the bottom edge of the PCB pattern to secure them, as shown in Fig.2B. Microwire arraying takes about ten minutes, but fixing the microwires solidly takes 30 minutes.

When the epoxy glue has solidified, the longer ends of the microwires are ordered connected to the corresponding pieces of copper. About 2 mm of insulation is removed from one end of each of the straightened microwires by briefly holding it in a small flame. A soldering iron is used to weld each microwire to the corresponding copper. Consider the six-channel microelectrode as an example: the first six copper dots on the top layer of the PCB pattern are used soldered to the six microwires. A stainless-steel wire is soldered to the ninth copper dot, as shown in Fig.2C. This wire is the reference end of the signal capture circuit. The tenth copper dot of the PCB top layer is connected to the ground end of the signal capture circuit and the body of the animal.

2.2.5 Packaging the microelectrode

When all microwires and other signal wires have been successfully soldered to the top layer of the PCB pattern, the surface of the PCB pattern is packaged using epoxy, as shown as Fig.2D. The soldering of the microwires probably takes half of an hour, and another half an hour is required for the packaging of the microelectrode to solidify.

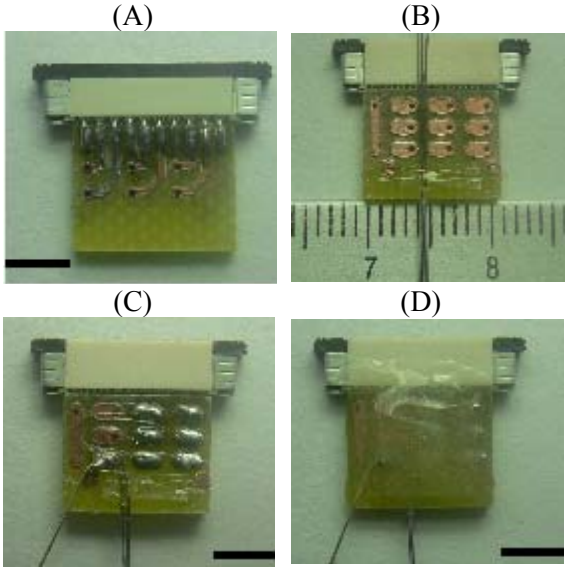

Fig.2 Fabrication of six-channel microwire array electrode for multi-site recording. Calibration bars of (A), (C) and (D): 5 mm.

If an eight-channel microelectrode is to be fabricated, then the microwires are welded to the dots of the PCB pattern from the first to the eighth. The microwire array electrode is required for use in a multiple single-unit recording experiment, then

given a slight modification of the PCB layout, the procedures are similar to those associated with the multi-site microelectrode. In Fig.3, the 16-channel electrode is a sample that is finished for use in a multiple single-unit recording experiment. The microwire array microelectrode comprises 16 wires that are aligned in series file, with an inter-electrode separation of 0.45-0.55 mm for a total horizontal span of 7.5-8.0 mm. The microelectrode in this style is suited to single-unit recording from a large brain region, such as the primary somatosensory cortex, the primary motor cortex, and the occipital cortex in the rat.

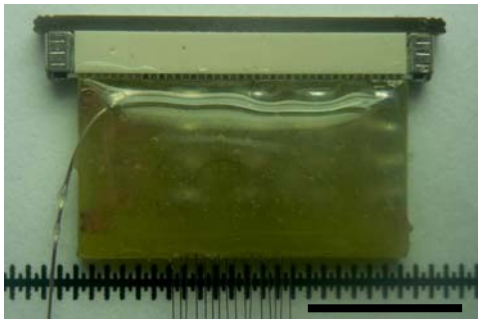

Fig.3 Assembly of 16-channel microwire array electrode for multiple single-unit recording. Calibration bars of 10 mm.

3 Results

3.1 Microelectrode testing

Electrochemical impedance spectroscopy (EIS) is performed on a microwire probe array in an artificial cerebral spinal fluid (ACSF) at room temperature. The composition of the ACSF is NaCl 6603 mg/L, KCl 223 mg/L, NaH_2PO_4 165.5 mg/L, $CaCl_2$ 220.5 mg/L, $MgCl_2$ 203.3 mg/L, $NaHCO_3$ 2520 mg/L and Dextrose 5405 mg/L [3]. An impedance spectrum analyzer IM6ex (IM6ex, ZAHNER-elektrik GmbH & Co. KG) is used. Thales software automatically supports all options and processes. A silver/silver chloride reference electrode and platinum counter electrode are utilized.

Measurements are made over a frequency range of 100 Hz to 50k Hz at open circuit potential with a sinusoidal perturbation voltage of 20 mV. The impedance spectroscopic measurements characterize the electrochemical properties of the electrode and ensure that the electrical integrity of the signal path is maintained after packaging and implantation in the brain. Figure 4 plots results for channel 4 of a six-channel microelectrode measured from the EIS. The impedance values of the channels associated with our proposed design, from EIS graph, are 200 kΩ, 220kΩ, 225kΩ, 225kΩ, 190kΩ and 215kΩ, respectively. A mean impedance of 212.5kΩ, standard deviation of 13.15kΩ and coefficient of variation of 6.18% are obtained for six-channel microwires electrode at 1 kHz. The phases of the channels are 50deg, 55deg, 47deg, 40deg, 57deg, and 52deg respectively. The average phase is 50.17 degrees; standard deviation is 5.58 degrees and coefficient of variation is 11.12%. Observe the coefficient of variation of impedance and phase that the presented microwire array electrode is suitable for recording electro-physiological activity.

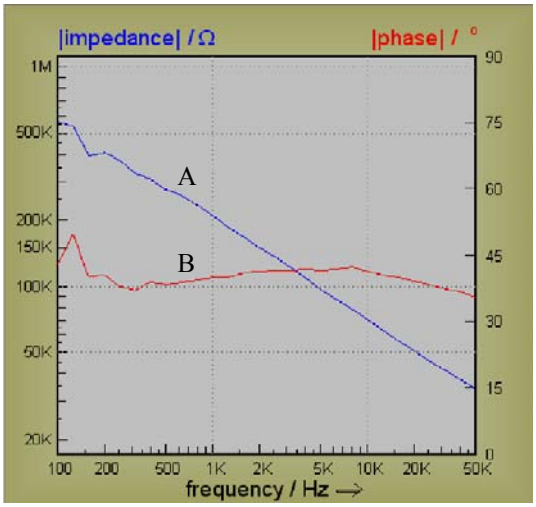

Fig.4 Relationships between impedance and frequency (mark A) and between phase and frequency (mark B) for channel 4 of a microelectrode, measured using EIS. The horizontal axle represents frequency; the left-hand vertical axis represents impedance and the right-hand vertical axis represents phase.

Regression of the impedance data is performed to obtain an equivalent circuit describing the physical nature of the electrode/electrolyte interface. Its impedance modeled by "Randles'" equivalent circuit represents the most appropriate circuit of microwire electrode. Taking channel 4 as an example, its equivalent circuit is shown in Fig.5 Parameters of the circuit reflect a liquid volume resistance Rs and an electrode-liquid interface represented by a double layer capacitance C connected in parallel with a charge transfer resistance Rw and a mass transfer impedance W. Electrodes such as those discussed in Moxon KA et al., 2004 and Nordhausen EM et al., 1996 have impedances from 80kΩ to 4MΩ, typically measured at 1 kHz in saline solution [4-5]. EIS testing

indicates that this improved microwire array electrode is suitable for recording the extracellular cortical signal.

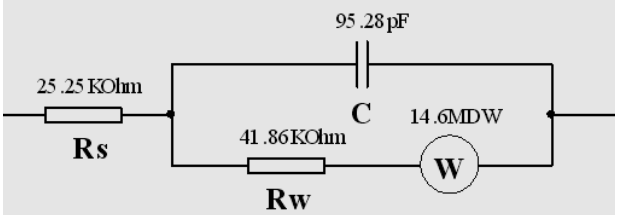

Fig.5 Randles' equivalent circuit of channel 4 showing the solution resistance Rs, double layer capacitance C, charge transfer resistance Rw and mass transfer impedance W.

3.2 *In vivo* application

3.2.1 Microelectrode implantation

Adult male Wistar rats (400–500 g) from the National Defense Medical Center Laboratory Animal Center were maintained in a colony room. All experiments were performed following the guidelines of the Institute of the National Defense Medical Center. All efforts were made to minimize animal suffering and the number of animals used.

The observation site was located in the right hind-leg region of the cortex. One small craniotomy was made to implant the microelectrode array. The coordinates of the craniotomy were, based on the atlas of Paxinos and Watson [6], as follows for the primary somatosensory cortex (SI); 1.5 mm posterior to the bregma, 3.0 mm lateral to the midline and 2.0 mm ventral to the skull surface. Arrays of Teflon-insulated tungsten microwires (50um diameter) were slowly lowered into the target areas. The microelectrode arrays were secured onto the cranium using dental cement and skull screws as anchors. Animals were administered penicillin before surgery to prevent infection and housed individually after surgery. All of the experiments were performed following the guidelines of the "Animal Experiments Committee of the National Defense Medical Center".

3.2.2 Intracortical recording

The proposed microelectrode was used to observe the neural signal from the primary somatosensory cortex (SI) that was evoked by mechanical stimulation of a brush. A six-channel electrode was used to record extracellular cortical evoked potentials in the right S1HL and a reference electrode was placed in the cerebellum region. To record the neuron-evoked potential, a brain signal capture and recording system was used. The recording system comprised a pre-amplifier, a filter with a band-pass of 300Hz–3,000Hz and a post-amplifier. The total gain of each channel was adjusted to 10,000. The sampling rate of recording was 30k Hz; the data acquisition system was based on a PC. In the experiment, only the evoked potentials under external stimulus were observed.

The responses of an anesthetized rat to stimulation the claw of the left hind leg by scratching using a brush are obtained. In Fig.6, the top window plot, Fig.6A, shows the period of 1 s; the middle window, Fig.6B, covers the period from 0.58 s to 0.76 s. The bottom window, Fig.6C, plots one section of the raw recording from 0.645 to 0.685 s. This section presents the potential evoked when the left back sole of the rat is stimulated. The improved microwire array electrode can be feasibly used to record the neural signal in vivo. Figure 7 presents the conscious rat in which the microelectrode has been successfully implanted.

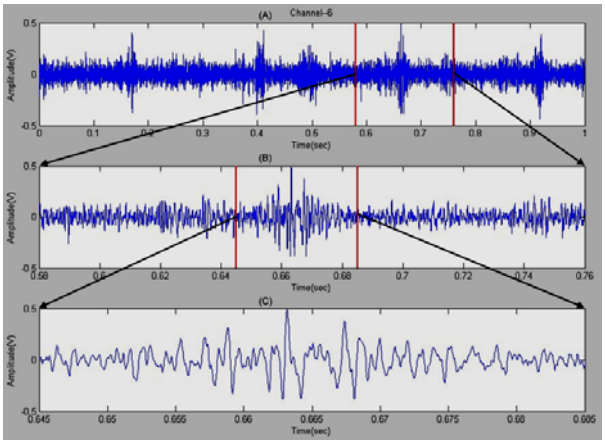

Fig.6 Evoked potential waveform when left back paw of rat is stimulated.

Fig.7 Conscious rat with successfully implanted microelectrode.

4 Discussions

A microelectrode must frequently be inserted and

extracted from an overhead socket of a rat during experiments. This process can easily injure the animal and affect the quality of implanted microelectrode. In this study, a lithe FFC and a precise connector are used. It can be easily repeatedly plugged in and pulled out. A simple, flexible and economical microwire array electrode can reduce injury to the rat during an experiment to measure the extracellular cortical signal. The variation among the impedances of the channels of the microelectrode is important. Carelessly it can cause additional interference signals and induce the preamplifier go into saturation. Therefore, length, region of insulation and area of the contact-tip of each microwire must be carefully examined, during the assembly of a microwire array electrode. In this work, electrochemical impedance spectroscopy is performed on a microwire array electrode in artificial cerebral spinal fluid. The measurements reveal that the microelectrodes are suitable for recording electro-physiological activity. The microelectrode impedance data are regressed to obtain an equivalent circuit of the electrode/electrolyte interface.

The thing about the spacing of the electrode tips have been discussed in many literatures. An array of parallel wire electrodes is constructed by using two springs [7]. A special fabrication jig is built by draping the microwires over fine musical instrument wire [8]. Tsai and Yen (2003) described a simple method for constructing a variety of shapes and sizes of fabrication jig by two slim bamboos onto a piece of paper [9]. The presented method is that the PCB pattern provides an integrative shaping, flexible outline and flat surface to supply as the fabrication plate, facilitating the fabrication of the microwire array electrode. Especially while arranging microwires into a desirable configuration and soldering microwires to the corresponding copper. The spacing among the electrode tips and the inter-electrode separation can be adjusted under a dissecting microscope: spacing can be reduced and separation set more precisely. The dimensions and shape of this PCB pattern can be elastically adjusted based on the position of the implanting or the range of the recording. A researcher can conveniently design and revise it to meet the demands of differential experiment.

5 Conclusions

Advantages of the proposed microwire array electrode include the universal availability of its material, its cost-effectiveness, the general availability of the tools required to produce it, simplicity, convenience, ease of reproduction and flexibility in meeting the requirements of various types of research. The manufacture of electrode arrays takes approximately two hour. An assembled six-channel microwire array electrode has a mass of only 1.96 g. The material of the entire microelectrode costs less than US$ $1.5. The procedure described herein is relatively simple even for a novice worker to implement in-house. The presented microelectrode is successfully adopted to record the extracellular cortical signal. The PCB layout files and a small sample are available free of charge upon request: please provide an e-mail address and return postage.

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