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Highly-Accurate, Implantable Micromanipulator for Single Neuron Recordings

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Abstract— **A precise and implantable micromanipulator is presented for automatically advancing electrodes during single unit recordings in freely-behaving animals. The modular design and enhanced clamping mechanism with simple mechanical components are designed to provide reliable linear motion using a piezo motor with a stroke of 3 mm. To be specific, a closed loop control system, based on the position feedback from a magnetoresistive (MR) sensor, was implemented to overcome the non-linear characteristics of the piezo motor and to locate electrodes precisely at the targeted position with the accuracy of 1 µm, even under load. The weight of the micromanipulator is only 0.84 g when it is fully assembled with the MR sensor, PCBs, and connectors. In addition, a protective cover is employed to prevent breakage during semi-chronic recording. The positioning performance of the micromanipulator was tested at various loading conditions using various control methods. Finally, the activities of a single unit were isolated successfully using small step adjustments, such as 1 to 5 µm, in freely-moving mice.**

I. Introduction

Micromanipulators are widely used during extracellular, single unit recordings in neuroscience research. The micromanipulators have the ability to move electrodes up and down, thereby allowing the exploration of regions of interest in the brain. This capability increases the probability of measuring the activities of specific neurons. Although commercial micromanipulating devices, such as stereotaxic instruments, are capable of positioning electrodes manually or automatically with high precision in anesthetized animals, implantable micromanipulators can be used with animals that are awake and behaving freely. This latter capability is required to investigate the functions and roles of specific brain regions, such as sensory/motor correlations, learning/memory, and decision-making processes [1], [2].

Implantable micromanipulators have been employed commonly in relatively large animals, such as monkeys [3], pigeons, and rats [4] because of the minimal restriction associated with the size and weight of the recording devices. However, it has been a challenge to record neural signals from freely-behaving mice due to their relatively small size compared to the recording devices. Even so, several varieties of genetically engineered mice are preferred for use in studying many neurological disorders [5-7]. Therefore, lightweight, manual micromanipulators, which are operated by hand to advance the electrodes, have been developed in order to increase the probability of obtaining single cell recordings from the desired neurons in freely-behaving, small animals [5], [6]. However, the relatively rough adjustment of electrodes by rotating screws for micromanipulation does not allow single units to be isolated easily, which generally requires the adjustment to be less than 20 µm. In addition, the animals must be restrained while the electrodes are advancing, which can cause abrupt changes in the location of the electrodes due to the animal's struggling. Consequently, we are proposing micromanipulators that are capable of positioning electrodes automatically.

Generally, micromotor-based micromanipulators have been researched [8-11]. However, components such as micromotors, reduction gears, and screws for yielding linear motion are likely to increase the overall size of the micromanipulators. This, in turn, can lead to increases in the frequency of bumping inside the recording chambers, resulting in drifting of the electrodes. Also, the micromotors lack sufficient torque and accuracy in the advancement of electrodes, since small-step adjustments are likely to vary depending on the loads encountered inside the brain and the friction among the mechanical components.

Other researchers have studied micromanipulators that are based on piezoelectric actuators for precise manipulation [7], [12]. However, high costs and the significant weight of conventional piezoelectric actuators are major limitations, even for recording in rats [12]. Although PZT (piezoelectric ceramic material)-based micromanipulators have been used with mice, high voltages are required to operate the PZT stacks sequentially, which imposes difficulties in assembling unit PZT stacks [7].

Recently, a piezo-motor-based micromanipulator, which was highly precise, small, lightweight, cost-effective, and suitable for extracellular, single unit recording in freely behaving mice, was proposed [13]. However, even though a simple advancing mechanism with a plate spring and a piezo motor was accomplished, tuning the frictional force between the plate spring and the rod of the piezo motor was arduous. In addition, this micromanipulator must be retuned occasionally since the screw that holds the plate spring can loosen.

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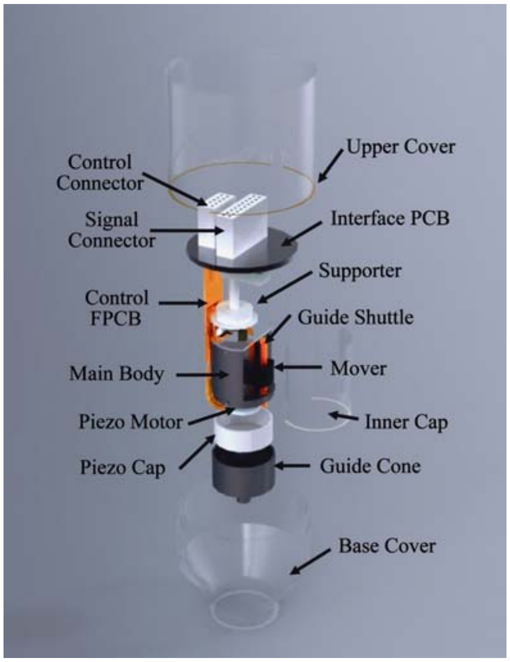

Fig. 1. Design of the proposed micromanipulator

Significantly, the linear motion by the flexural vibration of the piezo motor exhibits non-linear characteristics when several pulses are applied stepwise. For this reason, the pulse-based control method has difficulty in manipulating electrodes compared to the desired displacement. In addition, the actual displacement can be rather different from the targeted displacement when undesired loads occur, such as the clogging of electrodes or when the mechanical components abruptly encounter additional friction.

Therefore, a more reliable and controllable piezo-motor-based micromanipulator is proposed for use during semi-chronic, single unit recordings in freely-behaving small animals. We used the proposed micromanipulator to conduct micro-driving tests under various conditions and recorded extracellular, single unit activities in freely-behaving mice. The results of these tests confirmed that the proposed micromanipulator provides enhanced manipulation performance.

II. Semi-Chronically Implantable Micromanipulator

An innovative micromanipulator for single unit recordings in a freely-moving small animal is proposed. The proposed

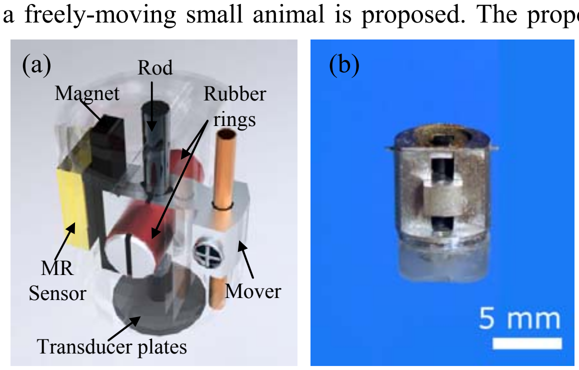

Fig. 2. Design and fabrication of micro-driving unit

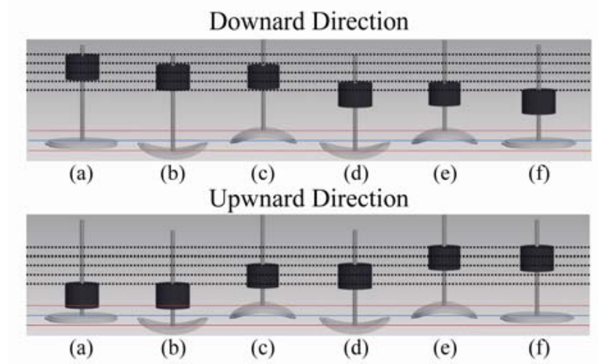

Fig. 3. Principle of micromanipulation

micromanipulator, shown in Figure 1, can be semi-chronically implanted, and the implantation is simple to accomplish.

A. *Design and Fabrication of the Micro-driving Unit*

The advancing mechanism in the micro-driving system for the movement of the electrodes is accomplished by a piezo motor (TULA35, Piezoelectric Technology Co., Ltd.), a pair of movers, and rubber rings, as shown in Figure 2 (a). The mover locked onto the rod of the piezo motor is driven by the difference between frictional and inertia forces, in accordance with the principle in Figuire 3.

From the initial position shown in Figure 3 (a), when voltage is applied slowly to piezoelectric transducers, causing the transducer plates to bend downward, the mover also moves down without sliding (Figure 3 (b)). This occurs because the static frictional force between the rod and the mover is greater than the inertia force. Then, when the voltage is eliminated or applied abruptly to the inverse direction, the mover maintains its position due to the inertia force (Figure 3 (c)). By repeating this sequence (Figures 3 (d-f)), the mover on the rod goes down. In a similar manner, when the voltage increases rapidly, the mover holds its position even though the plates are bent downward (Figure 3 (g)). Then, by decreasing the applied voltage or by slowly applying the voltage in the inverse direction, the mover follows the movement of the rod (Figure 3 (h)). By repeating several sequences, the mover moves upward (Figures 3 (i-k)).

In the fabrication of the micro-driving unit, the rod of the piezo motor was clamped onto a pair of v-grooved movers, which were held by rubber rings in order to generate a uniform holding force for the movers. Thus, this clamping mechanism provides robust and reliable motion to the movers without difficulties, such as the need for re-tuning (tightening a screw) in order to yield the appropriate holding force to the plate spring. Then, the mover is guided linearly through a slotted main body instead of using a guide pin to prevent the mover from rotating.

The modular design, such as the cylindrical package of the micro-driving unit, allows the reliable advancement of the electrodes and reduces the weight of the micro-driving unit to 0.36 g. Thus, the unit meets the overall requirement for the design of a micromanipulator for small animals, i.e., it is

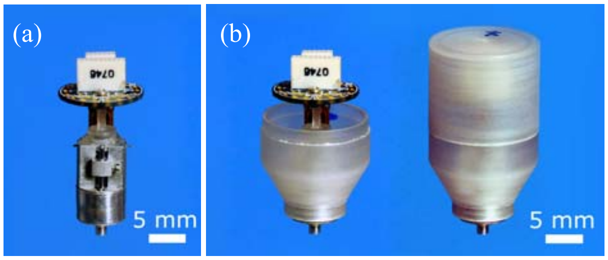

Fig. 4. (a) Fabrication of the proposed micromanipulator, (b) Micromanipulator with the protective covers

small, has a very low weight, provides precise advancement of the electrodes, is stable, is simple to install, and has a low cost [4]. In addition, the magnetoresistive (MR) position sensor used in the device (KG0924, Shicoh Engineering Co., Ltd.) is integrated on the wall of the main body in order to measure the current displacement of the mover-carrying electrodes. This arrangement allows the generation of sine and cosine signals according to the linear motion of the mover.

B. *Assembly of the Micromanipulator*

For semi-chronic implantation, the guide cone is assembled with the micro-driving system; thus, the micromanipulator is easily implanted in the heads of animals, allowing the bundle of electrodes to be aligned coaxially on the anchor of the cone. In addition, the replaceable guide shuttle allows the micromanipulator to be reused easily by installing new electrodes.

The interface PCB and connectors (NPD-18DD-GS for neural signal acquisition and NPD-10DD-GS for motion control, Omnetics Connector Corp.) are held in place by a supporter. The connector that transmits neural signals is compatible with the commercially available headstage (HS-16-CNR, Neuralynx, Inc.) as shown in Figure 4 (a). Finally, the covers for the top of the device and its base protect the micromanipulator from external impact and against water infiltration, as shown in Figure 4 (b). Therefore, the proposed micromanipulator is highly suitable for semi-chronic and repetitive use for in vivo, single unit recordings.

C. *Control System for the Micromanipulator*

For the advancement of the electrodes attached to the mover, a control system that is based on a digital signal processor (DSP) (TMS320F2808, Texas Instruments Inc.) and that provides five basic functions is used. These five functions are the generation of pulses, sensing and analyzing the output of the MR sensor, control of position feedback, communication with a PC or external panels, and display of status information on an LCD, as shown in Figure 5.

The piezo motor enables the mover to move upward and downward when pulses are applied to the piezo motor at a frequency of 110 to 130 kHz, a voltage of 10 to 30 V, and a duty ratio of either 75% or 25% for upward and downward directions, respectively.

First, the induced sine and cosine signals from the MR

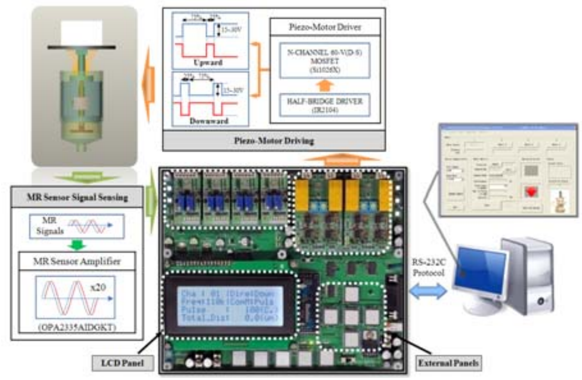

Fig. 5. Control diagram of the micro-driving system and fabricated controller

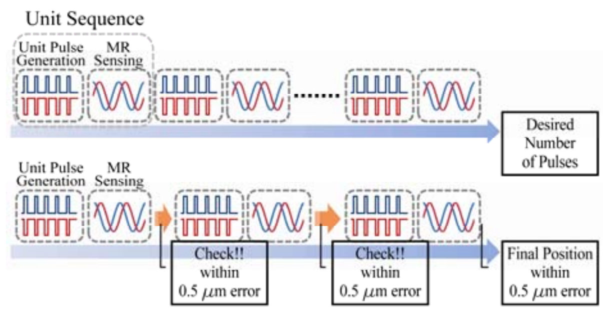

Fig. 6. Sequence of pulse and displacement-based control methods

sensor are amplified. Then, depending on the movement of the magnet attached to the mover, the phase difference is calculated and converted to displacement. Initially, the sensing procedures are calibrated to ensure accurate position sensing. The MR signals are acquired while the mover scans the whole range of the stroke. The maximum and minimum amplitudes of sine and cosine signals are obtained, and, then, the ratio of the peak to peak amplitudes of the two signals and the offset value are calculated. These calibration factors compensate for the calculation of the phase difference.

In order to position the electrodes accurately even when they are subjected to loads, the feedback control algorithm for the control of the motion of the mover is implemented on the DSP. First, for a pulse-based input, a small set of 2 – 10 pulses, is applied to the piezo motor. Then, the MR sensor signals are acquired consecutively 50 to 200 times with 40 kHz sampling, and the signals are averaged and calculated to the displacement of the mover. This sequence is repeated until the number of the applied pulses reaches the desired number. During the control, the position sensor operates only to monitor where the electrodes are located during movement. Alternatively, for displacement-based control (feedback control), sequences similar to the pulse-based sequences are repeated until the mover converges to within 1 μm of the targeted position, as shown in Figure 6. As a result, unlike other motorized micromanipulators, the proposed micromanipulator can position the electrodes on targeted regions successfully, even though undesired loads may be encountered due to the clogging of the electrodes or abrupt changes in friction may be incurred by the mechanical components [7-13].

The micromanipulator is operated primarily via RS-232C serial communication between the control board connected to the interface PCB and the PC. In addition, most functions can also work properly with a combination of the external control panels and the LCD, even without the PC.

III. Results

A. Micromanipulation Performance

The positioning performance of the micromanipulator was tested by measuring the displacement of the mover with a laser position sensor (Laser Doppler Vibrometer, Polytec, Inc.).

First, the resolution of the micro-driving system was defined empirically by applying the smallest step input capable of driving the mover reliably. The mover was able to advance with the resolution of 100 nm on average when two pulses were input 30 times at a driving voltage of 20 V.

In order to verify the micro-driving performance according to control methods and loading conditions, the displacement for each input was measured 30 times at a voltage of 20 V. In the case of pulse-based control at free load conditions, inputs of 10 to 200 pulses were transmitted to the piezo motor 30 times, and the average gain and standard deviation were analyzed. These values are summarized in Table 1. Then, the same inputs were applied under load while moving the guide shuttle inside the 0.3% agarose gel. For the displacement-based control (feedback control), targeted displacements, such as 1, 3, 5, 10, and 20 μm, were applied as inputs 30 times under free load and the same loading conditions as the pulse-based input.

As shown in Table 1, the standard deviation of the average gain did not vary significantly during either pulse-based or displacement-based control, even under loading conditions. However, the ratio of the gain to the free load condition decreased an average of 16.2%, and the maximum decrease was 27.6% for the pulse-based control when the guide shuttle moved inside the agarose gel. In contrast, there was no significant difference in the gains as a function of loading

TABLE I
MICRO-DRIVING PERFORMANCE ACCORDING TO CONTROL METHODS AND LOADING CONDITIONS

Input Type		Free Load		Under Load (Agarose Gel)		
		AVG.[a]	STD.	AVG.[b]	STD.	AVG. Diff. (a-b)
Pulse Input (Open Loop) (N)	10	1.102	0.030	0.950	0.021	0.152
	30	3.247	0.039	2.605	0.091	0.642
	50	4.776	0.229	4.656	0.342	0.120
	90	9.705	0.171	7.030	0.189	2.674
	180	21.400	0.342	20.155	0.493	1.245
[a]Disp. Input (Closed Loop) (μm)	1	1.029	0.050	1.093	0.029	0.064
	3	3.143	0.082	3.216	0.243	0.072
	5	5.305	0.225	5.184	0.319	0.120
	10	10.265	0.430	10.092	0.368	0.174
	20	20.263	0.759	20.217	0.671	0.046

AVG. = Average gain of displacement, STD. = Standard deviation to average gain, AVG. Diff. = Difference between average gains under free load and loading condition (Unit: μm)

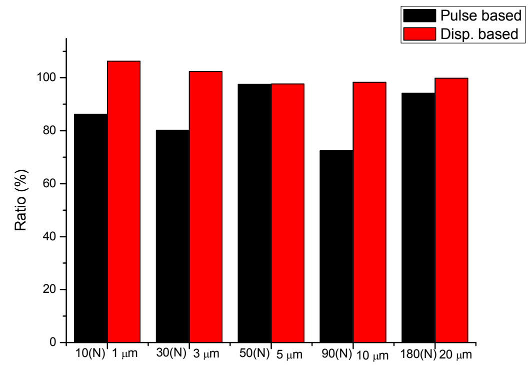

Fig. 7. The ratio of the average gain under load to free load according to control methods

conditions in displacement-based control, as shown in Figure 7. Furthermore, the accuracy of the MR position sensor was assessed as 225 nm through a comparison of the displacement between the MR sensor and the laser vibrometer when a stepwise displacement of 5 μm was applied 20 times, up to 100 μm. Consequently, these results showed that the electrodes can be advanced precisely within the accuracy of 1 μm to the targeted position for isolating single units.

B. Single Unit Recordings in Freely-Moving Mice

In order to evaluate single cell isolation performance in freely-moving mice, genetically-engineered, wild type hybrid mice (B6x129PLCβ1) were used. First, the bundle of tetrodes made with four twisted nichrom electrodes (0.0005 in, Kanthal Precision Technology) was loaded on the micromanipulator in a way similar to loading a manual micromanipulator. The micromanipulator was located on the thalamic region (target coordinates: AP -1.58, ML 1.8, DV 2.8 mm) by a stereotaxic instrument while the mouse was anesthetized. After recovery from the surgery, the mouse had

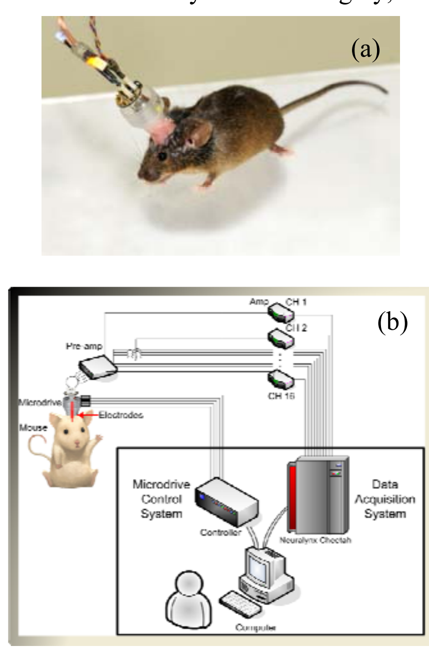

Fig. 8. (a) The freely behaving mouse with the micromanipulator mounted on the head, (b) The system configuration of the neural signal recording system and micromanipulator

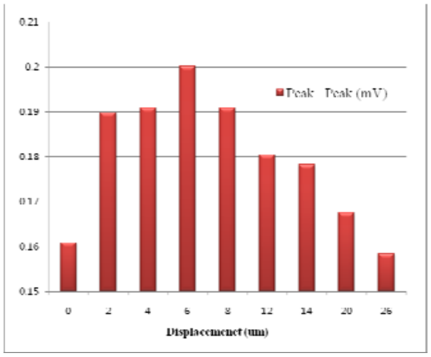

Fig. 9 (a) The change of action potential from a single neuron with regard to the advancement of electrode by micromanipulation in a mouse brain

no difficulty in lifting up its head and freely behaving, as shown in Figure 8 (a). Then, the micromanipulator advanced the bundle of electrodes with a small, stepwise adjustment, such as 1 to 5 μm, to search for the desired single units, at which point the neural signals were recorded.

For recording neural signals, a unity gain preamplifier (HS-16-CNR, Neuralynx, Inc.) was interfaced with the signal PCB of the micromanipulator. Then, the neural signals, via the tethers (cables) of the preamplifier, were amplified with a gain of 4,000 to 10,000 on the main amplifier (Neuralynx, Inc.). After the neural signals were sampled at 30.3 kHz and filtered between 300 and 9,000 kHz, as shown in Figure 8 (b), they were digitized via the data acquisition system (Cheetah System, Neuralynx, Inc.). The acquired signals are separated into signals of individual neurons using a spike-sort program (Spike Sort 3D, Neuralynx, Inc.).

Figure 9 shows, for the proposed micromanipulator, the change of action potential associated with the advancing electrodes. This result indicates that the small, controlled advancement of the electrodes made it possible to isolate single neurons. Practically, the single unit (clustered with the red), which cannot be distinguished commonly by manual micromanipulators, was isolated by a 2-μm advancement of the electrodes, as shown in Figure 10 (a). Finally, the proposed micromanipulator was able to classify seven single unit spikes on one tetrode, as shown in Figure 10 (b).

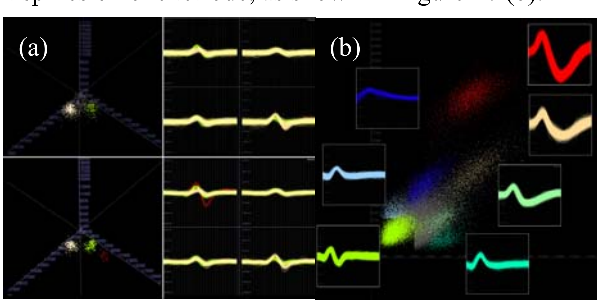

Fig. 10 (a) Single cell isolation with the small step adjustment, (b) Isolated single cells in mice thalamic region by advancing of the bundle of tetrodes

IV. Conclusion

A semi-chronic, implantable automatic micromanipulator based on a novel micro-driving system with a piezo motor was developed and tested. The micro-driving system was made possible by an enhanced clamping mechanism for the piezo motor and by feedback control using an MR position sensor. The modular design, including the guide cone, shuttle, and protection covers, allows the micro-driving system to be simply and semi-chronically implanted in small animals. The micro-driving performance was verified by measuring the displacement according to the desired number of pulses and the targeted displacement under free and loading conditions. The resolution of the micro-driving system based on the piezo motor and the accuracy of the MR position sensor was sufficient to control the advancement of electrodes at an accuracy of 1 μm or less. Furthermore, the electrodes can move precisely to the targeted position without loss, even under load, due to the feedback control. Finally, the single units can be isolated with the small-step adjustment, and a number of single unit activities were classified successfully with the micromanipulator in the thalamic region of freely-behaving mice.

Acknowledgment

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