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A Stereotaxic Instrument for Precise Localization of Honeybee Brain

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Abstract—**This paper presents a stereotaxic instrument which consists of a fixation unit and a three dimensional localization unit for the honeybees' flight control experiments. The fixation unit is a custom clip, designed for keeping the honeybee's head still during electrode implantation. The localization along the x- and y-axis is achieved by two micrometers with the precision of 10 μm, which are assembled as an X-Y sliding plane, while the localization along the z-axis is implemented by a micromanipulator whose operations can be controlled by a computer. Experiments are designed to verify the precision of this stereotaxic instrument. The preliminary results show that this instrument provides a good platform for carrying out further research on the honeybees' behaviors induced by electrical stimulus.**

I. INTRODUCTION

It is well known that despite of their small sizes and relatively simple nervous systems, insects possess the capability of foraging, communication and even more complex abilities, such as learning, memorizing, and cognition, which used to be regarded as the characteristics of higher organisms [1, 2]. Thus, investigations on insects whose brains contain much fewer neurons benefit our understanding of more complex brains. Behavioral experiments show that insects' seemingly intricate behaviors follow certain routines and the patterns of them can be understood by systematic observation and analysis. In order to gain deeper insights into the behaviors including navigation, learning, and cognition, the underlying neural pathways have been studied to investigate the inner mechanism of their reaction to external stimulus [3]. With the knowledge of insects' nervous systems and technologies in electronics, insect machine interface (IMI) is developed to control flight behavior by applying electrical signals to specific regions in the brain and the muscles. The combination of the insect's excellent ability of flight and the electronic components makes the IMI a great substitute for the micro air vehicles (MAVs) [4, 5]. A. Bozkurt et al. and H. Sato et al. have implemented IMIs based on moths and beetles respectively [6-10].

The IMI also provides a novel approach to explore the relationship between electric stimulus and the corresponding responses. Many studies have focused on the behaviors induced by external stimulus including visual stimulus, tactile stimulus, olfactory stimulus, etc. [11, 12]. One of the most studied behaviors is the visual processing ability of insects. In flies, optomotor response has been fully studied with respect to the neural pathway and its dominant neurons [3]; Optic flow has been substantiated as the main principle during the navigation of honeybees [2]. Although there have been remarkable results unraveling the corresponding neural pathway of certain behaviors, most parts of the underlying neural mechanisms still remain unknown. During the exciting exploration, electrical stimulus is regarded as an efficient approach to analyze the neural signal transmission of interest, and utilized to control the insects' flight.

In our previous work, we selected honeybees as the insect platform to build IMI [13], considering that honeybees had been investigated broadly as a model organism and were easily bred. In our prior experiments, the implantation of electrodes was assisted with a stereotaxic instrument used for rats, which was not accurate enough for experimental requirements. Moreover, because of the honeybee's tiny size, no suitable instruments were available, making the experimental operations inconvenient and inefficient. Thus, we present a stereotaxic instrument that enables fixation of a honeybee's head and three dimensional localization of specific brain region. The fixation is implemented by holding the membranous neck of a honeybee, which is a segment between the head and the prothorax, within a custom clip. The localization unit works with the precision of 10μm which is sufficient for differentiating adjacent brain regions. Each of the structural components is detachable, making this instrument flexible and operable.

The remainder of this paper is organized as follows. In section II, we describe the configuration and the characteristics of the stereotaxic platform. Experiments and results are shown in section III, followed by the discussion in section IV.

II. MATETIALS AND METHODS

The rectangular coordinates system is used in the instrument for spotting the honeybee's brain. This stereotaxic instrument consists of a fixation unit and a three dimensional localization unit. The usage of it makes the electrode implantation feasible.

A. Rectangular Coordinates

The stereotaxic instrument locates the electrode sites in a honeybee brain using the rectangular coordinates system (Fig. 1). As shown in the schematic diagram, the rectangular coordinates system consists of x-, y-, and z-axis. The median ocellus is referred as the origin point and the surface of the head as the X-Y plane, while the z-axis is perpendicular to

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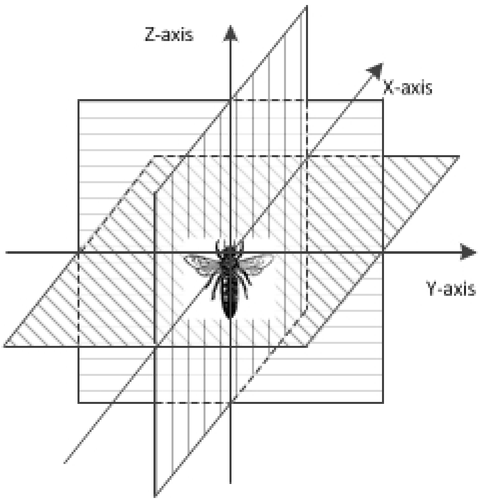

Fig. 1. Rectangular coordinates system adopted in the stereotaxic instrument. The median ocellus is referred as the origin point and the surface of the head as the X-Y plane. The stereo coordinates of specific brain region within a honeybee's head can be easily acquired with the brain atlas.

the head surface. Thus, the stereo coordinates of certain brain region can be acquired by measuring the distances along the axes directly, other than additional conversions in the spherical coordinates system. Besides, the atlas of a honeybee brain is usually depicted within the rectangular coordinates system, enabling the localization of specific brain region realized by means of it.

According to the 3D reconstructions of the NMR imaging of the honeybee brain with nominal resolution of $15.6 \times 15.6 \times 30\ \mu m^3$ [14], the precision of 10 μm is enough to locate main structures inside the brain, such as optic lobe, mushroom body, compound eyes, etc.

B. Fixation unit

The fixation unit is a custom clip for holding the honeybee's head still (Fig. 3). The clip consists of two semioctagonal stainless steel sheets, each coupled to a block. When the two sheets are butt-jointed with each other, the screw running through both blocks connects the two separate sheets to a complete octagon. There is a round aperture at the center of the entire combination. The honeybee is fixed by locking the slender membranous neck, which is the linkage between the head and the prothorax, within the central aperture. The thickness of the custom clip is 1 mm and the diameter of the central aperture is 2 mm, which are just suitable for locking the neck [15]. Meanwhile, the other parts of the body are still free to move without being influenced by the fixation. After that, the adhesion mixed by wax and rosin is smeared around the membranous neck for better immobilization.

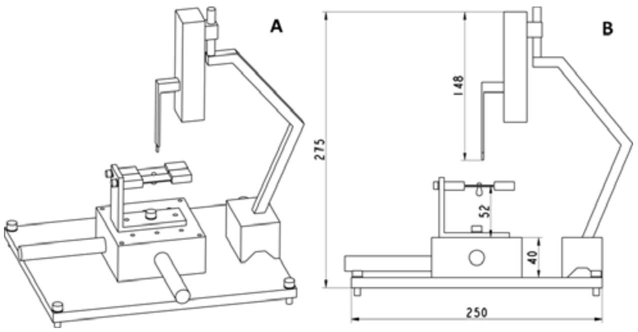

Fig. 2. Schematic structures of the stereotaxic instrument. (a) Three-dimensional view from the lateral side. The platform consists of an X-Y sliding stage, a micromanipulator, a custom clip, a magnetic base, and a metal base. (b) Two-dimensional view from the lateral side. A honeybee is fixed within the custom clip. The unit of the numerical value is millimeter. The height of the platform ensures that the experimental operations can cooperate with a microscope.

C. Localization unit

The main structures of the localization unit include an X-Y sliding stage and a micromanipulator along the Z-axis (Fig. 2 and 3).

An X-Y sliding stage serves as a stand for the fixation of a honeybee and controls the head's movements in the horizontal plane. Above the sliding stage, there is a mounting bracket fixed to it enabling the clip to connect to the bracket. So the movements of the fixed honeybee in the horizontal plane can be achieved by the movements of the sliding stage in the X-Y plane. Two micrometers are clung to the sides of the sliding stage, each responding to one direction respectively. That is, the distance offset on the x- or the y-axis can be measured up to 10μm.

A micromanipulator works along the Z-axis to control the movements in the vertical direction. The micromanipulator is a part of a patch clamp system, which is already used in the electrode implantation of rat navigation experiments [16]. The core component ensuring its precision of 10 μm is also a micrometer and there is a step motor inside the micromanipulator transforming electrical signals to angular displacements and sequentially driving the micrometer to move along the Z-axis. A signal generator (PowerLab System, ADInstruments Pty. Ltd.) promotes the step motor by electrical pulses, which are defined in terms of the pulse number, the pulse width, the frequency and the voltage. Each electrical pulse makes the micrometer within the micromanipulator move by one step. The pulse number controls the number of steps; the pulse width controls the time that the effective phase of each step persists; the frequency controls the steps over one second; the voltage controls the advancement of per step. Under appropriate settings, we can accomplish desirable implantation ensuring high precision and avoiding injuries to the brain due to quick insertion.

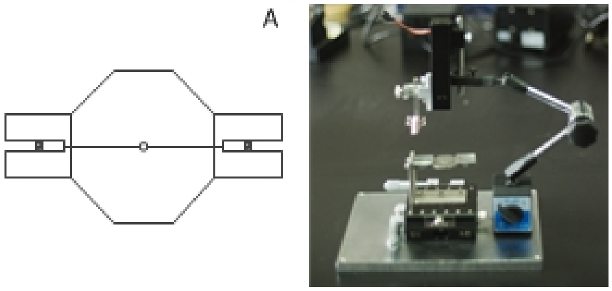

Fig. 3. (a) Structures of the custom clip. The thickness of it is 1 mm and the diameter of the central aperture is 2 mm. It consists of two semioctagonal sheets, and the screws running through both blocks connect the separate parts to a complete octagon. (b) Physical structures of the stereotaxic instrument. Each component of it is detachable, offering well convenience.

The electrode wire is clamped to the end of the micromanipulator so as to make it direct along the Z-axis. The micromanipulator is linked to a magnetic base with a bending arm, whose adjustable range is 300mm, via a cylindrical pin. The magnetic base has a switch to determine whether to turn on the inner magnetic device or not. Both the magnetic base and the X-Y sliding stage install on a metal base that weighs more than 6 kg to afford stable support.

D. Operations of this stereotaxic instrument

Operations with this instrument consist of two steps: coarse adjustments and then fine adjustments. After a honeybee is immobilized with the custom clip, coarse adjustments are first performed by moving the magnetic stand until aligning the electrode wire and the ocellus in the same z-axial line. Fine adjustments by the two micrometers make specific brain region right below the electrode wire. Then, z-axial adjustments are implemented by the micromanipulator that manages the implantation controllably. This instrument cooperates with a microscope (SZ61, Olympus Corporation.) to monitor the process of the electrode implantation.

III. EXPERIMENTS

The experiments of this study aim to verify the precision in the X-Y plane for this stereotaxic instrument. We conducted two sets of experiments, one for the x-axis and the other for the y-axis. A scale ruler, length of 1 mm and minimal scale of 10 μm, was used as a reference. The scale ruler was fixed using a bracket beside the X-Y sliding stage so as to make the scale ruler and the surface of the honeybee's head lie in the same horizontal plane (Fig. 4). A digital camera (DFK 21BU04, The Imaging Source Asia Co. Ltd.) was connected to the microscope to capture the images presenting in the visual field of the microscope. Since only the X-Y plane was considered, the micromanipulator and the magnetic base were not adopted in these experiments.

We selected a spot on the surface of the honeybee's head within the microscopic field indicating the reference point (Fig. 4). The adjustments of the micrometers caused the honeybee's head to move accordingly. We modulated the micrometers by several steps. Each step marched 10μm and then digital photos of each result were taken at a time. After that, by comparing the modulation of the micrometers and the displacements of the reference point, we could test the precision in the X-Y plane.

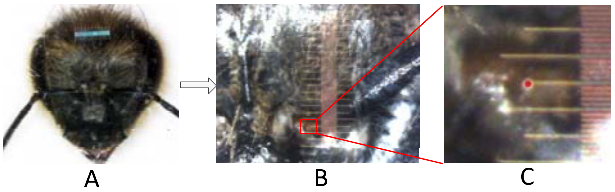

Fig. 4. (a) A honeybee's head together with the scale ruler in the visual field of a microscope. (b) A portion of a honeybee's head while testing the exactitude on the Y-axis, and (c) a magnified part of (b). The red dot represents the inner edge of the honeybee's left antenna base as the reference dot.

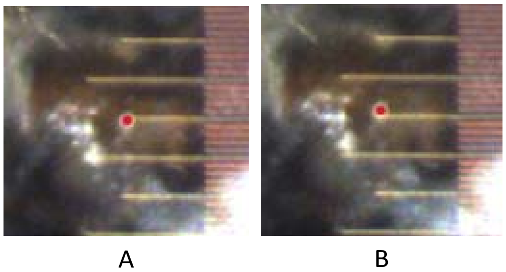

Fig. 5. (a) Reference point represented by a red dot before modulation in the y-axis. (b) Displacement of 10 μm shown after 1 step modulation.

Each set of experiments consisted of eight trials with different modulated steps: 1, 3, 5, 8, 10, 15, 20, and 30 steps. The results of the 1 step experiment in the y-axis are shown in the figure (Fig. 5). The movement of 10 μm can be clearly observed from the figure with comparison to the scale ruler. This demonstrates that the precision of the stereotaxic instrument can be up to 10 μm along the y-axis. A similar 1 step experiment on the x-axis shows that the exactitude can be up to 10 μm along the x-axis as well.

Larger steps experiments are performed for error analysis. There are eight groups of comparisons for both the x- and y- axis. The ideal displacements represent the measurements of the scale ruler and the actual displacements represent the modulation of the micrometers (see Table I). By comparing the ideal displacements and the actual displacements, these experimental results imply that the stereotaxic platform indeed provides precise localization in the X-Y plane.

As for the measurement of the precision along the z-axis, there are already successful products using micromanipulators for localization [17]. Furthermore, we have successfully applied the same micromanipulator for the electrode implantation of rat brain [16].

IV. DISCUSSION

In this paper, we built a stereotaxic instrument assisting the fixation of a honeybee and the electrode implantation. Experiments showed that this stereotaxic platform could modulate with the exactitude of 10 μm which is sufficient for the localization of main structures inside a honeybee's brain. However, there are still limitations of this stereotaxic instrument. During the fixation of the honeybee, the surface

TABLE I

Experiment		Trial							
		1	2	3	4	5	6	7	8
Error analysis on x-axis	Ideal (μm)	10	30	50	80	100	150	200	300
	Actual (μm)	10	30	51	81	102	152	203	303
Error analysis on y-axis	Ideal (μm)	10	30	50	80	100	150	200	300
	Actual (μm)	10	30	50	81	102	152	202	302

Error analysis on the x- and y- axis. The ideal displacements represent the measurements of the scale ruler and the actual displacements represent the modulation of the micrometers.

of the head may not be parallel with the horizontal plane. To improve this, the clip can be made rotatable to provide angular modulations for better implantation. And gravity sensors and angle sensors can be added to measure the oblique angles. Digital cameras would help provide accurate measurement and calibration based on image processing methods.

Moreover, the coupling of step motors to the micrometers of the X-Y sliding stage will make the localization accomplished automatically to reduce the errors introduced by manual operations.

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