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Design of a twin tetrode microdrive and headstage for hippocampal single unit recordings in behaving mice

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Abstract

A new, easy to construct electrode, microdrive and headstage for electrophysiological recording system which is specifically adapted for freely behaving mice is described. The system uses printed circuit boards and light, flexible cables to enable the animal's free movement for behavioral testing. A clip attachment system permits rapid and secure connection of the headstage and cables to the microdrive assembly on the animal's head. The current system provides eight recording channels, but the design can be modified to accommodate additional channels.

Keywords: Microdrive; Multielectrode single unit recording; Tetrode; Hippocampus; Mice

1. Introduction

Current advances in genetic engineering have rendered the mouse a prime choice of species for animal model studies, and genetically modified mice constitute, inter alia, valuable models of human pathologies. Naturally this has led to an increasing number of researchers studying mice, which, for electrophysiologists who are interested in measuring neuronal activity in freely moving animals (McHugh et al., 1996; Rotenberg et al., 1996; Cho et al., 1998; Buzsaki et al., 2003), raises significant technical difficulties due to their small size. The main concern for recording from mice during behavioral testing is not to disturb their free movement. Since behavioral experiments need a relatively large number of animals to be studied, we have developed a simple recording device that does not require specific skills or sophisticated tools for its construction in large numbers.

Here, we describe the components of a simple, compact and light recording system we have designed, and which constitutes an improved version of existing technology mainly developed for rats, monkeys, and more recently for mice (Bilkey and Muir, 1999; Korshunov, 1995; Nichols et al., 1998; Venkatachalam et al., 1999; Szabo et al., 2001). We have focused on optimizing the recording system by developing the design of the apparatus in an integrated manner. The first modification was to use a type of connector which constitutes and also supports the structure carrying the microdrive base (Fig. 1-6–9). The benefit of this assembly is to reduce weight and size. The second modification was to simplify the microdrive (Fig. 1-1), that is reduced to a set screw pushing a piston-like holder that protects the top of the cannulae containing electrodes. The third modification, a consequence of the preceding ones, concerns the manner in which the wires of the electrodes travel from the microdrive to the connector. The cumbersome, fragile and vulnerable externally exposed loop of electrode wires in most current devices, is reduced to a flexible spiral located around the cannulae (Fig. 1-1), and placed within, and thus protected by walls between which slides the cannulae holder (Fig. 1-8). The fourth modification concerns a headstage that results from a fusion of the support (Fig. 1-2, 3) for integrated circuits (Fig. 1-4, 5, 13) (i.e. impedance reducing pre-amplifiers), and of the body of female type connector with null insertion force. The fifth modification concerns the

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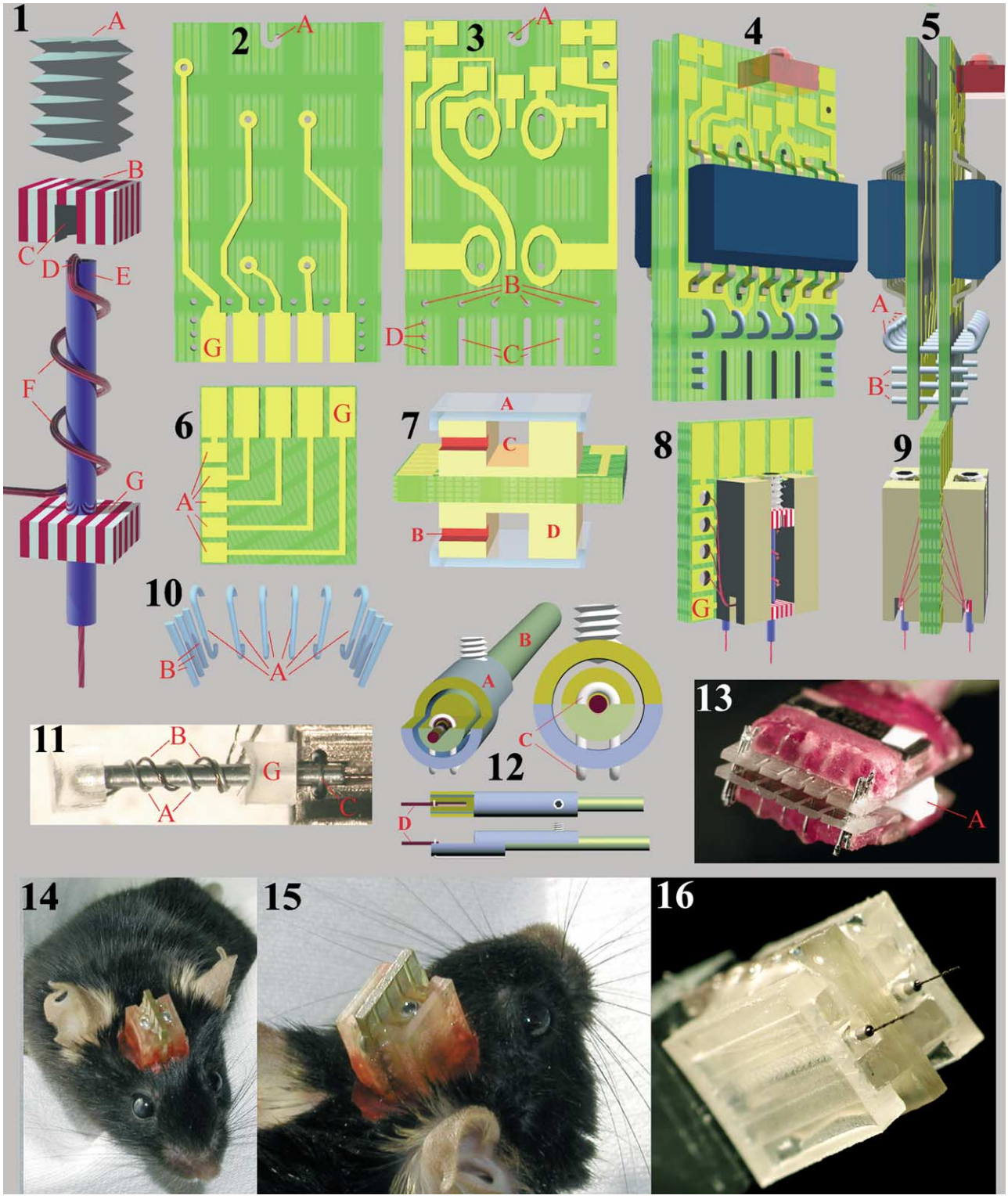

Fig. 1. Plate showing the small parts and tools used for the construction of twin tetrode miniature and headstage as well as photographs of a mouse carrying implants. Generally, composing parts are presented on the left side of the figure, and final products are shown on right side of the figure. The headstage is represented on top, and electrodes/microdrive is shown on bottom to demonstrate the way they should be connected. The two pieces of printed circuits (2, 3) are assembled using pieces of stainless steel wires (10) to form a pincer (4, 5) that gently pinches the double-sided connector of the electrode/microdrive miniature (7, 8, 9) to establish electrical contact during recording. For manipulating fragile electrodes, a tool (11, 12) is used to hold the cannula temporally until it is inserted into the groove of the microdrive. Photographs of constructed miniature (16) and headstage (13) as well as implanted C57BL/6 mouse (14, 15) demonstrate the compactness of the devices. For more details, see text.

particular care given to the electric cables connecting the headstage to the rotating commutator. And finally, the sixth, which is not just a modification but a necessity, concerns the use of a motorized commutator that we designed, but is not the object of the present report, and that is able to respond to the very weak torque that the cables of the headstage transmit.

2. Materials and methods

We describe here the construction of microdrive/electrodes that are permanently implanted onto mice, and the headstage that needs to be connected to mouse's implant during recording trials. In the final section we describe their use and present results of recordings made with the new device.

2.1. The miniature connector

The connector (Fig. 1-6) is a rectangular double faced piece of printed circuit (0.8 mm thick, 7 mm wide, 8 mm long) in which five lines constituting (computer board like) connectors were printed on each side. For construction of this printed circuit (and others described later), an inkjet printer (Epson Stylus Color 860, black and white, Epson photo glossy paper setup 1440 dpi) was used to print, onto standard transparencies, the mask applied to pre-sensitized printed circuits during the ultra-violet insulation (2–3′, see maker's advice and do tests). After UV light exposed parts are removed in an 0.1 N sodium hydroxide solution, engraving takes place in a bath of ferric chloride (45°) shaken time to time by hand for about 30 min, depending on the room temperature.

2.2. The microdrive

The displacement mechanism of the microdrive is a set screw A (Fig. 1-1A) of 1.45 mm diameter (Small parts, Miami, FL, Ref: B-SSX-080/1) that progresses along a 5.6 mm long groove, C (Fig. 1-7C, 8) with rectangular section 1.4×1.3 mm, located in a block D (Fig. 1-7D) of epoxy resin (cross-section, 1.7×4 mm) attached to the connector. The connector part of the miniature is gold plated to ensure good long-term electrical contact. Turning the set screw pushes on a piston-like small cubic part B (Fig. 1-1B) of the same section of the groove, that carries and protects the top of the cannulae D (Fig. 1-1D) from which the tetrode emerges. The free extremity of the cannulae emerges through a guide G (Fig. 1-1G) which is a small part with a circular window, that closes the bottom border of the groove (Fig. 1-8), to which it is glued. Finally, a cap A (Fig. 1-7A, 16, not shown in Fig. 1-8, 9) is placed on the groove and fixed with glue. Note that the groove's cross-section is not square to decrease the mechanical constraint imposed by the screw onto the cap. The parts (microdrive body, cap, piston and guide) are constructed by molding with epoxy resin (Ciron S.A., Barsac, France). More precisely, a model (original prototype) is made of PVC, with a classical manual drilling and milling machine (Cincinnati Milacron type P.F. or any other machines that yield 0.02 mm precision or better) and then a negative copy is molded in soft RTV silicone rubber (Ciron S.A.). We tried, but with poor results, to use a harder silicone rubber used by dentists to make impressions for artificial teeth. Finally, drops of epoxy resin were poured into the silicone rubber mold, and were hardened in a chamber heated to 50 °C for 10–12 h. The microdrive body (Fig. 1-7) mold includes a site for the connector (Fig. 1-6) which will be placed there after the epoxy resin had been poured into the corresponding mold. Should epoxy resin be deposited on the connector parts, it is easy to clean this off by heating and scratching gently. Slight irregularities or defects in these molded pieces are in fact useful. These irregularities create, when we adjust the piston to the groove, a slight friction and compression which avoid any mechanical play. The mechanical constraint is well tolerated by the epoxy molded groove because of the epoxy's elasticity.

2.3. The tetrode

Four 25 μm formvar insulated Ni–Cr (A-M systems, Carlsborg, WA) microwires are twisted together (Reece and O'Keefe, 1989), and are inserted into the cannulae (27 gauge stainless steel) onto which the guide, a small rectangular partition G (Fig. 1-1G, 11G) has previously been positioned. When exiting from the cannulae at the top end E (Fig. 1-1E), the tetrode is glued to the cannula and folded (Fig. 1-1D), and then, the cannula is glued to the piston B (Fig. 1-1B). The piston is equipped with a notch C (Fig. 1-1C) through which the tetrode exits without mechanical constraint. An uninsulated wire A (Fig. 1-11A) of the same alloy as the cannulae, (to avoid possible junction potentials) if possible, whose tip protrudes 100–200 μm from the cannulae is used as a ground electrode, and travels together with the tetrode wires B (Fig. 1-11B) which were finally to be soldered to fifth connector G (Fig. 1-8G). Once the glue is dry, the tetrode and ground wire are looped loosely around the cannula. This assembled part is then gently placed into the groove described in the previous section. Making the wire spiral is easy but nonetheless delicate operation since only a small portion of cannula remains available to be clasped during this process (Fig. 1-11). For this reason, we constructed a tool (Fig. 1-12ABC) that firmly holds the cannula (Fig. 1-11, 12D) without damaging the tetrode wires protruding from the cannula, via a tungsten wire of 0.1 mm diameter (Fig. 1-11C, 12C) that

is held tight by parts A and B (Fig. 1-12AB). The microwires were individually separated as they exit the epoxy microdrive blocks through the window B (Fig. 1-7B, 8, 9), and are then soldered into their corresponding attachment sites A (Fig. 1-6A, 8, 9) on the 5-pin connector on each side of the printed circuit. A protective cover A (Fig. 1-7A, 16) is finally placed to protect the cannula and assembled parts, and a small amount of epoxy glue is applied over soldered areas to insulate them, as well as at the junction of the cover and main pieces to hold them all together and also to render the electrodes waterproof.

2.4. Electrode implantation

The electrodes described here were destined to be implanted in both left and right dorsal hippocampi of mice, such that the spacing between the two tetrode containing cannulae was 2.8 mm. Using stereotaxic surgery, the electrodes were implanted to a depth, 1500 μm below the cranium (in the neocortex) at 2.0 mm posterior to Bregma, and 1.4 mm lateral to the midline suture (Fig. 1-14, 15). After mice recovered from surgery, the electrodes were gradually descended into the hippocampus 40–80 μm (one full turn equals 300 μm) daily for cell screening.

2.5. The headstage

The headstage (Fig. 1-4, 5, 13) is made of two small pieces of printed circuit boards (9 mm $W \times 11$ mm $L \times$ 0.4 mm thick) (Fig. 1-2, 3) that, once assembled by a hinge A (Fig. 1-5A, 10A), act as a pinch clamp (Fig. 1-5) whose inner sides are in contact with (male) connectors of electrodes and mobile outer portions support small format pre-amplifiers (TL 084C) to reduce impedance. As for the miniature, the connector parts are gold plated. Fig. 1-2, 3 shows recto and verso sides of only one of the two printed circuits constituting internal and external parts of the pincer, the other printed circuit being the mirror-image of the first. Slits C (Fig. 1-3C) between the connector parts for electrical contacts of the headstage, are made with a 0.3 mm milling tool, to make fingers among which the pinching pressure will be distributed and, thus, ensure reliable electric contacts by correcting or compensating any defects on the surface of the miniature connector. The hinge that links these two printed circuits is made of six small pieces (Fig. 1-10B) cut from a stainless steel wire of 0.3 mm diameter. They are inserted through the holes B (Fig. 1-2, 3B) and their extremities curved like a hook, to be anchored in dental cement (Fig. 1-13). Before dental cement placement a 0.8-mm-thick piece is wedged between the two printed circuits to ensure a space equal to the miniature connector thickness, 0.8 mm. The dental cement which covers the hooks and the operational amplifier must ensure a good rigidity to prevent solder breaking between pre-amplifiers and printed circuit, and to restrain, and restrict the flexibility to the hinge. On both sides of the placement of the miniature connector (Fig. 1-2, 3D, 5B), three stainless steel wires (0.3 mm in diameter) B (Fig. 1-4, 5B, 10B, 13) enable a fine adjustment of the electrical connections between headstage and miniature. These stainless steel wires are mobile in their holes, and squeezing of their extremities is sufficient for holding them in place. Finally, a piece of elastic material A (Fig. 1-13A) will be inserted between the two pieces of circuit boards to exert necessary force such that squeezing pressure ensures good electrical contact between connectors (electrodes and headstage).

2.6. The pre-amplifier

We used quadruple (field effect transistor input) operational amplifiers (TL 084C, Texas Instruments) and output was wired to the negative input in order to obtain a unity gain voltage follower with an output impedance which is theoretically null. Many operational amplifiers of this kind exist. Criteria for choosing these pre-amplifiers are high input resistance 1 GΩ, bias current lower than 50 pA, input capacity lower than 10 pF, low noise, and if possible low power supply.

2.7. The headstage cables

The headstage cables for electrical signals as well as power supply and ground were made of insulated 50 μm copper wires. In addition, a 100 μm tungsten wire hanged on the J shaped slot (Fig. 1-2A, 3A) cut in the printed circuit of the headstage, was used to augment the strength and elasticity that copper wires lack. Before the copper wires were placed and soldered, the tungsten wire was twisted 30–50 turns and once the two ends of the copper wires were soldered, the tungsten wire was released that had as an effect of its elasticity, to twist the copper wires around the tungsten wire and to make them bound together at equilibrium. They were then soaked into epoxy resin for protection. Prior to the total polymerization of epoxy resin, the wires were wound up around a 8 mm diameter metal rod protected by silicon tube, before a final polymerization at 50 °C for 12 h. The shape of resulting spiral of the cables might be unpredictable and different from its initial form, but its lightweight, elasticity and solidity was satisfactory enough for the mouse recording.

2.8. The commutator

Without torque-feedback commutator the light wire bundle would be quickly destroyed by a moving mouse. Using principles of Fee and Leonardo (2001), we

constructed one such assisted commutator, based on a low cost 18 channel commutator (Litton, LeChesnay, France). Commutators, similar to ours, which are able to respond to the very weak torque that the cables of the headstage transmit, are commercially available (Dragonfly, Ridgeley, West Virginia for example).

3. Results

Photographs of the assembled miniature (Fig. 1-16), headstage (Fig. 1-13) as well as C57bl/6 implanted mouse with the electrode/microdrive assembly (Fig. 1-14, 15) shows the compactness of our device. The miniature has a dimension of $7 \times 6.4 \times 8$ mm, and has a mass of approximately 0.29–0.32 g. The headstage has a mass of 1.00–1.20 g without cables.

Using our miniature/microdrive and headstage system we have successfully recorded single unit activity from the dorsal hippocampus in freely behaving mice. Signals from single wires were amplified 10 000 times and band filtered between 600 and 6 kHz, and digitized at 28 kHz. An example of bursting complex spike activity as well as spike wave form for tetrode is shown in Fig. 2A and B. The cellular activity was stable, and apparently the same wave forms could be obtained at least during a prolonged period of 1 week, and in some cases for a period of up to several months. Fig. 2C shows a sample of continuous recording with 1–50 Hz band pass and sampled at 2 kHz, showing slow wave rhythmic (theta) activity of the hippocampus in mice. Notice that the increase of theta frequency related to movement onset, is devoid of artifacts often caused by mouse movement, skull bone deformation or connector part displacements. We attribute the good quality of the recording to the very low level of the mechanical constraints imposed to the mouse by this low weight recording device.

4. Discussion

We describe in this paper the construction of a light and compact electrodes and headstage for chronic multielectrode recordings of extracellular unit activity in normal and transgenic mice. Special effort was made to reduce the size and weight of the entire device so that they do not disturb the free movement of mice. The setup described here is not only simple and easy to construct, but has the flexibility needed in most electrophysiological laboratories. This was made possible due to the use of printed circuit boards and new methods of assembling different essential parts for electrodes and headstage, on the one hand, and the use of flexible and light cables as well as motor assisted commutator, on the other hand. These devices have successfully been tested for recording from hippocampal place cells during open field exploration, in transgenic mice which were smaller and more fragile than normal mice (Jeantet and Cho, 2002).

It should be pointed out that the entire design was constructed intuitively and may be further optimized. In

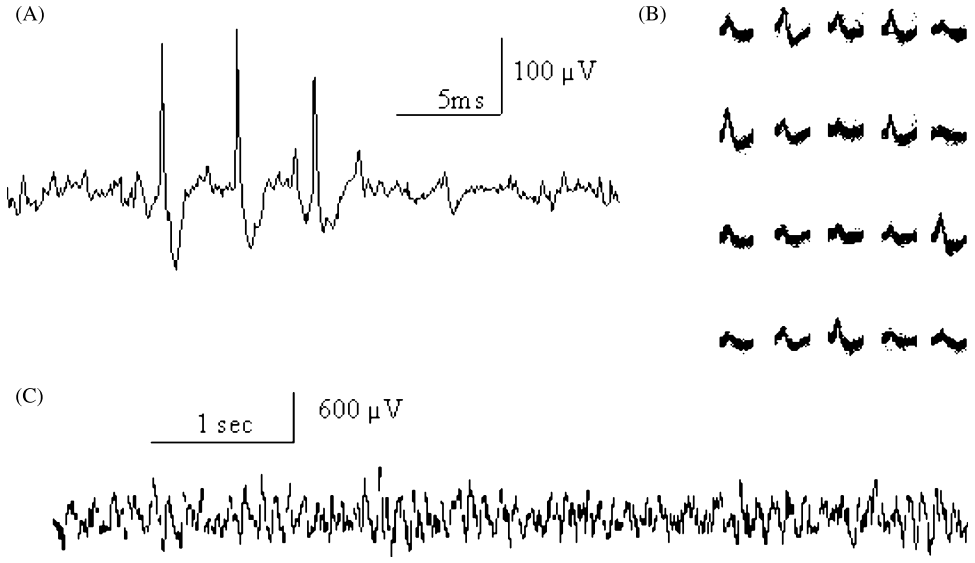

Fig. 2. Recording results of single unit and slow wave rhythmic activity of the hippocampus in freely behaving mice. (A) Continuous recording of a hippocampal complex spike cell exhibiting bursting firing (recording obtained with band filters 600–6 kHz, (B) image digitized at 28 kHz), and (B) spike wave forms for simultaneously recorded cells from a tetrode. (C) Represents hippocampal EEG recorded with band filters between 1 and 50 Hz and digitized at 2 kHz.

addition, our present system could easily be adapted for different anatomical structures, and multiple drives can be placed close to each other in larger animals. The only major constraint is the size of screws, which are an essential constituent of the microdrive, and the minimal spacing (i.e. 1.4–2.00 mm) between tetrode placements is limited by the diameters of the screws. Using this principle for a compact and light recording device, several improvements can be envisaged. For example, it is possible to upgrade to four tetrodes system using 16 single channel FET pre-amplifiers instead of operational amplifiers, with which the final size and weight of the new electrodes/microdrives would increase only 10–20% of the current two tetrodes recording system. A micro inchworm motor in the wall's groove at the guide level, could ensure the cannula's displacement, or alternatively, one can conceive a passive microdrive whose displacement mechanism would be situated on a headstage like system remotely controlled to adjust electrodes placement. However, such improvements would require sophisticated skills and high level technologies and that is exactly what we have tried to avoid here.

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