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A method of extracellular recording of neuronal activity in swimming mice

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Abstract

The design of a removable miniature microdrive-headstage waterproof assembly for extracellular recordings of single unit activity with high-impedance electrodes in swimming mice is presented. The assembly provides perfect protection of the critical components and electric contacts from water. Neuronal activity may be recorded even if the animal is diving and swimming under the water surface. The advantages of this construction include simple installation and removal of the electrodes, rapid attachment of the assembly to the animal's skull, and rapid removal after recording. The device provides precise vertical positioning of the electrode without rotation or lateral shift, stable recordings of single units for several hours and the possibility to change the penetration track many times in the same animal. The assembly weight is less than 160 mg. This work is the first successful attempt to record neuronal activity in mice performing spatial task in water maze.

Keywords: Microdrive-headstage waterproof assembly; High-impedance electrodes; Single unit activity; Swimming mice

1. Introduction

Since 1981 the submerged platform water escape task or Morris water maze has become one of the most widely used tests for estimation of spatial memory in rodents (Morris, 1981, 1984). Combinations of different influences (stimulation, lesions or protein-synthesis blockades) with the water maze task further prove it to be an effective paradigm for investigation of brain functions. Especially, Morris water maze task has been recognized as an effective test for measuring genetic variations of spatial memory in mice (Nakazawa et al., 2002, 2004), but all attempts to record the neuronal activity in mice performing spatial task in water maze were not successful.

Recording neuronal activity in mice during swimming causes several technical challenges. The construction should be leak-proof because water can easily make short circuit between the contacts. If the recording device fixed onto the skull is heavy, swimming animal wastes much energy to hold the head above the water surface. Thus, the construction should also be as small and as light as possible. These requirements increase the complexity of the device; thus, it is difficult to make a large number of them for implantation in many animals.

Water maze spatial tasks require the activity of several brain structures. Since the hippocampus plays the prominent part in spatial behavior it is usually investigated in these kinds of experiments. Unfortunately, hippocampal pyramidal cells are located very close to each other, thus analysis of neuronal activity in this brain structure requires efficient isolation of action potentials from different cells. A high-impedance electrode can record the activity of a single neuron even in brain structures with high cell density, using only one channel of amplification. We choose this technique, because it makes weight savings. Also, a high-impedance electrode must be removed from the brain after the experiment in all cases; thus, one removable device may be used for a large number of animals.

A design for a miniature, removable, waterproof microdrive-headstage assembly for recording neuronal activity with

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high-impedance electrodes in swimming mice is described. The construction provides stable recordings of neuronal activity even if the animal is diving and swimming under the water surface.

2. Materials and methods

2.1. Electrodes

High-impedance electrodes (1–5 MΩ at 500 Hz) are fabricated from tungsten wire with a diameter of 100–130 μm, sharpened electrolytically in KNO_2 solution and insulated with lacquer or glass. Also we used tungsten electrodes in glass insulation manufactured by Alpha-Omega (Cat.#: 367-070615-00, Alpha-Omega, Nazareth, Israel, O.D. 120 μm, S.D. 50 μm, angle 60°, impedance 1.5 MΩ at 1 kHz).

2.2. Microdrive-headstage assembly

The main challenge in recording neuronal activity in swimming animals is to protect the critical components from water. Since the connectors increase the weight and can be scarcely protected from water we exclude them from construction. In our device the preamplifier is directly electrically connected to the microdrive, and both are located inside the waterproof housing. The device consists of two separate parts; the base, which is attached to the animal's skull with dental cement, and a removable waterproof microdrive-headstage assembly. The removable assembly involves a microdrive, a preamplifier and a waterproof housing.

2.2.1. Microdrive

We use the microdrive for high-impedance electrodes (Korshunov, 2006). This design seems to be the most lightweight one published to date. Construction was slightly modified to adopt it for recording in water. As atonement for this, the depth of electrode penetration is limited by 4.0 mm. Other characteristics are kept the same.

The components and the cross-sectional views of the assembly are shown in Fig. 1. Since the microdrive construction was described earlier (for more details see Ref. Korshunov, 2006), we focus here only on the modifications. Two components (1 and 4; numbers in parentheses refer to Fig. 1) of the microdrive were modified in comparison with the previous design. The positions of grooves in the core component (1) were slightly changed (see Fig. 1). This allows us to reduce the height of the core component and keep the same range of precise positioning of the electrode. Since the heaviest component of the microdrive becomes shorter, the weight is also reduced.

New driving tube (4) has a hole on the top with diameter of 1.2 mm. When the microdrive is assembled, the gag (plexiglass) with diameter of 1.2 mm is glued inside the driving tube (see Fig. 1) with dichloroethane. This prevents vertical displacement of the driving tube along the core component and also provides waterproofing. The gag has a screwdriver slot for a small screwdriver, which is used for rotation. Thus, we exclude the rubber band, which was used for manual rotation in the previous design and also make weight savings. The total weight of this new microdrive version is less than 35 mg.

2.2.2. Preamplifier

We use two-channel impedance-reducing preamplifier to obtain differential recording. Two unpacked field-effective transistors (KP-201E-1, Russia) were glued to a miniature (0.5 mm × 2.4 mm × 5.6 mm and less than 19 mg in weight) printed circuit (8) and soldered to the conducting tracks. We choose this technology instead of integrated circuit, because this construction is smaller and lighter (each transistor crystal is only 0.8 mm × 0.8 mm × 0.6 mm and less than 1 mg in weight). Also transistors allow reduction in the quantity of separate wires in the cable (9) to four; this makes the cable more flexible. For more details see the paper (Korshunov, in press). The contact wires (made from stainless steel wire of 100 μm diameter; SS wire, A-M Systems Inc., not insulated) were soldered to input conducting tracks. The printed circuit is covered with black lacquer to protect transistors from light and damp. The total weight of whole-assembled preamplifier (without a cable) is 24 mg.

The cable (9) is made of fine copper wire of 150 μm diameter in formvar insulation. These three wires are spooled around the Teflon insulated copper wire of 0.4 mm diameter (MGTF, MP 16–11, 0.03 mm^2, TU16-505.759-81, Russia). The upper part of the cable is placed into a cotton sleeve (a cord for radio, diameter 0.8 mm, art. 602 t-r, GOST 22-173-76, Russia). In the lower part of the cable (the length of this part must be more than the water maze depth) fine wires were separated from Teflon insulated wire. Fine wires were put into a Teflon sleeve of 0.6 mm diameter. The wires were soldered to the printed circuit, and served as preamplifier outputs and power. Resistors were soldered to the opposite side of the cable and placed inside the output connector (for more details, see Ref. Korshunov, in press); this also makes weight savings. The Teflon insulated wire was soldered to a female pin and used for grounding the animal.

2.2.3. Waterproof housing

The microdrive and the preamplifier are put into the waterproof housing (6). The waterproof housing is machined from plexiglass in the form of a tube (see Fig. 1), which is tightly covered with the lid (7). The lid has two holes for the microdrive and the cable (9). The bottom part of the housing is machined in the form of a truncated cone (see Fig. 1). This permits attachment to the base (13), which has a complementary internal surface. The procedure for making the complementary surfaces was described elsewhere (Korshunov, 1995). The bottom part of the housing (6) has two eccentric holes for the microdrive and the contact tube. The wall of the bigger hole has a longitudinal slit. The diameter of this hole is slightly less (0.75 mm) than that of the core component (1). This provides rigid fixation of the drive in the hole by reversible deformation of the cone. When the microdrive is fixed in the hole, the contact wire (10) touches the core component (1) and provides electrical contact between the microdrive and preamplifier input. The hole with the smaller diameter (0.3 mm) is used to fix the contact tube (11) (a piece of 0.3 mm syringe needle). The upper part of the contact tube is insulated with a plastic sleeve (12). The fine (0.1 mm) stainless