Video Article

Acrylic Resin Molding Based Head Fixation Technique in Rodents

Mootaek Roh[1], Kyungmin Lee[1], Il-Sung Jang[2], Kyoungho Suk[3], Maan-Gee Lee[3]

[1]Department of Anatomy, School of Medicine and Brain Science and Engineering Institute, Kyungpook National University

[2]Department of Pharmacology, School of Dentistry and Brain Science and Engineering Institute, Kyungpook National University

[3]Department of Pharmacology, School of Medicine and Brain Science and Engineering Institute, Kyungpook National University

Correspondence to: Maan-Gee Lee at mglee@knu.ac.kr

URL: http://www.jove.com/video/53064
DOI: doi:10.3791/53064

Keywords: Neuroscience, Issue 107, Head fixation, Head restraining, Apparatus, Frameless, Dental cement, Molding, Neuroscience, Behavior, Rat, Mouse, Rodent

Date Published: 1/12/2016

Citation: Roh, M., Lee, K., Jang, I.S., Suk, K., Lee, M.G. Acrylic Resin Molding Based Head Fixation Technique in Rodents. *J. Vis. Exp.* (107), e53064, doi:10.3791/53064 (2016).

Abstract

Head fixation is a technique of immobilizing animal's head by attaching a head-post on the skull for rigid clamping. Traditional head fixation requires surgical attachment of metallic frames on the skull. The attached frames are then clamped to a stationary platform resulting in immobilization of the head. However, metallic frames for head fixation have been technically difficult to design and implement in general laboratory environment. In this study, we provide a novel head fixation method. Using a custom-made head fixation bar, head mounter is constructed during implantation surgery. After the application of acrylic resin for affixing implants such as electrodes and cannula on the skull, additional resins applied on top of that to build a mold matching to the port of the fixation bar. The molded head mounter serves as a guide rails, investigators conveniently fixate the animal's head by inserting the head mounter into the port of the fixation bar. This method could be easily applicable if implantation surgery using dental acrylics is necessary and might be useful for laboratories that cannot easily fabricate CNC machined metal head-posts.

Video Link

The video component of this article can be found at http://www.jove.com/video/53064/

Introduction

Head fixation or head restraining is a technique of immobilizing the animal's head by attaching a frame or plate on the skull for rigid clamping. The technique has been adopted for precise sensory stimulation, behavioral sensing with or without neural recording in awake animals[1-3] and for juxtacellular labeling during sleep-wake cycle[4]. It is also used for *in vivo* brain imaging[5,6]. Now head fixation technique is a commonly used tool in neuroscience and behavioral research.

Basically, traditional head fixation requires surgical attachment of metal head-posts on top of the skull[6-8]. The attachments are then clamped to a stationary platform, resulting in immobilization of the head. Neurophysiological research in awake animals such as neural and/or optical recording, brain circuitry intervention, brain imaging is accompanied by implantation surgery using dental acrylics. Therefore, it seems a simple task to implement the head fixation in those laboratories when it is experimentally necessary. The frames should be designed to be light-weight and small enough not to disturb animal's natural behavior in their home cages. It also provides mechanical rigidity when clamped to the stationary platform. It further has biocompatibility depending on experimental purposes. Therefore, it is technically difficult to design and fabricate such head-posts in general laboratory equipment.

In this study, a novel head fixation method is described, providing mechanical rigidity through convenient clamping without using metal head-posts. By using the method, head fixation could be done under general laboratory conditions, where implantation surgery is usually performed in rodents for brain study. The metal head-posts are replaced with acrylic resin cement molded partially by a custom-made head fixation bar and the molding process is done during implantation surgery. The fixation bar provides high spatial compatibility with other implants, such as neural recording probes, stimulation electrodes. Also it simplifies steps of clamping the animal's head on the fixation platform by removing screw-fastening. We used this method in the previous behavioral study and we concluded that this method is easy to implement and convenient in practical experimentation[9].

Protocol

Ethics Statement:
This experiment was approved by the Kyungpook National University Institutional Animal Care and Use Committee, and was performed according to Guide for the Care and Use of Laboratory Animals (National Institute of Health, 1996). After experiment, animals were sacrificed under carbon dioxide euthanasia.

Copyright © 2016 Journal of Visualized Experiments

1. Design of the Head-fixation Bar for Rats

NOTE: This step is a general guideline for a fixation bar design. Any modification about the dimension and material is possible depending on animals or implants. Designing a head-fixation bar using CAD software is recommended but not necessary.

1. Use CAD software to design a fixation bar.
    1. Design a fixation bar of a rectangular shape (123 × 35 × 6 mm) (**Figure 1A**). Make a rectangular notch (16 × 20 mm; port) in the middle of the fixation bar. Add slopes or indentations which serve as a guide rail on the borders of the port (**Figure 1A-a**, **Figure 1B**-arrows). NOTE: Ensure that the area of the port covers the estimated rectangle of rat's skull over the brain to allow vertical accesses using a stereotaxic manipulator to most of the brain areas through a craniotomy.
    2. Attach plates (50 × 20 × 6 mm) with two elongated anchoring holes on both sides of the fixation bar. Ensure that these holes pass screws of 6 mm diameter (**Figure 1A-d**). Make a hole of diameter 3 mm in front of the port for a locking hook (**Figure 1A-b**).
    3. Make a box having a floor (135 × 305 mm) and side walls (305 × 76 mm). Drill two 6 mm diameter holes as shown in **Figure 1A** (inset). Add supporting walls on the fixation bar (**Figure 1A-e**).

2. Manufacture the designed bar and box and assemble each of them using cyanoacrylate as shown in **Figure 1A** (inset). Assemble the bar on the box using screws of 6 mm diameter. Apply slope by adjusting the screw position of the bar for ergonomic head fixation (**Figure 1A-inset**). NOTE: For mice, the dimension of the fixation bar is 60 × 20 × 4 mm (consisting of two layers of 2 mm thick acrylic panels). The port is square of 8 mm width (upper) and 9 x 10 mm (lower) as shown in **Figure 1C**. Anchoring plates are the same size of rats'. The box plate and side walls are 100 × 72 mm and 100 × 50 mm, respectively.

2. Head Mounter Construction during Implantation Surgery

CAUTION: Be cautious when dealing with dental acrylic resin. Liquid may cause skin irritation. Put on a mask and wear gloves. Work in a well-ventilated area.

1. Preparation before surgery
    1. Tightly wrap the border of the port of the fixation bar using a paraffin film to prevent adhesion of applied acrylic resin to the bar (see **Figure 1B** and **Figure 2A**). Coat the boarder of the port covered by the paraffin film with a thin layer of acrylic resin.
    2. Use sterilized surgical instruments. Thoroughly disinfect stereotaxic frame and its surrounding using 70% ethyl alcohol.

2. Perform stereotaxic implantation surgery
    1. Anesthetize an animal by intraperitoneal injection of a cocktail mixture (2 ml/kg) of ketamine 10 ml (50 mg/ml), xylazine hydrochloride 1.5 ml (23.32 mg/ml), and saline 2.5 ml. Confirm that the animal fully anesthetized and does not respond to tail and toe pinches. Inject cocktail mixture of 1 ml/kg during surgery when necessary.
    2. Fix the animal to the stereotaxic apparatus. Apply ophthalmic ointment on eyes to prevent dryness during surgery. Shave and clean the scalp with 70% ethanol. Give subcutaneous injection of 2% lidocaine and incise the scalp along the midline of the skull. Completely scrape off periosteum. Rinse with saline several times.
    3. Make burr-hole drilling near the rim of the skull. Implant screws 4-5 turns (up to 1 mm depth) from the skull surface to prevent a breach of the dura mater. Make sure no leakage of CSF and blood on the skull surface after screw implantation. Rinse with saline and dry the skull surface using air-puff, especially around the screws.
    4. Coat skull surface with a thin layer of "dental adhesive (Super-Bond C&B)". Do not entirely cover the surface of the skull including the bregma if additional electrode implantation is scheduled. Use the bregma for marking craniotomy coordinates. Implant electrodes and/or microdrive and fix it on the skull by applying acrylic resin around it.
    5. Apply additional acrylic resin on top of the dental cement. Use appropriate amount of acrylic resin to leave sufficient fill-out space between bar-coated resin and initial mounting resin when fixating the screws and/or electrodes (**Figure 2A**).

3. Place head-fixation bar 5 mm or more above the skull surface and align its center (**Figure 2A**-Left).
4. Apply acrylic resin into the gap between the base of the skull and initial mounting resins. (**Figure 2A**-Center). Wait until the resin become hard.
5. Remove ear-bar and pull the molded mounter out of the fixation bar port. (**Figure 2A**-Right). Carefully apply additional acrylic resin around the edge rails of the molded mounter if necessary.
6. Rinse the wound surface of the scalp with sterile saline. Inject an antibiotic (enrofloxacin, 5 mg/kg s.c.) and an analgesic (butorphanol tartrate, 2 mg/kg s.c) immediately after surgery. House the animal individually in a single cage.

3. Habituating Animals to Head-fixation

Note: This step describes habituation schedules based on authors' empirically successful assessments. For an overview of habituation procedure for head-fixation, see Schwartz's[10] or Guo's[11] work.

1. Give sufficient recovery period (a week or more) to animals. On Day 1, Handle animals frequently on divided short sessions.
2. On the next day (Day 2), expose the animal to the fixation environment (box). Do not insert their head mounter into the port of the fixation bar. Leave animals to get accustomed to the environment. Securely grab the head mounter using hand for 1 to 2 sec and release it immediately. Repeat short term fixation by hand-grab several times.
3. On Day 3, habituate animals to head-fixation.
    1. Bring animals from the home cages and settle them in the box directing heads toward the port of the fixation bar. Grab the head mounter and insert it into the port of the fixation bar.
    2. Bind the mounter (animal's head) to the locking hook of the fixation bar using a rubber band. Stand for 5 min.

Copyright © 2016 Journal of Visualized Experiments

NOTE: Do not touch the trunk or tail of the animal after fixation, which leads the animal to squeak and struggle increasing animal's stress. When the animal continues struggling more than 1 min, release it and try later. From this step, animals show urine and feces excretion whenever exposed to head fixation but this gradually gets reduced as habituation proceeds.

3. Retrieve the locking rubber band and eject the mounter out of the port. Put the animals back to the home cage and provide a piece of cereal or a sunflower seed into the home cage as a reward. The next trial begins in 30 min. Repeat 3.4.1 to 3.4.4 five or more within a day.

4. On Day 4 to 7, expose the animal to head fixation as described in Protocol 3.4. Gradually increase the duration of head fixation (10 min, 20 min, 30 min, and 50 min as habituation proceeds).
NOTE: If animals do not feed the food reward, it can be a sign of a high level of stress. Do not increase the fixation time. If animals show repeated struggling behavior, habituate them for a longer period.
5. Check animal's body weight daily. Consider unsuccessful habituation when body weight is decreasing. Proceed to behavioral tasks with head fixation after successful habituation.
NOTE: Successful habituation facilitates subsequent behavioral training (**Figure 3E and F**).

Representative Results

This head fixation method is applicable to both rats and mice. Total eight rats received bilateral implantation of stimulating electrodes into the medial forebrain bundle. For four out of them, customized tungsten wire electrodes (microdrives) were additionally implanted into the M1 forelimb area (**Figure 2C**). For mice (n = 2), four stainless steel wire electrodes were implanted into frontal/occipital cortical areas. During surgery, head mounters were molded for head fixation in both rats and mice, as describe in Protocol section 2. After recovery from surgery, they were exposed to daily sessions of head fixation for either habituation or behavioral task. The molded head mounters stayed through the end of experiment (40.5 ± 1.73 (mean ± s.d.) days for rats and 81 ± 5.66 (mean ± s.d.) days for mice after surgery). Rats were exposed daily 40 to 60 min head fixation during behavior task; mice were exposed daily 20 to 40 min of behavior task.

Previously we used this technique to train rats push and pull a lever under head fixation. The reward was direct electrical stimulation of the medial forebrain bundle. Though it elicits exaggerated locomotor behavior, head fixation was firmly maintained without accidental mounter separation.

After habituation, docking the head mounter into the port of the fixation bar was completed in a few second by locking rubber band to the hook. When first introduced to the head fixation, animals presents struggling behaviors such as running in place, straighten out their legs (**Figure 3A and B**). However, such behaviors diminished with habituation. Successful habituation can be confirmed that animals show no more struggling behavior while head fixed (**Figure 3C and D**).

Copyright © 2016 Journal of Visualized Experiments

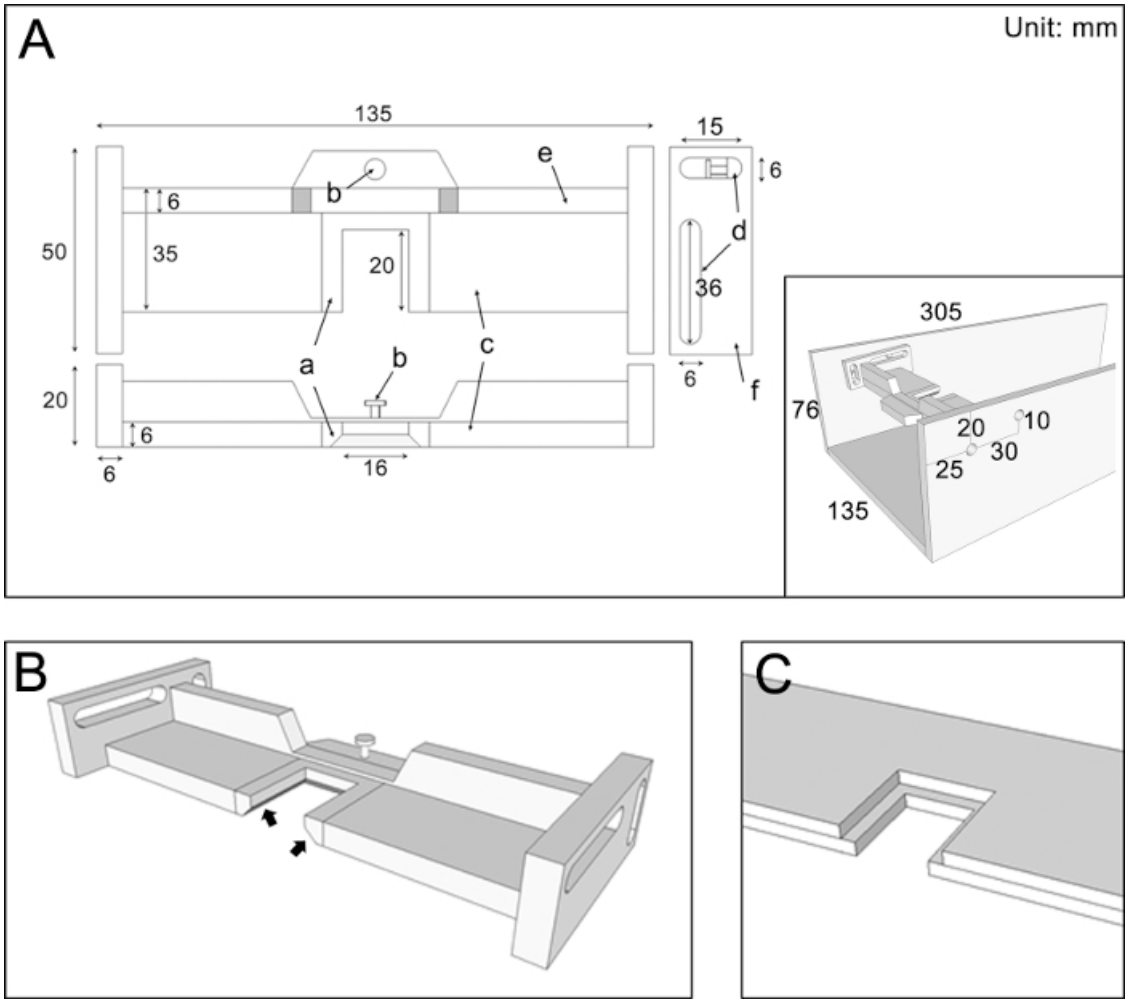

Figure 1. Design of the head-fixation bar.
(A) Component names of the head fixation bar. a) port; b) locking hook; c) fixation bar body; d) anchoring holes; e) supporting wall; f) anchoring plate. Design of the fixation box is depicted in the inset. Two holes of the side wall are used to ergonomically positioning the fixation bar. (B) Perspective view of designed head fixation bar for rats. Black arrows indicate the boundary of covering paraffin films. (C) The design of the port for head fixation in mice. (Modified from **Figure 1** of Roh *et al.*[9]) Please click here to view a larger version of this figure.

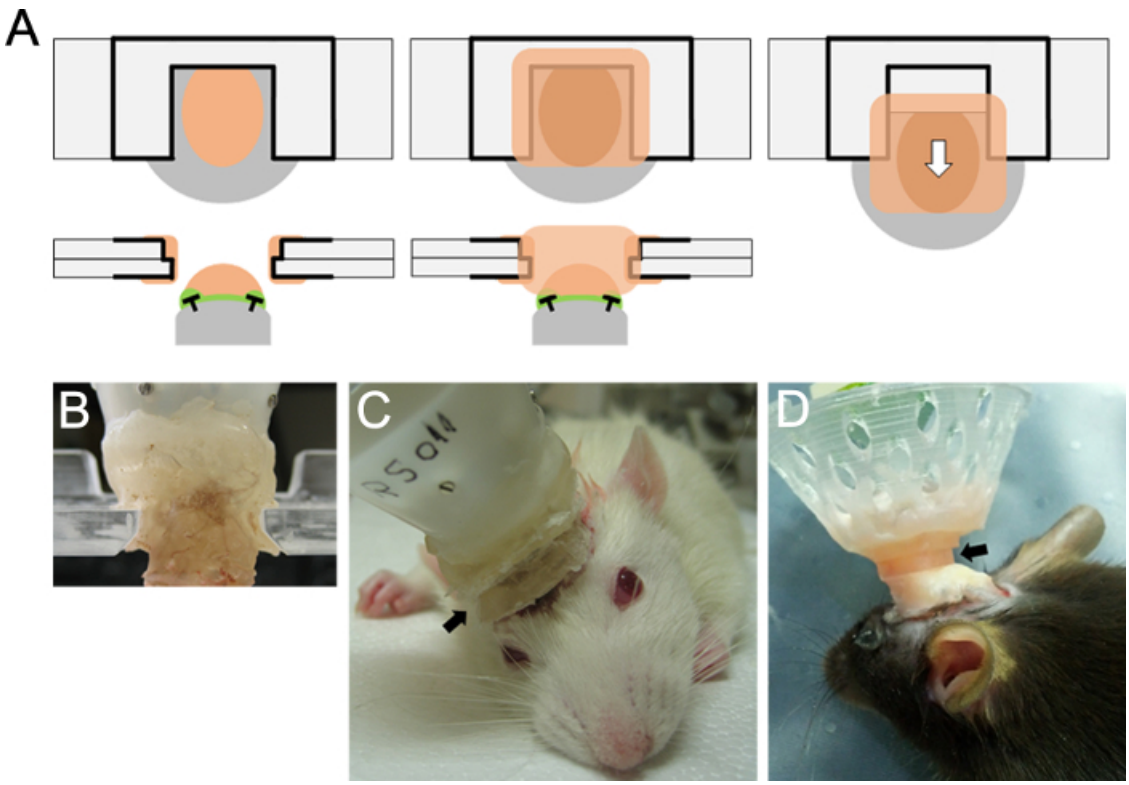

Figure 2. Construction of mounter molding during surgery.
(A) Critical steps molding the head mounter. (Left) Screws (T shapes in black) should be implanted perpendicular to the skull (gray) surface. A thin layer of dental adhesive resin cement (green) covers screws and the skull surface. (Center) After implantation depending on experimental purposes, additional acrylic resins (orange) are filled in the gap between initial resin coated on the port and base resins of the skull surface and becomes a part of the head mounter. (Right) After added acrylic resins are hard set, the head mounter can be released by sliding out of the port. Solid black lines indicate paraffin films. (Modified from **Figure 1** of Roh *et al.*[9]) (B) Constructed head mounter in rats is ready for releasing (after the step of A-Center). (C-D) Molded acrylic resins serving as a head mounter. After surgery, still under anesthesia, animals are released from the fixation bar. Black arrows indicate guide rails matching to the shape of the port. Please click here to view a larger version of this figure.

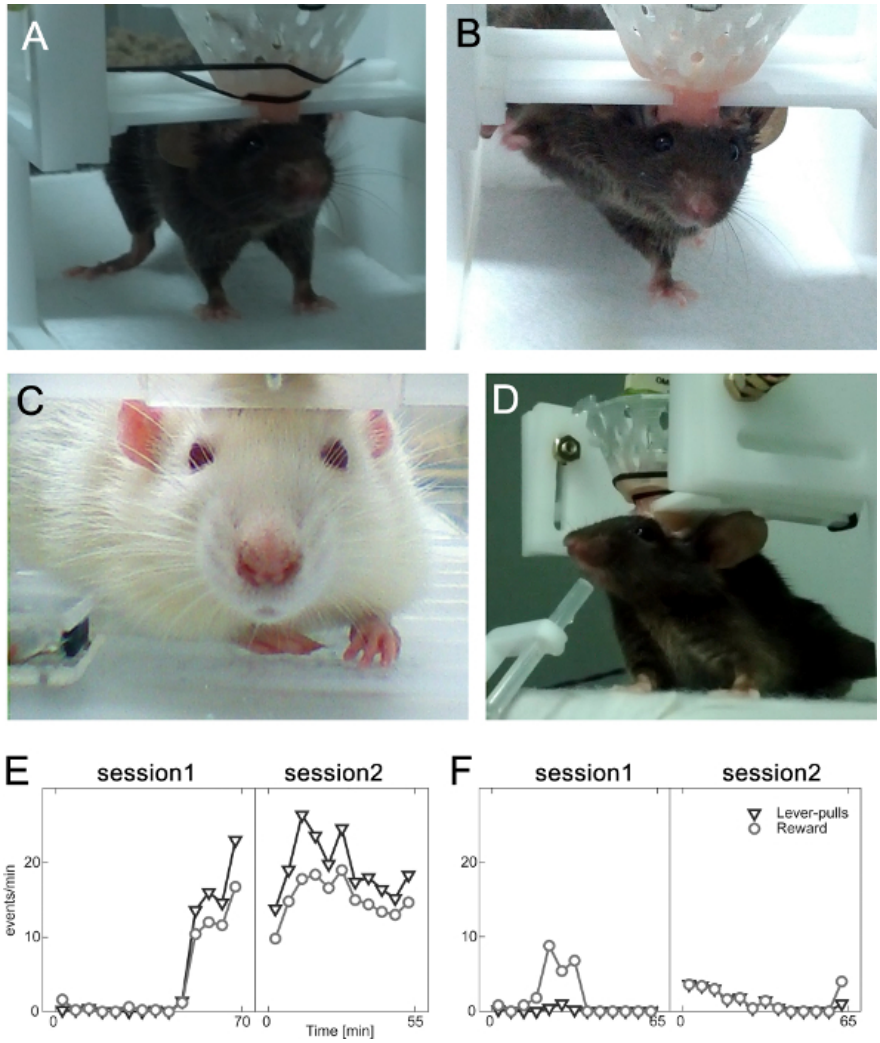

Figure 3. Head-fixed rat and mouse.
(A-B) When animals are initially exposed to head fixation, they emit struggling behavior. (C-D) As habituation proceeds, animals show less struggling behavior and usually sit down on the floor of the stationary platform. (E-F) Result of behavioral task performances from successful (E) and unsuccessful (F) habituation of head fixation environment (Modified from **Figure 3** of Roh *et al.*[9]). Reward number embraces both free (for shaping purposes) and contingent lever pull. In both sessions, insufficiently habituated rat shows poor performance with only rewarded by accidental lever pulls from non-contingent behavior. Please click here to view a larger version of this figure.

Discussion

This protocol demonstrates a simple and convenient head fixation method using materials at hand. For physically successful head fixation, the first critical steps during surgery is to rinse and dry the skull surface clearly before applying the dental adhesive resin cement. Second, this method does not uses screws implanting under temporal muscles and only depends on the screws on the skull, their perpendicular implantation against the surface of skull rim is crucial[10]. This reduces the possibility of accidental resin mounter separation from the skull, especially in rats.

The acrylic resin to build the head mounter is adhesive to the bar. We prevented this by covering the paraffin film over the port. However, this is an inconvenient procedure. One possible solution to improve this is to design the fixation bar with a metallic material. Furthermore, this should serve more stability in clamping than the acrylic bar by removing the gap occupied by the paraffin film. The described method might be inappropriate for brain imaging study because of small gap originated from paraffin film. This could also be resolved by metallic fixation bar or other method proposed elsewhere[12].

One of benefits of this method is flexibility (or compatibility). Since the area of the port covers vertical access routes into nearly most of the brain areas. This indicates that experiment-specific head frame design is not required. Once the fixation bar is designed, it can be used without any modification. Also, this method is immediately applicable in general laboratory condition. The head fixation without metal head-posts is achieved by molding the acrylic resin which is available in general laboratory performing implantation surgery. Fixation procedure is convenient to investigators without using screws and plates assembly[2,7,8].

Copyright © 2016 Journal of Visualized Experiments

Disclosures

The authors have nothing to disclose.

Acknowledgements

This research was supported by Basic Science Research Program through the NRF funded by the Ministry of Education, Science and Technology (2010-0022362) and (2012R1A2A2A02046812).

References

1. Mayrhofer, J. M. *et al.* Novel two-alternative forced choice paradigm for bilateral vibrotactile whisker frequency discrimination in head-fixed mice and rats. *J Neurophysiol.* **109**, 273-284 (2013).
2. Bryant, J. L., Roy, S., & Heck, D. H. A technique for stereotaxic recordings of neuronal activity in awake, head-restrained mice. *J Neurosci Methods.* **178**, 75-79 (2009).
3. Ollerenshaw, D. R. *et al.* Detection of tactile inputs in the rat vibrissa pathway. *J Neurophysiol.* **108**, 479-490 (2012).
4. Lee, M. G., Manns, I. D., Alonso, A., & Jones, B. E. Sleep-wake related discharge properties of basal forebrain neurons recorded with micropipettes in head-fixed rats. *J Neurophysiol.* **92**, 1182-1198 (2004).
5. Lutcke, H. *et al.* Optical recording of neuronal activity with a genetically-encoded calcium indicator in anesthetized and freely moving mice. *Front Neural Circuits.* **4**, 9 (2010).
6. Dombeck, D. A., Khabbaz, A. N., Collman, F., Adelman, T. L., & Tank, D. W. Imaging large-scale neural activity with cellular resolution in awake, mobile mice. *Neuron.* **56**, 43-57 (2007).
7. Kimura, R. *et al.* Reinforcing operandum: rapid and reliable learning of skilled forelimb movements by head-fixed rodents. *Journal of neurophysiology.* **108**, 1781-1792 (2012).
8. Hadlock, T., Kowaleski, J., Mackinnon, S., & Heaton, J. T. A novel method of head fixation for the study of rodent facial function. *Experimental neurology.* **205**, 279-282 (2007).
9. Roh, M., Jang, I. S., & Lee, M. G. Push-in Head Restraining Apparatus for Intracranial Self Stimulation Tasks in Rats. *Korean J Physiol Pharmacol.* **18**, 103-108 (2014).
10. Schwarz, C. *et al.* The head-fixed behaving rat--procedures and pitfalls. *Somatosensory & motor research.* **27**, 131-148 (2010).
11. Guo, Z. V. *et al.* Procedures for Behavioral Experiments in Head-Fixed Mice. *PLoS ONE.* **9** (2014).
12. Osborne, J. E., & Dudman, J. T. RIVETS: a mechanical system for *in vivo* and *in vitro* electrophysiology and imaging. *PLoS One.* **9**, e89007 (2014).

Copyright © 2016 Journal of Visualized Experiments