Journal of Neuroscience Methods 243 (2015) 63–67

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

journal homepage: www.elsevier.com/locate/jneumeth

Basic neuroscience

An inhalation anesthetic device for stereotaxic operation on mouse pups

Sachine Yoshida[1,2], Yuya Morimoto, Taishi Tonooka, Shoji Takeuchi*

Institute of Industrial Science, The University of Tokyo, Tokyo, Japan

HIGHLIGHTS

- The described anesthetic device for stereotaxic operation on mouse pups consists of polydimethylsiloxane (PDMS).
- The PDMS device is fabricated from 3D computer-aided design (CAD) data obtained from an actual mouse pup head.
- Day 10 pups were anesthetized and stabilized in a non-invasive manner and successfully injected using the PDMS device.

ARTICLE INFO

Article history:
Received 7 November 2014
Received in revised form 19 January 2015
Accepted 22 January 2015
Available online 31 January 2015

Keywords:
3D printer
CAD data
PDMS
Tracer injection

ABSTRACT

Background: Mouse pups are invaluable model animals for understanding the molecular and neural basis underlying behavioral development. Stereotaxic operations with anesthetic control are useful tools in systems neuroscience. However, there are no commercially available anesthetic or stereotaxic devices for mouse pups. Current devices have several problems such as invasive approach for stabilization, poor sanitary control, and less flexibility to combine other surgical apparatuses.
New Method: Here, we developed an inhalation anesthetic device equipped with stereotaxic function for mouse pups, by using polydimethylsiloxane (PDMS). PDMS is tolerant to heat and water exposure, and soft enough to cut or make a hole. The anesthetic and the stereotaxic parts were fabricated from the three-dimensional computer-aided design (3D CAD) data obtained from the head of a real mouse pup.
Results: To confirm its utility, a tracer was injected into the brain. We were able to anesthetize and stabilize pups at once in a non-invasive manner using the PDMS device. The histological staining revealed that tracer injection was successful. Our device was compatible with various types of commercial stereotaxic and anesthetic apparatuses via trimming and tube insertion, respectively.
Comparison with existing method(s): To our knowledge, this is the first report of a device that can stabilize the mouse pup's head with the non-invasive manner and functions as an inhalation anesthetic device that can be sterilized.
Conclusions: The present fabrication method will provide a handy and functional instrument for stereotaxic operations in animal models at various developmental stages.

1. Introduction

Mice are invaluable experimental animals for understanding the behavioral regulation at the molecular level. In the experimental systems neuroscience, stereotaxic surgical operations in adult mice are highly effective and have various applications such as site-specific brain lesions, targeted delivery of chemicals and vectors, and implantation of electrodes (Tsuneoka et al., 2013; Cetin et al., 2006). To perform these operations, adult mice are often anesthetized using barbiturates, and stabilized using commercially available stereotaxic apparatus. However, the stereotaxic operations in mouse pups that provide valuable information in the neural basis of behavioral development have technical difficulties in stabilizing the head and in controlling the dosage of the barbiturates. In pups, it is impossible to stabilize the head by using ear bars, since the external auditory meatus that accepts the ear bars in the adult are not fully opened yet. The skull is too fragile to stabilize tightly. In addition, the dosage control of barbiturate anesthesia is hard, unlike adult mice, because immature animals have lower levels

Abbreviations: BDA, biotynilated dextran amine; BLP, basolateral amygdaloid nucleus posterior part; CAD, computer aided design; LEnt, lateral entorhinal cortex; PDMS, polydimethylsiloxane; PMCo, posteromedial cortical amygdaloid nucleus; PND, postnatal day; Py, pyramidal tract.

* Corresponding author. Tel.: +81 3 5452 6650; fax: +81 3 5452 6649.
E-mail address: takeuchi@iis.u-tokyo.ac.jp (S. Takeuchi).
[1] Present address: Faculty of Medicine, Toho University, Tokyo, Japan.
[2] Present address: JST, PREST, Saitama, Japan.

http://dx.doi.org/10.1016/j.jneumeth.2015.01.025
0165-0270/

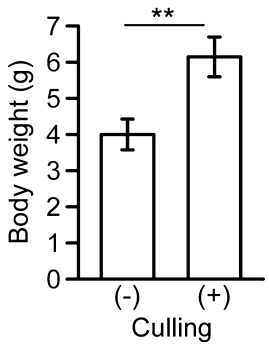

Fig. 1. Body weight comparison of PND 10 pups between without (−) and with (+) culling. All numerical data are shown as mean ± SD. ** $p < 0.01$. $n = 24$ (−), 15 (+). Coefficient of variation: 10.66 (−), 8.95 (+).

of serum albumin and body fat, which contribute to the diminished efficacy of the barbiturates (Flecknell, 1987). Consequently, there are no commercial inhalation anesthetic devices or stereotaxic instruments specialized for mouse pups that can stabilize the head in a non-invasive manner.

In this paper, we develop an inhalation anesthetic device equipped with stereotaxic function. To stabilize the pup's head in a non-invasive manner, we employ three-dimensional (3D) computer-aided design (CAD) data of an actual pup head and develop the anesthetic and stereotaxic parts. The present device consists of polydimethylsiloxane (PDMS), a silicone-based organic polymer, which is one of the most commonly used materials in bio-medical microdevices because of their easy fabrication process, biocompatibility, and low cost (Jose et al., 2007; Sia & Whitesides, 2003). We first conduct the stereotaxic operation on mouse pups using the PDMS device and evaluate its utility. Finally, we discuss advantages of this PDMS device and future applications of our method.

2. Materials and methods

2.1. Animals

All animal experiments were approved by the University of Tokyo and Toho University, and followed rules of the animal experiment committee of these universities. C57BL/6 mice were purchased from Sankyo Laboratory (Japan). Mice were maintained in clean cages with purified paper bedding (Alpha-Dri, Shepherd Specialty Papers, USA) under a 12-h light/dark cycle (lights-on 07:00) with food and water *ad libitum*. To reduce the body size differences, pups were culled to five at postnatal day (PND) 2 (Agnish and Keller, 1997). Body weight was measured at PND 2 and 10. At PND 10, pups with culling showed increased body weight and decreased variation coefficient compared to those without culling (Fig. 1). All experiments were performed between 8:00 and 11:00.

2.2. Fabrication of an anesthetic device with stereotaxic function

In this study, we developed an anesthetic device with brain stereotaxic function using 3D scanned data from an actual head of a mouse pup. The overall fabrication process is shown in Fig. 2. First, a 10-day-old pup (6.1 g) was scanned to acquire the 3D CAD data using a 3D scanner (Hamano engineering, Japan) (Fig. 2a). This data was incorporated into the design of a single flow channel for anesthetic gas using 3D modeling software (Fig. 2b). The actual information for dimensions of our device was provided in Suppl. Fig. 1. After fabricating 3D molds using a 3D printer (KEYENCE, Japan), we transcribed the upper and lower PDMS (Torey, Japan) replicas from the molds and bonded them using etching system

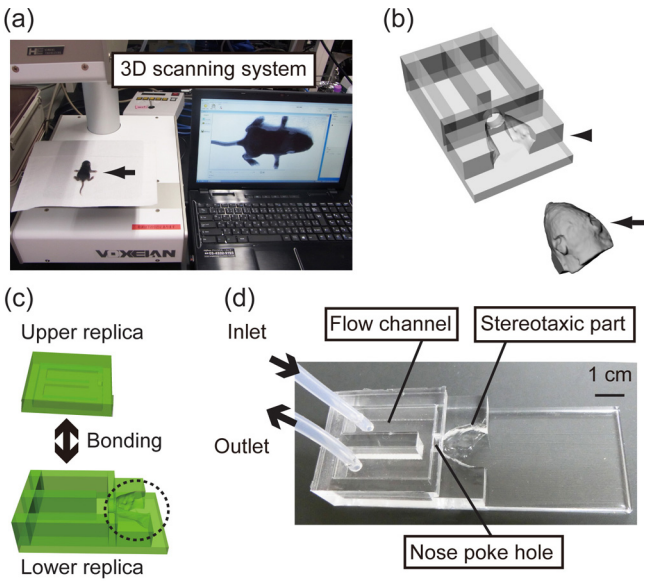

Fig. 2. Fabrication flow of an inhalation anesthetic device for mouse pups equipped with a brain stereotaxic instrument. (a) A day 10 pup (arrow) was scanned to obtain 3D CAD data. (b) The shape of the scanned head part (arrow) was subtracted from the design of the device (arrowhead). (c) After fabricating molds using a 3D printer, upper and lower polydimethylsiloxane (PDMS) replicas were transcribed and bonded. The dotted circle shows the structure based on a real pup head. (d) Two tubes are inserted to acquire inlet and outlet ports. The device consists of a single flow channel for anesthetic gas having a nose poke hole and a brain stereotaxic part.

FA-1 (Samco, Japan) (Oxygen gas: 20 mL/min, Power: 50 W, Time: 5 s) (Fig. 2c). Two holes (4 mm in diameter) were made using biopsy punch (Fig. 2d). Finally, two tubes were inserted into these holes to connect to a commercial inhalation anesthesia apparatus (Muromachi, Japan). To measure how much force was necessary to remove a pup's head from the PDMS device, a tail of pup anesthetized by sodium pentobarbital was pulled toward horizontal direction using a force gauge (IMADA, Japan) (Suppl. Fig. 2a).

2.3. Confirmation of anesthetic control by tail pinch

PND 10 pups were gently handled and placed into the PDMS device by the experimenter and exposed to 1, 2, or 4% isoflurane. The tails were pinched using tweezers every 15 s for up to 10 min. The time at which the pup stopped responding to the tail pinch was recorded.

2.4. Tracer injection into mouse pup brain using the PDMS device

PND 10 pups (6.1–6.2 g) were used for the brain microinjections. The PDMS device was incorporated within a commercially available stereotaxic apparatus (Narishige, Japan). First, the PDMS device (arrows, Fig. 5a) was set at an orthogonal orientation to the ear bars (arrowheads, Fig. 5a) in front. Next, the pup's nose and head were promptly placed into our device for head stabilization and anesthesia. After 2% isoflurane anesthesia, the skull was exposed and a hole was drilled. For tracer injection, biotin-conjugated dextran amine (BDA) (Molecular Probes, USA) was dissolved in 0.1 M phosphate buffered saline (PBS) (pH 7.4) to a final concentration of 5%. Glass micropipettes with a tip diameter of 50–60 μm were prepared for the BDA injection. BDA was injected iontophoretically (5 μA, 7 s on/7 s off for 5 min) at the stereotaxic coordinates A–2.5, L 3, V 4 on the right hemisphere. After injection, skin was sutured and returned to the home cage with mother and siblings. After 3 days (PND 13), pups were sacrificed to confirm the injection.