

Multiple Mouse Biological Loading and Monitoring System for MRI

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The use of mice to study models of human disease has resulted in a surge of interest in developing mouse MRI. The ability to take 3D, high-resolution images of live mice allows significant insight into anatomy and function. However, with imaging times on the order of hours, high throughput of specimens has been problematic. To facilitate high throughput, concurrent imaging of multiple mice has been developed; however, this poses further complexities regarding the ease and rapidity of loading several animals. In this study, custom-built equipment was developed to streamline the preparation process and to safely maintain seven mice during a multiple-mouse imaging session. Total preparation time for seven mice was ~24 min. ECG and temperature were monitored throughout the scan and maintained by regulating anesthetic and heating. Proof of principle was demonstrated in a 3-h imaging session of seven mice. Magn Reson Med 52:709–715, 2004. © 2004 Wiley-Liss, Inc.

Key words: multiple mouse; mouse imaging; MRI; anesthesia; mouse monitoring; small animal imaging

Recently, interest has grown in MRI of mice as it has become the mammal of choice to study functional genomics—the role of genes in the function of an organism. The mouse is an ideal vehicle for conducting genetic tests for a number of reasons: 1) there is 99% correspondence between the mouse and human genes; 2) among mammals, the mouse has a very short life cycle; 3) there are a number of inbred strains of mice; and 4) the genetic resources and tools to manipulate the genome in mice are well developed. Vital to the functional genomics agenda is MRI. As new models of human disease are discovered, *in vivo* characterization of function and anatomy is an absolute necessity.

Three-dimensional, high-resolution MR images of mice *in vivo* have allowed for further insight into anatomy and function. However, with imaging times on the order of hours, high throughput of specimens has been difficult. There have been a few attempts of imaging multiple animals (1–4). We have chosen to address this issue by implementing a system that images mice concurrently in shielded transmit/receive RF coils in a common magnet (5). In addition, we developed a multiple mouse loading

system to further increase throughput. This system allows all animal preparatory work to be performed independent of the magnet and a rapid loading system which simultaneously inserts all mice into their respective RF coils within the magnet bore, just prior to scanning.

In imaging live animals, several key features must be present throughout the imaging session: 1) a safe method of anesthesia, 2) environmental control, and 3) physiological monitoring. In addition, imaging multiple subjects poses further complexities regarding the ease and speed of preparation and the reproducibility of animal positions to facilitate image registration. Consequently, three major custom components were designed and fabricated: an induction chamber to facilitate preparation, a platform with embedded monitoring leads to standardize position, and a holding chamber to maintain anesthesia before the mice are inserted into the MRI scanner. In this article we present details of the designs and methods of our multiple-mouse loading system.

MATERIALS AND METHODS

All animal utilization protocols were approved by the Hospital for Sick Children Animal Care Committee, subject to the Canadian Council on Animal Care.

Mouse Loading System

The mouse loading system consists of two major parts: the “mouse hive” and the “loading array” (Fig. 1). The mouse hive’s main function is to position up to 19 Millipede RF coils (6) (Varian NMR Systems, Palo Alto, CA) in a hexagonal array inside the magnet bore. The loading array is designed to hold and transport multiple mice housed in 50-mL centrifuge tubes with holes drilled through their tips to allow entry of anesthetic gas. After the mice were anesthetized and interfaced to the monitoring equipment at a preparation area in the vicinity of the magnet, they were inserted into the modified centrifuge tubes and mounted onto the loading array. After mounting all mice the loading array was transported and inserted into the magnet and positioned on a rail system. The rail system allowed the array to couple with the mouse hive when pushed down the bore of the magnet.

When fully inserted into the magnet, the centrifuge tubes dock onto the anesthetic delivery system within the RF coils (Fig. 2). To ensure an airtight seal with the anesthetic delivery system, two types of nose cones were designed and fabricated: a rigid nose cone and a spring-loaded nose cone. The rigid nose cone (Fig. 2) is used only for the central position of the array and acts as a spacer to position the mice within the coils. The remaining posi-

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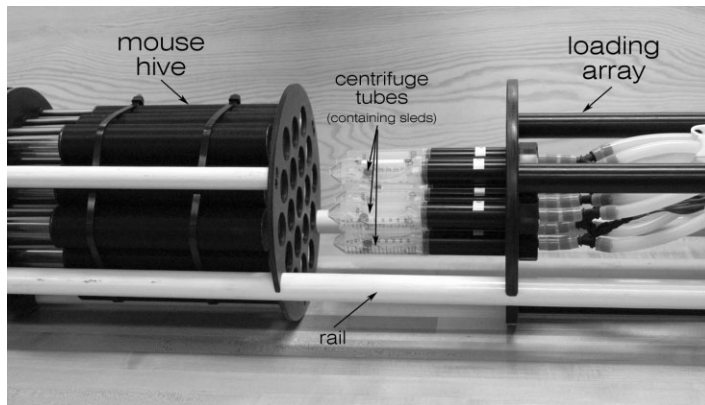


FIG. 1. The mouse loading system. The “loading array” and “mouse hive” connected with a common fiberglass rail system.

tions in the array are all outfitted with spring-loaded nose cones to compensate for any geometrical discrepancies and create airtight seals with their respective centrifuge tubes. Isoflurane mixed with oxygen is supplied from the mouse hive end to the specimen through a tube along the axis of each individual coil. This anesthetic gas mixture flows into the tubes, past the mice and is collected by a passive scavenging unit attached to the back of the loading array.

Induction Chamber

Since imaging times can take up to 3 h, minimizing animal preparation time is crucial to limit the mouse’s exposure to anesthesia. Hence, we developed a custom induction chamber to streamline the preparation process (Fig. 3).

The custom induction chamber creates a single environment for both induction and handling of multiple mice. Constructed from clear acrylic, the induction chamber features self-closing silicone iris ports to minimize anesthetic leakage and allows the user to access the internal environment without the need for special gloves. Compared to conventional mask and circuits for a single mouse, the induction chamber is large enough to house up to 20 mice and allows for free manipulation of the mice without the attachment of cumbersome tubes and masks. The unit is supplied with a constant flow of anesthetic gas which is collected using a passive scavenging system. Resistive heating elements are used to heat the floor of the chamber to maintain the animals’ body temperature during preparation.

A commercially available “glove box” can be modified to suit the above purpose, but our approach is more cost-effective and better suited for this application.

Sled

One of the most awkward and time-consuming aspects of preparing mice for MRI is the application of ECG electrodes and rectal temperature probes. In addition, many of the conventional electrodes, such as cuff and needle electrodes, were found to distort the animal’s posture, making it difficult to standardize positioning. Therefore, we devised a custom form-fitted positioning platform with embedded ECG and temperature probes called the “sled” (Fig. 4a) (patent pending).

The sled was constructed by generating a precise physiological plaster facsimile of a representative specimen in a favorable position (see Appendix for further details). Polypropylene sheets were then vacuum-formed and cut around the plaster facsimile to create thin, lightweight, autoclavable sleds. Nonmagnetic neonatal/pediatric ECG electrodes (ConMed, Utica, NY) were embedded into the sled to contact the chest and a thermocouple (Omega Engineering, Laval, Canada) was mounted in a similar fashion to measure skin temperature at the abdomen. A non-magnetic electrical connector mounted on each sled allows for easy sensor connection to the loading system. Motion restraints made from Velcro fasteners were used to limit movement of the head.

After removing the hair from a mouse’s chest, the mouse was positioned on a sled (Fig. 4b) and loaded into a modified 50-mL centrifuge tube.

Holding Chamber

As the mouse/sled/tube combination is moved from the induction chamber to be mounted on the loading array, an anesthetic atmosphere is essential. Hence, a separate

FIG. 2. Cross-sectional diagram illustrating the integration between the “loading array” and “mouse hive”.

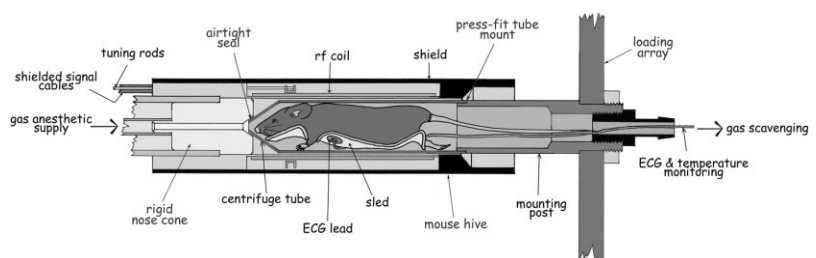
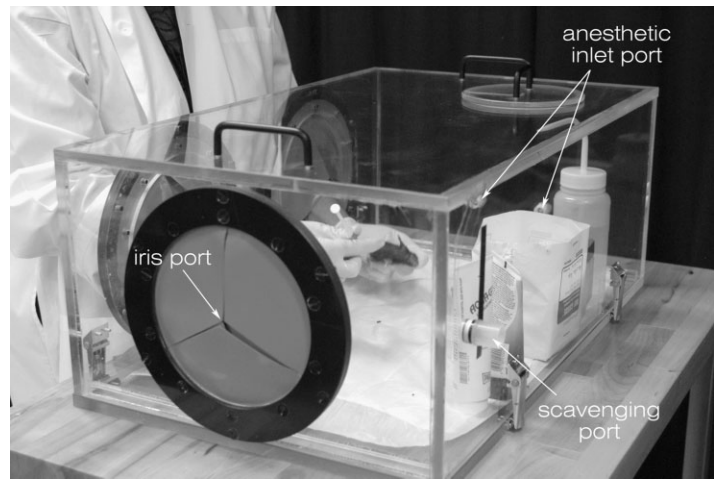


FIG. 3. The induction chamber.



“holding chamber” was developed to maintain anesthetic while awaiting the preparation of the remaining mice (Fig. 5).

Similar to the induction chamber, the holding chamber was constructed using clear acrylic and equipped with iris ports to allow easy access to its internal environment. An opening on one side of the holding chamber allows the loading array to be partially inserted and clamped to the side wall, thus creating a well-sealed volume. Anesthetic gas was continuously supplied to the chamber and collected using the passive scavenging system attached to the back of the loading array. As the centrifuge tubes containing mice are mounted on the array, their respective scavenging line is opened to allow the anesthetic to flow

through the 6.35-mm ($\frac{1}{4}$ ”) hole, into the tube and past the mouse.

Imaging Seven Mice In Vivo

The imaging of multiple mice was conducted using a 40-cm-diameter bore, 7 T magnet (Magnex Scientific, Oxford, UK) equipped with four parallel receivers (Varian).

Prior to the mouse imaging experiment the induction chamber was preheated, the mouse hive was placed inside the magnet bore, and the loading array was attached to the holding chamber. Seven mice were anesthetized in the induction chamber with a continuous flow of 3% isoflurane (AErrane (Isoflurane, USP) Baxter, Toronto, Canada). When the animals had no toe-pinch reflex, they were injected with 1/2 mL of saline in their abdomen (Baxter) to prevent dehydration and hair was removed from the chests (Nair, Carter-Horner, Mississauga, Canada) for the application of the electrodes in the sled. Conductive double-sided adhesive gel (Tyco Healthcare, Mansfield, MA) was applied to the ECG electrodes and the mouse was placed prone on a sled. Using restraints made from Velcro, each mouse’s head was strapped to its sled, then the mouse/sled combination was inserted into a modified 50-mL centrifuge tube.

After transferring the mice to the holding chamber, the monitoring leads on the sled were electrically connected to wires that run coaxially with the scavenging lines and their centrifuge tubes were press-fit onto the mounting posts of the loading array. Here, the prepared mice were maintained using 3% isoflurane while awaiting the preparation of the remaining mice.

Once all mice were mounted onto the loading system, the array was detached from the holding chamber and inserted into the magnet, where it was docked onto the anesthetic delivery system within the RF coils. A 1% isoflurane mixture with oxygen was used to maintain anesthesia during imaging. A single 28-pin CPC connector (Electrosonic, Toronto, Canada) was used to connect all monitoring leads to their respective monitoring devices.

Nonmagnetic T-type thermocouples affixed to the sled were monitored using a 16-channel temperature monitor and data logger (Topac, Hingham, MA). The mice’s body

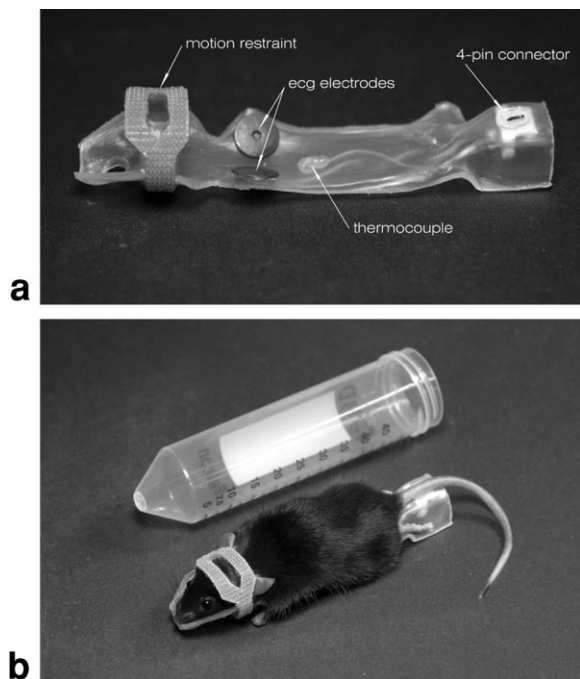


FIG. 4. **a:** The sled, showing embedded monitoring sensors and head restraint. **b:** An anesthetized mouse on a sled with the head restraint attached. The sled assembly easily slides into the centrifuge tube.

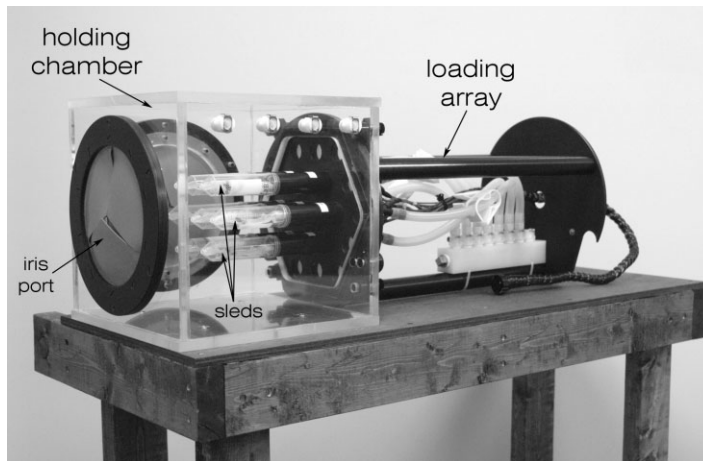


FIG. 5. The holding chamber with attached “loading array.”

temperatures were maintained between 34–37°C throughout the imaging process using a variable temperature, industrial-strength dryer (Edemco, Colorado Springs, CO). The warm air from the dryer was transferred and dispersed around the RF coils using a custom polycarbonate tube manifold.

The ECG and derived respiratory signal from each mouse was monitored using an ECG trigger unit (Rapid Biomedical, Wurzburg, Germany, or SA Instruments, Stony Brook, NY) and oscilloscope (Agilent, Palo Alto, CA).

We performed three sets of scans highlighting different contrast in the brain. The mice were i.p.-injected in the abdomen with Magnevist (1 mM/kg dosage gadopentetate dimeglumine, Berlex, Pointe-Claire, Quebec) 20 min prior to scanning. The first scan was a T_1 -weighted 3D spin-echo sequence with the following scan parameters: TR 200 ms, TE 12 ms, FOV 40 mm \times 20 mm \times 16 mm, 256 \times 128 \times 32 matrix, 156 μm \times 156 μm \times 500 μm resolution, NEX 2, total data acquisition time of 27 min 18 sec. The second scan was a diffusion-weighted 3D spin-echo sequence with the following scan parameters: 184 s/mm^2 b -value applied along the long axis of the mice, TR 1,117 ms, TE 21 ms, 156 μm \times 156 μm \times 500 μm resolution, NEX 1, total data acquisition time of 76 min 15 sec. The final scan was a T_2 -weighted multislice sequence with the following scan parameters: TR 6,200 ms, TE 20 ms, FOV 40 mm \times 20 mm, 256 \times 128 matrix, 156 μm \times 156 μm \times 1,000 μm resolution, NEX 3, total data acquisition time of 39 min 41 sec. The total scan time for the entire imaging session was under 2 h 45 min.

RESULTS AND DISCUSSION

With two people working, the preparation and loading of seven live mice took \sim 24 min in total, resulting in a time of about 3.5 min per mouse. Figure 6 shows a representative slice in all seven mice imaged with the three different imaging sequences. After imaging, all mice recovered well.

To minimize animal preparation time of multiple specimens, parallelization of processes is of utmost importance. For example, the development of the induc-

tion chamber allowed for the induction of multiple specimens simultaneously, and the sled has synchronized the application of the ECG and temperature probes while standardizing body positioning. Hence, the preparation and loading times should decrease on a per mouse basis as the number of specimens increase. Previous experiments conducted on four mice took \sim 16 min to prepare and load with one person, which results in a time of 4 min per mouse. Since most of the procedures can only be accomplished by one person, the preparation and loading times of one or two people are very similar. An estimated time for preparing and loading 16 mice solo would be \sim 45 min.

We have demonstrated a simple way of fabricating a customized sled for MR mouse imaging. For our purposes, we found that three sizes of sled can accommodate the majority of our specimens: small for 18–22 g mice, medium for 22–26 g mice, and large for 26–30 g mice. This technique is easily adaptable to other specimens such as rats and guinea pigs. However, the temperature monitoring may not work as well due to the larger size of the animal—since the mouse is so small, its cutaneous temperature follows the internal temperature very well. For larger subjects a rectal probe would likely be necessary.

In addition to simplifying the loading, the rigid sled also allows for reliable positioning, which aids in subsequent image analysis. If the animal is in the same position over several imaging sessions, tracking the desired region of interest is easier for either observer study or automated image processing schemes during longitudinal studies or comparative studies between different subjects.

Possible limitations of our system include the inability to individually control anesthetic and temperature for each mouse, even though there has yet to be a need for such functionality. If required, individual anesthetic control can be implemented with the addition of exclusive anesthetic vaporizers for each mouse. However, this would be an expensive endeavor. Individual temperature control would be much more difficult to implement due to space constraints in and around the array of the 3-cm-diameter birdcage coils.

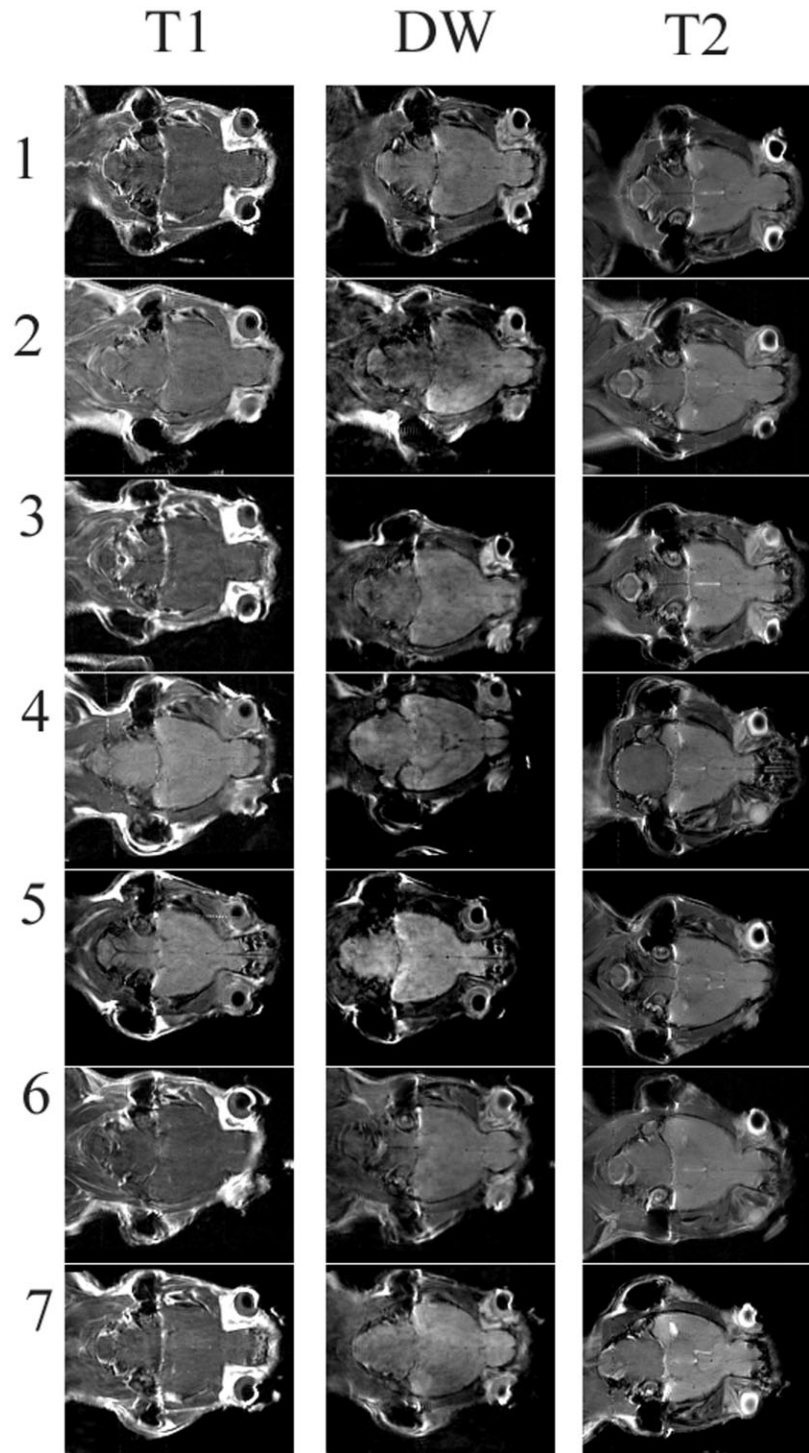


FIG. 6. Selected 2D slices from the whole-brain images of seven live mice. The three different imaging sequences are shown from each mouse brain. Left row: T_1 -weighted, middle row: diffusion-weighted, and right row: T_2 -weighted.

For future work, we plan to amalgamate the induction and holding chambers and to implement temperature feedback to maintain body temperatures at around 37°C. In addition, we will design multiple specimen cardiac and respiratory gating for in vivo whole-body imaging.

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APPENDIX

Making of Sleds

Fabrication of Plaster Molds

A representative mouse was sacrificed and placed supine in a 50-mL centrifuge tube (BD Biosciences Discovery Labware, Oakville, Canada). The mouse was arranged in a natural position and then frozen to ensure that the eventual sled allows for comfort and unrestricted respiration and to make a solid impression in the mold.

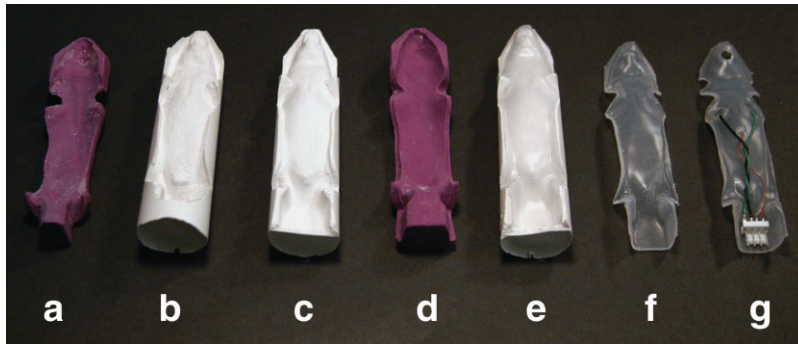


FIG. 7. The various steps involved with the construction of a sled. We show (a) the initial negative VPS mold of the frozen mouse; (b) the first positive plaster mold; (c) the sculpted plaster mold with a notch carved out as a placeholder for an electrical connector; (d) the final VPS mold taken from c; (e) the plaster mold with conformed plastic following vacuum forming; (f) the trimmed polypropylene sled; and (g) the final sled complete with monitoring devices attached (see Appendix for details).

Once frozen, a negative mold of the mouse was taken using VPS (vinylpolysiloxane) (Ash Temple, Concord, Canada) and after sufficient hardening the mouse was removed and the mold was cleaned and trimmed (Fig. 7a). Plaster of Paris was mixed and poured into the VPS form to create a precise physiological facsimile of the mouse (Fig. 7b). Fine details, like hair, were removed using sculpting tools to provide a smooth, generic mold of a mouse (Fig. 7c). At this stage, part of the plaster was also removed to accommodate an electrical connector for the monitoring equipment.

As described below, the plaster molds are destroyed during the vacuum process so a good negative VPS mold was necessary from which multiple plaster facsimiles can be made (Fig. 7d).

Method of Vacuum Forming

Vacuum forming is a standard plastic manufacturing process and a variety of equipment is commercially available. However, we adopted a simple, cost-effective method using two common appliances, an oven and a vacuum cleaner, along with two custom components, a vacuum platform and a holder frame (Fig. 8).

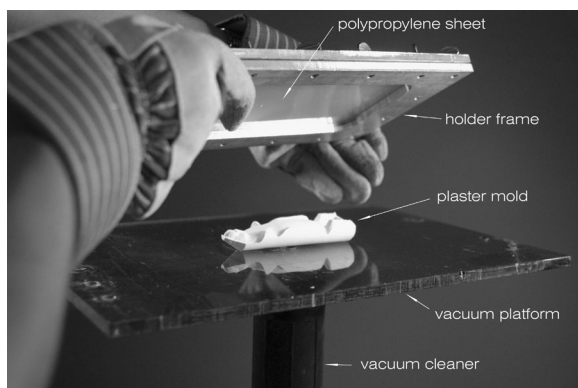


FIG. 8. The vacuum-forming process. The plaster mold is placed on top of the hole drilled into the vacuum platform. When vacuum is applied to the malleable polypropylene sheet, the plastic takes on the shape of the plaster mold (see Appendix for further details).

The vacuum platform was constructed out of a 12.7-mm (1/2") sheet of acrylic and served as an interface between the plastic and the vacuum cleaner. A 9.92-mm (25/64") hole was drilled into the acrylic and positioned over the vertically clamped vacuum nozzle. The plaster mold was placed directly over the hole before the preparation of the plastic sheet.

A 1.6-mm (1/16") sheet of polypropylene plastic (McMaster-Carr Supply, Cleveland, OH) was cut and clamped between two custom-made aluminum holder frames. The assembly was placed in the oven and heated until soft and translucent, $\sim 250\text{--}300^\circ\text{C}$. Once soft, the vacuum cleaner was turned on and the frame was quickly placed over the mold on the vacuum platform. With the frame pressed flush against the vacuum platform, the plastic immediately formed over the mold.

After allowing the plastic to cool, the polypropylene was removed from the frame and the plaster mold was broken away, leaving a thin plastic form of a mouse (Fig. 7e). Excess plastic was carefully trimmed using a drum sander and files (Fig. 7f), ensuring that the final geometry integrated precisely within the centrifuge tubes.

Integrating Monitoring Devices

In order to reduce animal preparation times, monitoring leads such as ECG and a cutaneous thermocouple were embedded onto the sled. The positions of the monitoring leads were determined and wire holes were drilled through the sled. After threading the wires through the holes, the ECG electrodes and thermocouple were fixed to the sled using hot glue (Arrow Fastener, Saddle Brook, NJ) and connected to a 4-pin AMP/Tyco Connector (Electrosonic, Toronto, Canada) (Fig. 7g). Finally, the connector was adhered to the space reserved on the underside of the sled (See Fabrication of Plaster Molds).

Motion Restraints

To reduce motion of the head during brain imaging, we restrain the head to the sled. This is done with straps made from hook and loop fastener material (Velcro, Fig. 2b). Notches are made in the sled to position the restraint at a desired location.

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