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Creation Of Resin Rodent Skulls To Reduce Animal Numbers For Stereotactic Surgery Practice

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Stereotactic brain surgery in laboratory rodents is a common procedure used for various applications in neuroscience research.^{1–3} This technique can be used to implant cannulas,^{2–4} electrodes,¹ or other devices such as optical fibers⁵ into specific brain regions to directly manipulate and/or record neuronal activity. One advantage of stereotactic placement of cranial implants is that it allows neuroscience experiments in unanesthetized animals that are alert and freely behaving. However, for long-term implants to be successful, lab personnel must be sufficiently trained in this challenging technique.

For novice users, the many steps of stereotactic brain surgery may seem overwhelming. To start, the rodent must be firmly secured in ear bars to completely immobilize and level the skull.⁶ An error in this step may cause inaccurate placement of the implant device resulting in inadequate data collection. Many implants also require a headstage to be permanently attached to the skull to protect the device or to enable attachment of wires to transmit signals to/from electrophysiology equipment.¹ Attaching headstages and implanting devices requires drilling into the skull to place anchor screws and applying dental cement to secure the headstage and implant in place.^{1,4} Dental cement must be applied in a time-dependent fashion at the proper consistency to mold the cement into crevices on the skull while remaining solid enough to create a steadfast foundation. Finally, if the cement is not applied correctly, the headstage will easily fall from the skull within a few days, leaving the rodent unusable for data collection.¹

Lab personnel must practice stereotactic techniques multiple times to become proficient before accurate data can be collected. This learning curve often results in a large number of rodents being euthanized purely for practice surgeries, inaccurate positioning of implant devices, or headstage attachment failure. In order to reduce the animals used in experiments, our lab has created a method for constructing resin rodent skull models to practice various stereotactic techniques. This method allows lab members to train and gain procedural memory for the many nuances in the individual steps of the surgery before attempting the technique on an animal. This approach aligns with the 3 R's of research, as it reduces the number of animals used for training purposes and replaces them with practice models.

To create the rodent models, an 8 ounce pack of Sculpey Mold Maker® (Polyform Products Company, Elk Grove Village, Illinois) was used to create multiple molds into which casting

resin could be poured. The mold maker was kneaded until softened and then formed into approximately $3 \times 3 \times 1.5$ cm blocks. A skull from a single rodent that is representative of the size and strain planned for future experiments was then gently and evenly pressed into the mold with the dorsal side down. The skull was immediately removed from the mold by grasping the incisors with forceps and retracting the skull outward. This approach left a detailed imprint of the rodent skull in the mold (Figure 1a).

After making several molds, Amazing Casting Resin (16 oz pack, Amazing Casting Products, Kalamazoo, Michigan) was mixed at a 1:1 ratio. Once a clear yellow liquid was achieved (~30 s), the resin was slowly poured into each mold and allowed to harden (Figure 1b). After approximately 10–15 minutes, the replicate skulls turned an opaque white color and were removed from the molds using forceps (Figure 1c). The resin skulls were wiped with a kimwipe to remove residual mold maker material from the skulls. Finally, excess resin was trimmed away using scissors, leaving multiple resin rodent skull models (Figure 1d).

With this method, at least 20 model rodent skulls can be created in less than an hour using a single kit. These skulls are inexpensive to make and can be utilized for many practice applications. Our lab personnel routinely use these models to practice manipulating and positioning the skulls in ear bars (Figure 2a), as well as to learn drill techniques for craniotomies and screw placement (Figure 2b). Lab members can also practice applying dental cement to attach 3-D printed, practice headstages (Figure 2c). An added benefit is the skulls can be soaked in acetone to remove dental cement, allowing for multiple practice trials per model.

In conclusion, resin rodent skulls are replicates of a specific rodent population and serve as an alternative tool for training lab personnel on stereotactic brain surgery techniques. This method drastically reduces the number of rodents euthanized for practice or due to procedural error. Additionally, the same materials can be applied to make skull replicates of larger research species in which stereotactic brain surgery is also performed.

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Figure 1. Creation of resin mouse skulls. (a) Mold with imprint of mouse skull. (b) Casting resin cured in the mold. (c) Resin skull released from mold. Note excess resin on the edges that can be trimmed away with scissors. (d) Multiple finished resin mouse skull replicates.

Figure 2. Practice applications for resin skulls: (a) ear bar manipulation, (b) drill techniques and screw placement, (c) headstage attachment with dental cement.