Journal of the American Association for Laboratory Animal Science
Copyright 2021
by the American Association for Laboratory Animal Science

Vol 60, No 5
September 2021
Pages 496–501

Association of Primate Veterinarians Cranial Implant Care for Nonhuman Primates in Biomedical Research

DOI: 10.30802/AALAS-JAALAS-21-000108

Purpose

Use of nonhuman primates (NHPs) in biomedical research may include performing cranial surgeries with chronic implantation of research devices. The Association of Primate Veterinarians (APV) supports the responsible use of NHPs in neurobiological research. Such research must meet specific criteria, such as the Institutional Animal Care and Use Committee (IACUC) review and approval, verification of the surgeon's skill and experience, and establishment of a close working relationship with institutional veterinary staff (Guide 2011). This document provides nonhuman primate researchers, IACUCs, and veterinary staff with guidelines for conducting research involving chronic cranial implants and for assessing their routine and non-routine care.

Background

Success in maintaining a chronic cranial implant in operational condition is a function of how the implant is placed and the types of materials used, coupled with the animal's physiology and healing responses. The laboratory animal veterinarian should interact closely with the research group to ensure the adequacy of surgical training and the use of optimal surgical technique, along with the use of appropriate methods to maintain the cranial implant postoperatively. Cranial implantation surgery must be conducted with consideration of normal host anatomy and physiology, as well as the maintenance of aseptic technique. Multiple survival surgical procedures are commonly used to stage placement of hardware (e.g., recording chambers, headposts) and cranial implants (e.g., epi- and subdural electrode arrays). Staging the invasive procedures until the data recording is required helps to preserve the integrity of the cranial implant and safeguard the health of the animal. For example, the headpost is implanted first, and then the animal is trained to perform the task while head-fixed. Once the animal is reliably trained, the recording chamber is implanted and a craniotomy created when ready to record. The IACUC must ensure the investigator provides a thorough description of all experimental and surgical procedures, which may include the surgical approach, total number of survival surgical procedures, implant maintenance, duration of experimental procedures, plan to address common complications, and humane endpoints prior to approval of experimental procedures (APV, 2020).

Background

Animal selection

A thorough assessment should be performed on animals prior to cranial device implantation to ensure that they will tolerate experimental procedures. Prior to instrumentation, research staff typically train animals to perform specific behaviors, including study-related tasks, pole and collar training, and restraint in a primate chair. Only animals that perform these behaviors consistently should be considered for instrumentation. The training should not cause distress to the animals. An animal that manifests abnormal behaviors such as lethargy, lack of interaction, increasing fear or anxiety, self-injurious behavior, or reduced food intake because of training is a poor candidate for implantation. Similarly, the behavioral and temperament characteristics of the animal should be taken into consideration in light of the skill and experience of the trainer and the study timeline. Animals that are fearful, excessively reactive, or unwilling to engage with humans may require counterconditioning, more time, and more patience during training to overcome their natural tendencies. If the trainer or study timeline cannot accommodate these, other animals should be considered (Bliss-Moreau & Moadab, 2016). The presence of cranial implants does not automatically exclude animals from social housing. Efforts should be made to establish stable pairs prior to instrumentation and maintain the pairs after implantation of devices.

Pre-surgical planning and procedures

There have been a number of recent refinements to ensure accurate placement of recording chambers for more precise experimental intracranial neuronal recordings as well as methods to reduce the risk of complications associated with cranial implants (e.g., infections, chronic inflammation, bone and tissue loss, etc.). For example, it is now possible to use pre-surgical imaging, such as computed tomography (CT) and magnetic resonance imaging (MRI), in combination with various software applications to create 3-D printed skulls of individual animals which aid in the design of cranial implant hardware for more precise placement of recording chambers and reduction of surgical time (Johnston et al., 2016; Overton et al., 2017). Clipping the hair liberally around the surgical site while avoiding clipper burns and cuts helps minimize unwanted irritation and infections. Small scissors or commercial depilatory products can be used in areas inaccessible to electric clippers. The skin must be surgically prepared and draped in a sterile fashion prior to initiating surgical procedures.

Surgical procedures

1. Cranial implantation surgeries must employ techniques minimizing trauma and preserving tissue architecture. Aseptic technique, appropriate instrument and suture use, isotonic fluid lavage, and skillful and gentle tissue handling is required
2. A neat and sterile cranial surgical site provides the best bonding surface, promotes bone remodeling, and facilitates anchoring of the cranial implant to the skull.
3. Use of a high-powered drill may lead to thermal cranial damage and secondary local bone necrosis with loosening of the screws and eventual implant detachment. Hand drills do not cause thermal damage, but their use can

lead to larger than necessary holes due to their increased instability. Ideally, piezoelectric drills are recommended for creating craniotomy sites since they cut only bone tissue (Johnston et. al., 2016). Continuous lavage with cold isotonic fluids during drilling or application of thin layers of exothermic compounds (e.g., polymethyl methacrylate, PMMA) may help prevent or minimize thermal damage to the bone and periosteum. This kind of damage is particularly important in younger or smaller NHPs with a thinner cranium.

Although PMMA has been used extensively in the past, there are newer strategies that have less potential for adverse outcomes, and their use is strongly encouraged over PMMA (refer to Refinements in Cranial Implants). In the human body the exothermic reaction of PMMA polymerization can reach temperatures of 82-86°C, so application of thin layers (5 mm or less) is critical. However, humans have a more extensive blood supply and larger exposed surface area to dissipate heat, making it likely that higher temperatures are reached for a longer period of time when used in smaller animals or applied in thick layers (Vaishya et al., 2013). In other reports, polymerization temperatures reached even higher, ranging 40-110°C, or even as high as 120°C (Dunne & Orr, 2002). The importance of controlling curing temperature cannot be overstated as heat in excess of 44°C may delay bone healing (Eriksson & Alberksson, 1983). Further, temperature in excess of 47°C for 5 minutes burns and permanently damages the bone. The resultant necrosis and structural compromise lead to potential implant instability and/or failure. Higher temperatures result in greater damage in less time (Lundskog, 1972).

Note: As there is risk of B-virus exposure from the aerosolization of CSF if the dura is breached, mucus membrane and respiratory protection must be worn by the surgeons and assistants.

4. Titanium or high-quality stainless steel orthopedic screws are often used to anchor cranial implants. Drilling pilot holes combined with the use of bone taps and blunt tipped screws minimize or even eliminate bone damage while contributing to implant longevity (Abee et al., 2012). Ideally, cranial MR images should be made before cranial implantations are performed to achieve the best outcome; therefore, a well thought out plan is essential. Stainless steel is a ferrous metal, which must not be placed in the magnetic field of a MR magnet; whereas, titanium, ceramic, and plastic-based implants provide an advantage over stainless steel, as they are non-ferrous materials that can be safely used in an MRI. However, they all cast shadows, and therefore can impact the quality of the images.
5. Hemostatic materials, such as Gelfoam®, effectively stop acute bleeding, but they must not be left inside the cylinder indefinitely. To remove Gelfoam® the cylinder should be filled with sterile saline for approximately 10 minutes to soften residual foam pieces and the process repeated, if needed. Forceful removal of Gelfoam® residue may produce additional hemorrhage and should be avoided. Bone edges are the most common source of bleeding within the cylinder, and hemorrhage can be controlled by sealing the edges with bone wax. The implanted cylinder may be opened for visual examination and carefully cleaned 1-2 days after surgery. After assuring adequate hemostasis, sterile saline should be placed in the cylinder followed by aseptic replacement of a clean, sterilized or disinfected cap. Steam sterilization, chemical sterilization (e.g., ethylene oxide), or high-level chemical disinfection with povidone-iodine scrub, alcohol, or 1:10 sodium hypochlorite solution are acceptable methods to process caps. Routine cleaning and maintenance of the inside of a cylinder/chamber protecting a craniotomy site is typically initiated within one week post-operatively. However, an undisturbed cylinder/chamber can be expected to remain sterile following aseptic surgery. Routine cleaning may be initiated when the cylinder/chamber is opened for recording.

Post-surgical procedures

While tending to newly placed or chronic cranial implants, one should be vigilant about potential pain. If there is any evidence of pain or distress associated with routine cleaning the underlying cause should be investigated, addressed, and appropriate analgesia given.

1. <u>Wound margin care</u>
   a. An uninfected surgical wound that is healing is best left alone for a period of 7-14 days post-operatively. Sterile saline rinses can be used if needed to clean the wound. Use of H2O2 is not recommended for 2-3 weeks post-operatively as it can interfere with the normal healing process. Dry, non-infected, hard crusts formed during normal healing may cause local irritation or pruritus, inviting self-trauma. Petroleum jelly or wet dressings applied every 2-3 days will keep the scabs soft and facilitate healing. There is no universally recommended frequency of cleaning. Rigorous or excessive cleaning can result in inflammation and infection. Wound margins should be closely inspected weekly and cleaned as often as needed.
   b. Re-growing hair should be carefully removed on an as needed basis. Scissors or small electric clippers are ideal for hair removal in conscious, chaired animals. Depilatory creams can be irritating and should be reserved for hair removal in sedated animals.

The wound margin adjacent to an implant requires regular observation and attention as it may become infected leading to suture loosening and skin dehiscence or necrosis, resulting in areas of skin devitalization or retraction away from the implant. Daily cleaning may be necessary as serous, serosanguinous, or purulent secretions will dry at the wound margin producing a protein-rich crust that may serve as a nidus of infection. Cleaning of the skin/implant interface involves gentle removal of loose crusts and unwanted hair with scissors and rinsing of wound margins. The following solutions should be considered for cleaning: sterile saline; chlorhexidine diacetate, 0.05% solution (1:40 dilution of stock chlorhexidine; Slatter, 2003); povidone-iodine, 1-2% solution (1:10-1:5 dilution of stock povidone solution); Dakin's solution, 0.5% sodium hypochlorite (particularly in the presence of necrotic tissue); Vetericyn Plus VF, 0.012% hypochlorous acid; or 1.5-3% hydrogen peroxide to remove dried blood and other secretions followed by copious saline irrigation. Water or sterile saline may be used as the diluent for any of these disinfectants.

Note: Hydrogen peroxide can cause tissue damage to open wounds. None of the listed compounds are effective indefinitely nor are they effective against all pathogens. A 7- to 10-day rotation of different disinfectants should be employed.

   c. Enzymatic debriding compounds facilitate the process by which devitalized tissue is softened or liquefied and removed.
   d. Infected sites should ideally be cleaned daily. Where mild, but chronic skin/implant problems are evident, twice a week inspection and cleaning 3-4 days apart are recommended. Culture and sensitivity should be done to

ascertain the nature of the infectious agent. The indiscriminate use of systemic or local antibiotics may contribute to the development of bacterial resistance and is strongly discouraged (APV, 2019; Lieberman et al., 2018; Woods et al., 2017).

2. Cranial headpost care

The skin may retract away from the headpost over a period of weeks to months post-operatively, and this is usually a gradual process. In the absence of local infection, skin repair surgery may be attempted. If skin retraction is significant, corrective surgery should be considered before the addition of bone cement.

3. Routine recording cylinder care

Most recording cylinders are anchored with screws and methacrylate products, and have a cap secured with 1-3 small screws. The inside of a chronic recording cylinder is not sterile, but it must be maintained aseptically. Recording cylinders are routinely opened in the non-sterile environment of the research laboratory or procedure room. Careful cleaning of the recording cylinder as described below has been demonstrated to minimize or prevent active cylinder infections. Ideally, no smell should be detectable in the recording cylinder, and the underlying dura should appear creamy white, smooth, and shiny.

a. The outside of the cylinder is typically contaminated and must be cleaned before the cylinder is opened for cleaning and/or recording. Povidone-iodine scrub (soap) should be used for the initial cleaning and rinsed with saline or 70% alcohol. Residual blood may be removed with 0.75-1.5% hydrogen peroxide (H2O2). Care must be taken to avoid contact between alcohol or H2O2 and viable soft tissues that are in the process of re- epithelialization.
b. Aseptic techniques must be used while opening a recording cylinder.
c. Uninfected cylinders should be cleaned twice a week. Sterile instruments (e.g., aspirator/suction tips, forceps) and supplies (e.g., gauze, gloves) should be used while working inside the recording cylinder. After cleaning, it is recommended the old cap be replaced with a clean, sterilized or disinfected cap. Steam sterilization, chemical sterilization (e.g., ethylene oxide), or high-level chemical disinfection with povidone-iodine scrub, alcohol, or 1:10 sodium hypochlorite solution are used successfully by various programs for cap processing.
d. Known or suspect infected cylinders should be cleaned frequently (Gografe & Niekrasz, 2009), regardless of whether animals are treated with antimicrobial agents. If there are multiple cylinders, they should be thoroughly cleaned sequentially rather than simultaneously. No materials (e.g., forceps, suction tips, etc.) should be shared between cylinders during multiple cylinder care. Uninfected cylinders should always be cleaned before known or suspect infected cylinders. Cleaning should always begin with a sterile saline lavage followed by suction. The dura must be carefully examined for the presence of focal infection, necrosis, cuts, or tears before any cleaning agents are applied. Disinfectants and antibiotics may contribute to unwanted toxic events that manifest clinically as neurological deficits. The following compounds have proven useful:

i. Use of a 0.75-1.5% H2O2 solution or a 1:1 mixture of H2O2 and povidone-iodine facilitates removal of biofilm and proteinaceous material from the interior surface of the cylinder wall.
ii. Rinsing several times with a dilute povidone-iodine solution at 1-2% (dilution is necessary for ionization of bound iodine). After cleaning, a few drops of 2% povidone-iodine solution may be left inside the cylinder.
iii. Some programs have reported no problems with the use of chlorhexidine for the routine maintenance of the inside recording cylinders; however, its use is controversial, as the compound has been demonstrated to have neurotoxic properties (Henschen & Olson, 1984; Perez et al., 2000; Lai et al., 2011). Manufacturers of chlorhexidine antiseptics include this warning on product labels, "Chlorhexidine should not be used to cleanse the skin prior to lumbar puncture; contact with the meninges should be avoided" (Prescribers' Digital Reference, accessed 9/10/20). Since other disinfectants (i.e., Dakin's solution, povidone-iodine) have been demonstrated to be efficacious for cylinder maintenance, the use of chlorhexidine should be carefully evaluated. At a minimum, care should be taken to evaluate the dural integrity prior to using chlorhexidine and to thoroughly rinse the cylinder free of the compound after each use. Consideration of disinfectant residue must also be given to hardware that penetrates below the dura, such as guide tubes. Leaving residual chlorhexidine in the cylinder for extended periods of time is also not recommended.
iv. A veterinarian should be consulted to address refractory infections. In this situation, Dakin's solution may be used as long as the integrity of the dura has not been compromised. Typically, it should remain in the cylinder for no more than 10 minutes followed by a thorough saline rinse. However, for especially refractory infections, Dakin's can remain in the cylinder overnight.
v. Chlorine dioxide is typically not used in routine cleaning, but it may be effective in short-term treatment of mycotic infections (Lee et al., 1998).
vi. In the majority of cases involving a durotomy or durectomy, the underlying cortex is covered with artificial dura combined with the use of silicone membranes, collagen matrix, or aliphatic polyether polyurethane sheets. Where the dura has been cut, it should be sutured to protect the cortex. The cylinder cleaning process is the same as with intact dura. It is critical to rinse with copious volumes of sterile water or saline if any disinfectant is used.

4. Granulation tissue

Granulation tissue (GT) formation is part of the normal healing process, but it is not always desired when maintaining chronic cranial implants. Budding GT on the wound margin and the dura is typically highly vascular and bleeds easily, oozes serum, and may interfere with healing if it becomes infected. Dural GT that has not been removed on a regular basis may bleed and eventually result in dural fibrosis. Thick granulation tissue pads can harbor bacteria and become a source of chronic chamber infections.

a. 5-Fluorouracil (5-FU) may be helpful in reducing or delaying the GT growth (Spinks et al., 2003). 5-FU is an antimetabolic, antimitotic agent that reduces tissue regrowth, vascularization, and bacterial overgrowth by interfering with nucleic acid synthesis, thus preventing mitosis. 0.5-1.0 ml of 25 mg/ml aqueous 5-FU can be instilled into the cylinder three times weekly to bathe the dura for 5 minutes. At the end of 5 minutes the cylinder should be rinsed with copious volumes of sterile saline. 5-FU must never be used on compromised dura as subdural leaks may contribute to complications. 5-FU decreases fibrinolytic activity and enhances the risk of thromboembolic events (Kessler & Rosengart, 1994). Care must be used when handling 5-FU

because it is a known carcinogen, and appropriate safety measures should be implemented per institutional guidelines.

b. Early GT deposits may be removed using suction. Local anesthesia can be provided via instillation of 0.25-0.5 ml of 1-2% lidocaine, 0.25% bupivacaine, or a 50:50 mixture for a few minutes before removal. Post-procedural systemic analgesics should be considered.

c. Chronic growth of GT typically leads to the formation of a firm fibrous layer requiring "dural scraping," which must be conducted under general anesthesia with the post-operative use of analgesics. GT deposits on the wound margin may be addressed by surgical debridement followed by a V-plasty, regular cleaning, treatment of local infections, and chemical or electrical cauterization under systemic or local anesthesia.

5. Treatment of infections

Polymicrobial bacterial colonization is common in cranial implants, and the formation of biofilm significantly complicates treatment strategies. Microbiota analysis has suggested that anaerobic bacteria comprise a large percentage of the microbial community in infected recording cylinders. Additionally, bacterial communities in infected cylinders are more similar to the skin margin and oral communities rather than feces (Lieberman, 2018). Assessment of the skin/implant interface can help determine if the infection is superficial or originating from under the cranial implant. In addition, the inability to retain fluid within the recording cylinder is often the result of open tracts between the cylinder and wound margin. The ideal interface should be smooth, and free of "pockets" and abrupt changes in the contour of the implant. Reshaping the interface and performing a V-plasty should be considered. Culture and sensitivity of purulent exudate should be used to guide antimicrobial therapy in conjunction with cleaning/debridement of the area.

Infections inside recording cylinders are common and can be prevented and treated with careful cleaning and maintenance as outlined. Systemic antibiotics should be reserved for treating cylinder infections in which the dura or bone are severely compromised, when clinical signs of meningitis are present, or where the infection has been unsuccessfully treated with frequent cleanings and use of disinfectant solutions. Indiscriminate use of antibiotics can result in bacterial resistance and additional problems (APV, 2019; Lieberman et al., 2018; Woods et al., 2017). The use of disinfectant solutions within the cylinder (e.g., povidone-iodine, Dakin's solution) has been adopted by some research laboratories with the intent to limit polymicrobial bacterial colonization. These solutions have been used as part of cleaning regimens and have been left in the chamber after cleaning for extended periods to treat chronic infections. Chronic infections should always be treated in consultation with a veterinarian, and antibiotic selection based on culture and sensitivity results.

Due to the formation of biofilm, which can be difficult to fully penetrate with either disinfectant solutions or antibiotics, removal of the implant may be the best option when the dura or bone are significantly compromised, or when repeat episodes of meningitis are observed. Taking into consideration IACUC approval and the welfare of the animal, reimplantation may be considered once the infection has resolved, and the bone has completely resossified.

Long term monitoring of animal health

As NHPs may be maintained on neurobiological research studies for multiple years, routine health monitoring is critical. Long-term cranial implants can induce chronic inflammation and infection leading to systemic sequelae such as amyloidosis and hematologic changes (Frydman et al., 2017). Routine physical examinations, blood work, and careful monitoring of the animal and any changes in the appearance of the cranial implant are recommended.

Considerations for cranial implants in New World monkeys

Most of the above recommendations may be similarly applied to both Old and New World primates; however, there are a few special considerations to keep in mind when working with New World primates or primates smaller than 5 kg in general.

1. The size and weight of the implant should be scaled down with consideration given to the surface area of the skull that will be taken up by the implant as well as the final weight of the implant and all components. A good rule-of-thumb is that the implant generally should not weigh more than the head to which it is attached (roughly 8-10% of animal body weight).
2. The final implant should allow for normal postural adjustments and species-typical behaviors. It may also be helpful to modify the home environment to accommodate the implanted animal (e.g., making the opening to the nest box larger or removing enrichment items that could snag the implant).
3. The IACUC should carefully consider the scientific justification for implant size which would interfere with species-typical behaviors or weigh more than 10% of the animal's body weight. It is warranted to determine whether these issues may be remedied through redesign using lighter-weight materials (see next section on Refinements to Cranial Implants). Alternatively, a period of relative confinement in a smaller enclosure may allow the animal time to adapt to the weight of the implant and re-learn balance without the risk of falling. Staging the addition of components to the implant over time may allow the animal to adjust to the weight of the implant gradually.
4. Another important consideration for New World species – especially marmosets and tamarins – is the relatively thin skull and the proximity of the lissencephalic cortex beneath it. As mentioned above in the section on Surgical Procedures, heat generation may be a problem for activities such as drilling or the curing of various resins and cements. Heat generation may be even more detrimental to smaller New World species due to diminished bone thickness, smaller total blood supply, and the ease with which heat may be transferred through bone to the underlying cortex. Not only is the bone itself more vulnerable to thermal injury, but there is also the potential for thermal injury, inflammation, and obstruction of blood flow in the nearby cortex. Using materials that cure at a lower temperature or limiting the amount/thickness of exothermic substances like PMMA are strategies to reduce thermal injury; see the next section on Refinements for suggestions on alternatives.
5. Adding to this hazard is that fluids for lavage to remove excess heat can saturate the fur of a smaller animal and lead to intraoperative hypothermia. The use of waterproof drapes or suction to divert lavage fluids away from the animal should be considered.
6. Finally, New World species appear to be more susceptible to cortical injury and consequences such as cerebral edema, focal traumatic brain injury, and ischemic reperfusion injury either from the thermal injury referenced above, or from the insertion of chronically implantable devices such as guide cannulas, optic fibers, multichannel electrodes,

or any other device that penetrates the cortex. While such procedures are routinely performed in macaques and mice, and appear to be well-tolerated in these species, marmosets (and potentially other related New World primates) appear to be more vulnerable to the consequences of such manipulations. Care should be taken to ensure that device insertions are completed as atraumatically as possible. The duramater should be incised to allow easy passage of the device without snagging or compressing the dura and adjacent cortex. The device should be inserted slowly and gently, allowing the brain parenchyma to relax around the implant as it is advanced. Rapid motions should be avoided which compress and then allow abrupt rebound of the tissue. Finally, the number of devices that are implanted in a single procedure should be limited. Investigators are encouraged to consult with their veterinarians regarding pre-treatment of animals with anti-inflammatory medications, such as steroids and/or non-steroidal anti-inflammatory (NSAID) medications, to help prophylactically address cerebral edema and neuroinflammation. Other strategies that might be considered are the use of hyperventilation to lower PACO2 and cause cerebrovascular constriction to help limit cerebral edema, and osmotic therapies such as mannitol or hypertonic saline infusions.

Refinements to cranial implants

Recent refinements in both the manufacturing and implantation of cranial implants have proven beneficial in lengthening the lifespan of the implant and decreasing the potential for adverse effects in the animal. They can be attached to the cranium without using methacrylate products and should be considered. 3D printing utilizing MRI/CT imaging techniques and the use of metal alternatives, such as carbon-reinforced polyether ether ketone (PEEK), have led to improvements in the overall fit of the implant to the skull as they can be customized to each animal (Mulliken et al., 2015; Overton et al., 2017; Chen et al., 2017). Further work has led to the development of an MRI-compatible, acrylic-free implant consisting of a combination of glass-PEEK coated with hydroxyapatite and ceramic screws (Ortiz-Rios et al., 2016). Titanium implants attached to the skull using specialized titanium bone screws and hydroxyapatite have led to better osseointegration, improved implant stability, and easier maintenance of skin/implant margins (Adams et al., 2007; Adams et al., 2011). These refinements should be given preference as they eliminate the use of PMMA, which is known to cause bone necrosis, tissue damage, and implant instability (Johnston et al., 2016; Adams et al., 2011).

References

1. **Abee, C, Mansfield, K, Tardif, S, Morris, T (eds.)** (2012). Nonhuman Primates in Biomedical Research, 2nd ed. American College of Laboratory Animal Medicine Series. Academic Press.
2. **Adams, DL, Economides, JR, Jocson, CM, Horton, JC.** (2007). A biocompatible titanium headpost for stabilizing behaving monkeys. J Neurophysiol 98(2):993-1001. doi: 10.1152/jn.00102.2007. Epub 2007 May 23. PMID: 17522180.
3. **Adams, DL, Economides, JR, Jocson, CM, Parker, JM, Horton, JC.** (2011). A watertight acrylic-free titanium recording chamber for electrophysiology in behaving monkeys. J Neurophysiol 106(3):1581–1590.
4. **Association of Primate Veterinarians** (2020). Humane endpoint guidelines for nonhuman primates in biomedical research. J Am Assoc Lab Anim Sci 59(1): 6–8.
5. **Association of Primate Veterinarians** (2019). Guidelines for MRSA infections in nonhuman primates in biomedical research. J Am Assoc Lab Anim Sci 58(3):285-288.
6. **Bliss-Moreau, E, Moadab, G** (2016). Variation in behavioral reactivity is associated with cooperative restraint training efficiency. J Am Assoc Lab Anim Sci 55(1):41-49.
7. **Bourne, GH** (1975). The Rhesus Monkey, Vol. 1 Anatomy and Physiology. New York: Academic Press.
8. **Chen, LM, Heider, B, Williams, GV, Healy, FL, Ramsden, BM, Roe, AW** (2002) A chamber and artificial dura method for long-term optical imaging in the monkey. J Neurosc Methods 113:41–49.
9. **Chen, X, Possel, JK, Wacongne, C, van Ham, AF, Klink, PC, Roelfsema, PR** (2017). 3D printing and modelling of customized implants and surgical guides for non-human primates. J Neurosci Meth 286: 38-55.
10. **Dunn, NJ & Orr, JF** (2002). Curing characteristics of acrylic bone cement. J Mater Sci 13:17-22.
11. **Eriksson, RA & Alberksson, T** (1983). Temperature threshold levels for heat induced bone tissue injury: a vital microscopic study in rabbit. J Prosthet Dent 50:101–107.
12. **Frydman, GH, Marini, RP, Bakthavatchalu, V, Biddle, KE, Matpalani, S, Vanderburg, CR, Lai, B, Bendapudi, PK, Tompkins, RG, Fox, JG** (2017). Local and systemic changes associated with long-term, percutaneous, static implantation of titanium alloys in rhesus macaques (Macaca mulatta). Comp Med 67(2):165-175.
13. **Gardiner, TW & Toth, LA** (1999). Stereotactic surgery and long-term maintenance of cranial implants in research animals. Contemp Top Lab Anim Sci 38(1):56-63.
14. **Gografe, S & Niekrasz, M** (2009). Care of chronic cranial implants in nonhuman primates, Nov 2008 – April 2009 survey of US institutions. 37th Annual APV Workshop, November 4-7, 2009, Broomfield, CO.
15. **Henschen, A & Olson, L** (1984). Chlorhexidine-induced degeneration of adrenergic nerves. Acta Neuropathol 63:18-23.
16. **Kessler, CM & Rosengart, A** (1994) Hemostasis and Stroke. CRC Press.
17. **Johnston, JM, Cohen, YE, Shirley, H, Tsunada, J, Bennur, S,Christison-Lagay, K, Veeder, CL** (2016). Recent refinements to cranial implants for rhesus macaques (Macaca mulatta). Lab Anim 45:(5): 180-186.
18. **Lai, P, Coulson, C, Pothier, DD, Rutka, J** (2011). Chlorhexidine ototoxicity in ear surgery: review of the literature. J Otolaryngol Head Neck Surg 40(6):437-40.
19. **Lee, G, Danneman, P, Rufo, R, Kalesnykas, R, Eng, V** (1998). Use of chlorine dioxide for antimicrobial prophylactic maintenance of cephalic recording devices in rhesus macaques (Macaca mulatta). ContempTop Lab Anim Sci 37(2):59-63.
20. **Lieberman, MT, Van Tyne D, Dzink-Fox, J, Ma, EJ, Gilmore, MS, Fox, JG** (2018). Long-term colonization dynamics of Enterococcus faecalis in implanted devices in research macaques. Appl Environ Microbiol 84:e01336-18.
21. **Lieberman, MT** (2018). Characterization of polymicrobial infections in macaques with chronic cranial implants and evaluation of alternative antimicrobial strategies. Massachusetts Institute of Technology, PhD Dissertation.
22. **Lundskog J** (1972). Heat and bone tissue. An experimental investigation of the thermal properties of bone and threshold levels for thermal injury. Scand J Plast Reconstr Surg 9:1-80.
23. **Mulliken, GH, Bichot, NP, Ghadooshahy, A, Sharma, J, Kornblith, S, Philcock, M, Desimone, R** (2015). Custom-fit radiolucent cranial implants for neurophysiological recording and stimulation. J Neurosci Meth 241:146-154.
24. **National Research Council** (2011). Guide for the Care and Use of Laboratory Animals (8th edition). Washington DC: National Academy Press.
25. **Niekrasz, MA & Wardrip, CL** (2012). Surgery in Nonhuman Primates. In Abee, CR, Mansfield, K, Tardif, S, & Morris, T (eds). Nonhuman Primates in Biomedical Research: Biology and Management, 2nd edition., pp. 339-358. Amsterdam: Elsevier.
26. **Ortiz-Rios, M., Haag, M, Balezeau, F, Frey, S, Thiele, A, Murphy, K, Schmid, MC** (2018). Improved methods for MRI-compatible implants in nonhuman primates. J Neurosci Methods 308:377–389. https://doi.org/10.1016/j.jneumeth.2018.09.013.

27. **Overton, JA, Cooke, DF, Goldring, AB, Lucero, SA, Weatherford, C, Recanzone, GH** (2017). Improved methods for acrylic-free implants in nonhuman primates for neuroscience research. J Neurophysiol 118: 3252-3270.
28. **Perez, R, Freeman, S, Sohmer, H, Sichel, JY** (2000). Vestibular and cochlear ototoxicity of topical antiseptics assessed by evoked potentials. Laryngoscope 110(9): 1522-7.
29. **Slatter, D** (2003). Textbook of Small Animal Surgery, 2nd edition.
30. **Spinks, RL, Baker, SN, Jackson, A, Khaw, PT, Lemon, RN** (2003). Problem of dural scarring in recording from awake, behaving monkeys: a solution using 5-fluorouracil. J.Neurophysiol 90(2): 1324-1332.
31. **Vaishya, R, Chauhan, M, Vaish, A** (2013). Bone cement. J Clin Orthop Trauma. 4(4):157-163. doi:10.1016/j.jcot.2013.11.005.
32. **Woods, SE, Lieberman, MT, Lebreton, F, Trowel, E, de la Fuente-Núñez, C, Dzink-Fox, J, Gilmore, MS, Fox, JG** (2017). Characterization of multi-drug resistant Enterococcus faecalis isolated from cephalic recording chambers in research macaques (Macaca spp.). PLoS ONE 12(1):e0169293.