Published in final edited form as:

Exp Neurol. 2007 May ; 205(1): 279–282.

A Novel Method of Head Fixation for the Study of Rodent Facial Function

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Abstract

The rodent vibrissal system offers an excellent model for the study of both sensory and motor function. It has been widely employed to gather data pertaining to sensory and motor function involving the 5th and 7th cranial nerves and the central nervous system. Existing methods of head fixation for precise measurements of ocular and vibrissal function involve exposing the cranium and applying dental cement from which two or more threaded rods emerge. This common approach is suboptimal, requiring a relatively complicated implantation procedure, and results in a large, chronic interface between the scalp and environmentally-exposed implant material attached to the skull. Here we describe a head fixation device that is inexpensive, easy to build, less prone to infection, preserves access to the cranial midline, and permits repeated measurements over many months.

Introduction

The rodent vibrissal system has been an extremely valuable model for studies attempting to elucidate mechanisms of learning, processing sensory information, and understanding facial sensorimotor reflexes and pathways (Bermejo et al. 1996, Nguyen et al. 2005, Crish et al. 2003, Ahissar et al. 2003, Kleinfeld et al. 1999). The rodent eye blink model has also been employed to elucidate the relationships between the 5th and 7th cranial nerves (Gong et al. 2003, Zerari-Mailly et al. 2003). Most investigators who examine facial movements employ either pharmacologic sedation (Wiegand et al. 1991), or some type of head fixation device to minimize motion artifact (Bermejo et al. 1996, Lee et al. 2003, Sachdev et al. 2000, Hosoi et al. 2003). The most commonly employed head fixation device involves removal of the soft tissues overlying the cranium, and securing a “helmet” of dental cement onto the bone, with several bolts protruding for fixation into an external device. Significant shortcomings of this approach include chronic granulomatous tissue reaction around and under the cement, and severe infection leading to dislodgement of the head mount (Zeigler, H.P., personal communication). While recent research describes a method of allowing the animals to move freely, and

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performing complex subtraction algorithms to calculate vibrissal movement and position (Knutsen et al. 2005), this approach requires intensive computer data programming and processing. The purpose of this study was to create a simple head fixation device that 1) minimizes the skin – foreign body interface, 2) is easily and reliably secured to the skull, and 3) is relatively small, light weight, and made of biocompatible materials. In particular, improving implant biocompatibility and reducing infection at the implant/tissue interface should permit essentially indefinite repeated measurements of ocular and vibrissal function.

Materials/Methods

Design of implantable head-fixation device

The head-fixation implant was designed as a single piece that fit onto the calvarium of the adult rat. A computer model (Rhinoceros Ver 3.0, Robert McNeel & Associates) of the implant was first created in reference to photographs of skulls from adult Fisher and Wistar rats. The implant had an external curvature following the lateral and posterior borders of the superior skull surface (see figure 1) and contained 8 screw holes (1.35 mm ID) for attachment to the skull that were positioned to provide firm attachment to all of the major cranial plates, while avoiding penetration of cranial sutures and dural sinuses. Four holes were positioned above the lateral edges of the temporal and parietal cranial plates. An additional 4 screw holes were provided by pairs of lateral and posterior ‘tabs’ or extensions bent inferiorly to match the lateral and posterior curvature of the adult rat skull, respectively. These screw hole tabs were bent by holding the implant in a small iron c-clamp that was filed so that the clamp edges matched the width and curvature of the adult rat skull, and tapping the tabs with a small hammer until they rested on the clamp edges (thus conforming to rat skull shape). After the initial tab bending, small adjustments in tab angle were made with hand pliers to accommodate variability in adult rat skull size as needed during surgery. These hand adjustments sometimes resulted in tabs snapping off as the titanium became brittle with repeated bending, which occurred less frequently if the tabs were heated with a propane torch during bending. The number of potential adjustments for particular implants is limited due to the loss of flexibility in the tabs with repeated bending, so tab angle for individual implants was measured to best match skull size to implant size in subsequent animals when reusing the implants.

External attachment with the implant for head fixation was provided by two extensions that exited the caudal scalp and terminated in rings (2 mm ID) oriented horizontally behind the pinna. These rings were positioned just lateral to the caudal skull and neck. Therefore, the rat head could be held securely by lowering the implant’s rings onto vertical posts that were separated sufficiently to straddle the rat’s neck.

The computer representation of the implant was made into a physical prototype by printing the design onto paper and using it as a template for carving the implant’s 3-D shape from a 1.5 mm thick sheet of microcrystalline wax. The wax version of the implant was then cast in white gold by a jeweler (Allan Leavitt, Boston, MA) using the lost wax method, and used in a preliminary implantation study. Results from the first implantation (see Results section) indicated that the posterior attachment points (rings) needed greater separation in order to better accommodate the caudal head and neck between the vertical posts, and that two additional attachment points (rostral to the pinna) were needed to more evenly distribute the strain on screw attachment points with the skull. The computer representation was then modified and sent to a machine shop (Whitman Tool & Die, Whitman MA) for wire EDM fabrication from surgical-grade 99.2 % commercially pure titanium (figure 1). The devices were autoclaved in preparation for surgical implantation.

Implantation surgery

Seven 250–400 g female Wistar rats underwent surgical implantation of the head fixation devices. Briefly, after the induction of anesthesia via an intramuscular injection of ketamine (60mg/kg) and medetomidine (0.5 mg/kg) mixed in saline, a midline incision was made in the scalp, and two small incisions corresponding to the location of the implant's posterior extensions were made (figure 3A). A subperiosteal plane was developed over the calvarium as far anterior as the nasofrontal junction. The sterile implant was then secured to the calvarium with surgical titanium screws (1.3 by 4 mm; Synthes CMF, Needham MA), and the skin closed (figure 2). Animals were monitored daily for indications of infection, implant extrusion, unusual cage behavior, and changes in behavior during handling. The animal receiving the initial gold prototype was sacrificed at 60 days, and the subsequent six animals receiving the titanium implants were survived for 16 weeks. Experimental procedures were conducted in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, and the Animal Welfare Act (7 U.S.C. et seq.), and an animal use protocol approved by the Massachusetts Eye and Ear Infirmary Animal Care and Use Committee.

Testing protocol

Animals were handled daily for 2 weeks prior to and 1 week after the implantation of the fixation devices. Then, starting on postoperative day 7, animals were removed from their cages, acclimated to the testing site, and placed into a Decapicone® plastic rodent restrainer (Braintree Scientific Inc., Braintree MA). The head was placed between two threaded posts secured to a platform (figure 3), the rings of the right and left posterior extensions positioned onto the posts, and nuts were applied to hold the implant rigidly, thus securing the head in a fixed position. The animals remained in the head fixed position for a ten minute period during each testing session, during which they were rewarded with chocolate drink (Yoo-hoo, NY, NY) through a tube leading to their mouth. Testing proceeded on a daily basis for the ensuing week, and then weekly for the ensuing 2 months, and once again the following month prior to sacrifice.

Postmortem Evaluation

For assessment of the depth of screw penetration and its potential effects on the central nervous system, two animals were sacrificed via perfusion using normal saline followed by 10% formalin. The brain was removed from the skulls and examined grossly for dural penetration. A coronal segment of the tissue corresponding to the locations of the overlying screws in one of the brains was then serially sectioned and H&E stained.

Results

There were no intra-operative complications, and none of the animals experienced postoperative infection or extrusion of the implant over the 4 month study period. In all cases, the titanium plate successfully secured the rodent head in a fixed position for the acquisition of facial movement data. There was no significant breakdown of skin overlying the implanted head plate in any of the animals, despite head fixation testing on an at least weekly basis for most of the study period. There was no significant scarring or granulation at wound sites. Weight gain was appropriate when compared with control animals.

Gross and histologic examination (N=2, N=1, respectively) of skull and brains revealed that although the tips of the screws typically penetrated the brain, there was an absence of fibrosis, granulation tissue, or foreign body reaction. Specifically, brain tissue at the screw penetration locations appeared healthy and without chronic inflammation in H&E stained sections (figure 4).

Discussion

The facial nerve has long been used to study neuronal plasticity and regeneration in the peripheral nervous system, and much effort has been devoted to establishing an ideal animal model for clinically relevant studies of its function. Previous experimental models have used the rat, cat, rabbit, and guinea pig facial nerve (see Moran and Graeber 2004 for review). The rat has emerged as a particularly useful model for studying facial nerve function, since details of its gross and microscopic neural anatomy are well documented (Mattox et al. 1987; Guntinas-Lichius et al, 2005, de Faria et al 2006), and the nerve supports the rat's elaborate and highly quantifiable vibrissae movement (Bermejo et al 1996, 2002, 2004). Furthermore, because the rat transmits sensory input through the trigeminal system and provides motor output to the vibrissal system via the facial nerve, it has been used to describe complex sensorimotor behaviors and define anatomic reflex loops. Several neurosurgical treatment options (Dort et al. 1994, Hadlock et al. 2004) and neurotrophic agents (Murray et al. 1993) have been investigated in rat facial nerve model, making it an important tool for studying human facial nerve lesion, repair, and regeneration.

Although there are electrophysiologic models for the assessment of facial nerve activity in the rat, behavioral parameters such as eye and whisker movements remain superior indicators of global function. Monitoring of these facial movements has been complicated by inherent motion artifact introduced by head movement. Investigators have addressed this by attempting to prevent motion through head fixation, or by gathering data despite head movement and subsequently "subtracting" the head movement artifact. Both of these methods have produced reliable methods of recording of facial nerve activity in awake, behaving animals, but both approaches have shortcomings as mentioned above.

In an effort to improve the fixation approach, we have described a novel device for head fixation that allows precise, repetitive measurements of both ocular and vibrissal function that can be stably maintained for months. The use of a biocompatible implant largely eliminates the tissue reaction at the skin interface, as well as the infection risk of a more exposed implant. Since the weight of the implant (600–700 mg) represents only 0.3 % of the weight of the animal, it is unlikely to cause a change in head, neck, or facial function given its relatively small mass. In addition, the posterior extensions do not appear to interfere with the normal range of head/neck motion, and the external hardware attachment points behind the pinna leave the entire face surface free for study of all facial nerve functions. Moreover, unlike standard head fixation with dental acrylics, this implant leaves the superior surface of the cranium accessible for neurosurgical manipulation.

In summary, titanium implant head fixation offers a valuable tool in assessing facial function in the rat model and represents a significant simplification over currently employed head fixation techniques. Major advantages include 1) fabrication that is rapid and inexpensive, 2) implantation using a relatively simple and rapid surgical procedure, 3) lack of threaded regions on the implant that would have the potential to wear over time, and 4) the potential for long-term residence time due to effective skull attachment and minimal surface area of exposed wound edge at the device exit locations. Beyond application in rats, this general head fixation approach would likely prove useful in any small animal model for investigators studying precise facial sensorimotor behavior.

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1.

A) The implant outline is shown adjacent to (B) a superior and (C) lateral view of a titanium implant secured to an adult female Wistar rat skull using 9 screws. Note how the screw locations avoid the cranial sutures which are indicated by black dots in (B). The implant outline shows the shape prior to bending the tabs for the 4 lateral screw locations. The scale bar applies for A and B, but not the perspective view in C.

2.
Rostral and caudal views of a rat with a shaved head 10 weeks after implantation, showing a healthy tissue interface at the four implant extension locations. The posterior extensions typically fell directly behind the pinnae, as shown.

3. An adult rat is shown in the restraint apparatus with the implant's external extensions secured onto threaded vertical posts (embedded in gray epoxy) using 4 large aluminum nuts. The rat's body is restrained in a tapered plastic sleeve and held in a plastic half-pipe using black Velcro straps located at the forelimbs and hindlimbs.

4.
H&E section of brain (500 micron scale bar shown) at one of the implant screw penetration sites. Note extent of lesion, though the absence of chronic inflammation or granulomatous reaction.