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A simple, inexpensive method for subcortical stereotactic targeting in nonhuman primates

J. Nicole Bentley[a], Siri S.S. Khalsa[a], Michael Kobylarek[b], Karen E. Schroeder[b], Kevin Chen[a], Ingrid L. Bergin[c], Derek M. Tat[b], Cynthia A. Chestek[b], Parag G. Patil[a,b,*]

[a] Department of Neurosurgery, University of Michigan, Ann Arbor, MI, United States
[b] Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI, United States
[c] Unit for Laboratory Animal Medicine, University of Michigan, Ann Arbor, MI, United States

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ABSTRACT

Background: Many current neuroscience studies in large animal models have focused on recordings from cortical structures. While sufficient for analyzing sensorimotor systems, many processes are modulated by subcortical nuclei. Large animal models, such as nonhuman primates (NHP), provide an optimal model for studying these circuits, but the ability to target subcortical structures has been hampered by lack of a straightforward approach to targeting.

New Method: Here we present a method of subcortical targeting in NHP that uses MRI-compatible titanium screws as fiducials. The in vivo study used a cellular marker for histologic confirmation of accuracy.

Results: Histologic results are presented showing a cellular stem cell marker within targeted structures, with mean errors ± standard deviations (SD) of 1.40 ± 1.19 mm in the X-axis and 0.9 ± 0.97 mm in the Z-axis. The Y-axis errors ± SD ranged from 1.5 ± 0.43 to 4.2 ± 1.72 mm.

Comparison with existing methods: This method is easy and inexpensive, and requires no fabrication of equipment, keeping in mind the goal of optimizing a technique for implantation or injection into multiple interconnected areas.

Conclusion: This procedure will enable primate researchers to target deep, subcortical structures more precisely in animals of varying ages and weights.

1. Introduction

The ability to perform stereotactic targeting to various brain structures in large animal models has wide applicability across multiple areas of neurophysiologic, anatomic, and neuropathology research. For example, microelectrode recordings in cortical areas have enabled substantial progress in the development of brain-machine interfaces (Flesher et al., 2016; Gilja et al., 2012; Gilja et al., 2015; Hochberg et al., 2012; Hochberg et al., 2006; Jarosiewicz et al., 2015), have led to a better understanding of primary sensory (Ahissar et al., 1992; Gray et al., 1989; Maldonado et al., 2000) and motor systems (Riehle et al., 1997), and facilitated studies in stem cell transplantation (Lee et al., 2015) and tumorigenesis (Selek et al., 2014). However, there is a need to perform similar neurophysiologic and neuropathologic studies in subcortical areas as well (Buzsaki, 2004; Quiroga and Panzeri, 2009). While injection and implantation studies are widely performed in rodent models using atlas-based approaches (Bakhurin et al., 2016; Lin et al., 2006), the ability to use atlases for subcortical targeting in nonhuman primates (NHP) is limited by inaccuracies of this approach (Daye et al., 2013; Frey et al., 2004). Whereas rodent brain structures are relatively constant in size and location, NHP brains can be highly variable (Deogaonkar et al., 2005; Francois et al., 1996; Miocinovic et al., 2007), especially as the animal ages (Alexander et al., 2008; Koo et al., 2012; Matochik et al., 2000). Currently, much of the subcortical targeting being performed in NHP relies on histological (Paxinos et al., 1999; Saleem and Logothetis, 2012) or magnetic resonance imaging (MRI) atlases, in which up to 6 mm of anatomic variability in brain structures was seen (McLaren et al., 2009).

Acknowledging this anatomic variability, many laboratories have implemented other methods such as head-holding chairs (Frey et al., 2004), which requires extensive fabrication of bulky parts, or MRI-based targeting using non-ferrous compatible headframes (Bjarkam et al., 2009; Li et al., 2013; Chen et al., 2015). However, these MRI-compatible frames necessitate that the animal maintains its exact

* Corresponding author at: Department of Neurosurgery, University of Michigan, 1500 E. Medical Center Dr., SPC 5338, Ann Arbor, MI, 48109-5338, United States.
E-mail address: pgpatil@med.umich.edu (P.G. Patil).

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position between imaging and surgery, a difficult task during transport, and is a limitation that introduces error. Additionally, use of these frames requires that imaging and surgery be performed in sequence, leading to long surgical and anesthetic times. As an alternative approach, skull-mounted cylinders or chambers are frequently used for single-unit subcortical recordings and enable serial electrode insertions (Galashan et al., 2011), but this technique is not conducive to multi-electrode array implantation, such as with macro-microelectrodes (Adtech, Racine, WA) or bundles of tungsten wires that require larger connectors or headstages. Due to these anatomic and procedural limitations, a method of individualized subcortical targeting is needed without the constraints of using specialized and expensive equipment. This is of special importance in large animal models, as there is a heightened need to mitigate risk from surgical procedures.

Here, a method is described that uses a simple, fiducial-based approach that is a substantial improvement over previous methods. It requires no customized parts or fabrication of devices, relies on widely available equipment, is inexpensive, and is easily adapted to any laboratory with MRI access. Briefly, the method involves implantation of MRI-compatible titanium screws around the skull in a minor procedure. The screws serve as fiducials, which are uniquely defined, immobile reference points that can be located both in physical space and imaging space. Importantly, fiducials must be distributed widely over the skull due to the creation of a centroid by their configuration, the center of which should approximate the target as closely as possible. Co-registration of imaging and surgical spaces is then performed, which enables the selection of a target on the image and the transformation of its coordinates into surgical coordinates. Results are presented from an in vivo application using a cellular marker for histologic confirmation of targeting accuracy, with a discussion of the elements that were crucial for success.

2. Materials and methods

2.1. Animals

All animal experiments were performed in accordance with animal use protocols submitted to and approved by the University of Michigan Institutional Animal Care and Use Committee. All animals were rhesus macaques at the end of their experimental lifetimes for other studies. Two NHP had deep structures targeted with stem cell injections: 2 injections were performed in Monkey F (19-year-old male, 12.6 kg; thalamus, hippocampal injections) and 6 were performed in a second animal, Monkey B (25-year-old female, 8.4 kg; bilateral thalami, hippocampi, and substantia nigra injections). An additional 10 animals (aged 5–32 years) at their experimental endpoints and that were otherwise to undergo euthanasia were used for optimization of fiducial design, MRI parameters, and surgical technique. One of these 10 animals (Monkey S; 20-year-old male, 12.5 kg) had data available for comparison of atlas-based and MRI-based approaches using a slightly different technique, prior to optimization of this method.

2.2. Fiducial placement

The overall technique begins with fiducial placement. After several design iterations, the optimal markers were self-drilling titanium screws (Biomet HT X-drive screw #95-6104, Warsaw, IN) measuring 1.5 mm in diameter and 4 mm in length. The implantation procedure was begun by first sedating the animal with telazol 4 mg/kg intramuscular (IM). The animal was then intubated and sedation maintained using gas anesthesia (isoflurane 1.25–3%). Titanium screws were implanted in a procedure lasting approximately 20 min, and the animal was imaged with MRI immediately following this, with imaging lasting approximately 45 min. Additional time was needed for transport to an off-site MRI scanner, totaling approximately 2-2.5 h for the entire procedure. Additional analgesia was given postoperatively using carprofen (2 mg/kg) subcutaneously, given at 12 and 24 h.

The fiducial implantation procedure began with the animal in the prone position and the head supported on a stack of towels. To facilitate visualization, the head was first shaved. Betadine was used for antisepsis at the desired fiducial sites. Sterile drapes were applied around the head and the scalp was infiltrated with bupivicaine 2.5% with epinephrine 1:200,000 for local anesthesia at the proposed sites of implantation. Antibiotics were administered prior to incision (cefazolin 25 mg/kg). Fiducial locations were bilateral supraorbital ridges, two pairs of parietal sites bilaterally (asymmetrically placed), bilateral occipital nuchal ridges, and bilateral zygomatic roots just anterior to the external auditory meatus. The first site of fiducial placement was incised via a stab incision using a #15 surgical blade. The bone was cleared of soft tissue and periosteum using blunt dissection with a 4 × 4-cm gauze on the end of a hemostat. Once the bony surface was cleared of tissue, a drill bit (Biomet 1.5-mm HT X-lock short-drive blade #15-1194, Warsaw, IN) was used to implant a self-drilling screw with a hand-held screwdriver until flush with the skull surface (Biomet twist-drill handle #01-7390 or #01-7164, Warsaw, IN). Following placement, the skin incision was closed over the screws with a non-absorbable monofilament nylon suture (1–2 sutures per site, using 3-0 Ethilon©, #1673H, Ethicon, Somerville, NJ). Alternatively, an entirely subcutaneous closure could be performed using an absorbable monofilament suture, such as a 4-0 Monocryl© (#Y218H Ethicon, Somerville, NJ), using a subcuticular stitch. This process was repeated for the remaining fiducial locations.

The most challenging screws to place were at the zygomatic roots and external occipital protuberances. The zygomatic root can be palpated on the skin and is defined as the superior and anterior border of the external auditory meatus, which provides a relatively flat plane for screw placement as it slopes upward to and is continuous with the squamous temporal bone. The skin incision was made overlying this prominence, and the dissection was directed through deeper layers of muscle until the bone was reached. Though not required, monopolar electrocautery can be used to clear the bone of temporalis muscle and to provide hemostasis. The external occipital protuberances serve as attachment sites of the occipital musculature and are also easily palpated on the scalp. Incisions were made over these bilaterally, off of midline, and blunt dissection was used to clear the bone of soft tissue using a hemostat and gauze. Additional fiducials may be implanted over the head as needed, keeping in mind that asymmetric placement over the head will ensure confirmation of side on imaging.

The procedure in Monkey S was similar to this optimized procedure, however, screws were housed in a plastic anchor and fiducial locations did not include zygoma and occipital protuberances.

2.3. Imaging

Following fiducial placement, an MRI was obtained, however, there is no requirement that the fiducial implantation and imaging be performed the same day, as fiducials are entirely implanted and do not dislodge from the bone. For an independent imaging session, sedation can be administered with intermittent bolus doses of telazol IM (4 mg/kg). If being performed on the same day as the definitive procedure, inhaled or continuous IV anesthetic can be given using a portable MRI-compatible ventilator (ModuFlex Compact SN 4086, Dispomed, Joliette, Quebec, Canada) with an isoflurane vaporizer (InterMed Penlon Sigma Delta SN D0610 0110, Penlon Limited, Abingdon, Oxon, United Kingdom).

Animals were positioned supine on the MRI table. During the procedure, the animals' heart rate, respiratory rate, and oxygen saturation were monitored. Imaging was performed at our institution on a General Electric® MR750 3.0 T system using primarily a sagittal T1-weighted image (flip angle 12, echoes 1, TI 450, bandwidth 31.25, FOV 19.2, slice thickness 0.5 mm, 384 × 384 matrix, NEX 2, phase FOV 1). Screws are more easily seen on T1 imaging, however, in order to maximize