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Stereotactic neurosurgical planning, recording, and visualization for deep brain stimulation in non-human primates

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Abstract

Methodologies for stereotactic neurosurgery and neurophysiological microelectrode recordings (MER) in non-human primate research typically rely on brain atlases that are not customized to the individual animal, and require paper records of MER data. To address these limitations, we developed a software tool (Cicerone) that enables simultaneous interactive 3D visualization of the neuroanatomy, neurophysiology, and neurostimulation data pertinent to deep brain stimulation (DBS) research studies in non-human primates. Cicerone allows for analysis of co-registered magnetic resonance images (MRI), computed tomography (CT) scans, 3D brain atlases, MER data, and DBS electrode(s) with predictions of the volume of tissue activated (VTA) as a function of the stimulation parameters. We used Cicerone to aid the implantation of DBS electrodes in two parkinsonian rhesus macaques, targeting the subthalamic nucleus in one monkey and the globus pallidus in the other. Cicerone correctly predicted the anatomical position of 79% and 73% of neurophysiologically defined MER sites in the two animals, respectively. In contrast, traditional 2D print atlases achieved 61% and 48% accuracy. Our experience suggests that Cicerone can improve anatomical targeting, enhance electrophysiological data visualization, and augment the design of stimulation experiments.

Keywords

Stereotactic neurosurgery; surgical planning; microelectrode recording; visualization; software; deep brain stimulation; monkey

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1. INTRODUCTION

Non-human primate models of deep brain stimulation (DBS) for Parkinson's disease provide unique opportunities to study the therapeutic mechanisms of DBS in vivo (Hashimoto et al., 2003; Elder et al., 2005). The therapeutic benefits of DBS are dependent on accurate placement of the electrode in the appropriate neuroanatomical target. As a result, stereotactic neurosurgical navigation systems for human (clinical) applications continue to evolve (Finnis et al., 2003; D'Haese et al., 2005). However, similar image-guided systems for non-human primate research are lacking. Therefore, we developed computerized techniques to address several limitations in traditional non-human primate stereotactic neurosurgery and microelectrode recording (MER).

One limitation in non-human primate stereotactic surgery is the use of standard 2D brain atlases for identification of the initial anatomical target location for DBS electrode implantation (or any similar procedure). This approach is prone to error because the brain size, shape, and location of subcortical structures can vary between animals (Percheron and Lacourly, 1973; Francois et al., 1996; Deogaonkar et al., 2005). There have been numerous attempts to refine non-human primate neurosurgery with the integration of magnetic resonance imaging (MRI) and population-based brain atlases (Saunders et al., 1990; Alvarez-Rojo et al., 1991; Rebert et al., 1991; Nahm et al., 1994; Asahi et al., 2003; Frey 2004; Francois et al., 1996; Deogaonkar et al., 2005; Christensen et al., 1997). Unfortunately, most of these advances have not achieved wide scale acceptance into practice.

Limitations also exist in the techniques used to collect and analyze MER data. Microelectrode recordings are accompanied by multiple sources of error such as brain shift, electrode deflection, improper electrode zeroing and microdrive imprecision. These issues can cause discrepancy between the expected electrode location in the brain and the experimenter defined classification of the recorded neural signal. Brain atlases are commonly employed to provide anatomical guidance in interpreting (classifying) the MER data because of variations in neural firing characteristics. Unfortunately, traditional 2D brain atlases only provide information in the sagittal or coronal plane, limiting opportunities to determine the electrode location along oblique trajectories. In addition, MER data are typically saved as paper records, limiting options for data visualization.

Another limitation in current DBS practices is the lack of opportunity to visualize the predicted spread of stimulation for a given DBS electrode location in the brain. The fundamental purpose of DBS is to modulate neural activity with applied electric fields. Therefore, the ability to predict the spread of stimulation, prior to permanent implantation, would provide additional information for identification of the optimal electrode placement in the brain. And, following implantation, predicting the spread of stimulation could make the identification of therapeutic stimulation parameters a more straightforward process.

To address these limitations we developed a software system (Cicerone) for stereotactic neurosurgical planning, neurophysiological data collection, and DBS visualization. The system was developed for monkey DBS studies, but it can be applied to a range of procedures requiring stereotactic localization and neurophysiological mapping of subcortical structures in non-human primates. Cicerone provides interactive 3D visualization of co-registered MRI/CT images, subject-specific 3D anatomical brain atlas, and neurophysiological MER data. The software can be used to define a pre-operative target location and trajectory for the DBS electrode placement and help select the location on the skull for chamber placement. Entering microdrive coordinates and MER data during recording sessions enables real-time interactive visualization of the electrode location in the brain. Cicerone also provides tools to compensate

for stereotactic inaccuracies, thereby improving the match between the MER data and the brain atlas defined neuroanatomy. In addition, the user can simultaneously visualize the DBS electrode and its predicted stimulating effects on the surrounding neural tissue. As a result, stereotactic placement of the DBS electrode can be optimized prior to permanent implantation using the combination of anatomical, neurophysiological, and electrical data. The goal of this study was to demonstrate the utility of Cicerone for DBS electrode implantation in two rhesus macaques, targeting the subthalamic nucleus (STN) in one animal and the globus pallidus (GP) in the other.

2. METHODS

The Cicerone software was written using VTK (Visualization Toolkit; Kitware, Clifton Park, NY) and Tcl/Tk (Tool Command Language; <http://tcl.sourceforge.net>) making it portable across platforms, including Windows.

2.1 Subject-specific 3D brain atlas

Cicerone uses a 3D brain atlas that can be modified to fit the neuroanatomical profile of a specific animal. The standard atlas was created from the University of Washington digital brain template atlas of the longtailed macaque (*Macaca fascicularis*) (Martin and Bowden, 2000). This was accomplished by outlining individual structures in each coronal atlas slice, spaced at 1 mm intervals. The 3D volumes were created by interpolating between these contour lines using the graphical modeling program Rhinoceros v3.0 (McNeal & Associates, Seattle, WA). With appropriate modifications this atlas can be used for several other non-human primate species, including rhesus macaque (*Macaca mulatta*) (see Discussion).

In this study we addressed two methods (linear scaling and nonlinear warping) for adapting the standard 3D brain atlas to a specific subject. The simple approach was to scale the standard 3D atlas along three axes, mediolateral (ML), anteroposterior (AP) and ventrodorsal (VD), to match the subject's MRI and/or CT scans. This was accomplished in Cicerone with a simple graphical user interface that allows for manual scaling and repositioning of individual nuclei to better fit imaging and/or electrophysiological data. The landmarks we used to create the linearly-scaled custom atlas were the anterior (AC) and posterior (PC) commissures, lateral ventricles and ML, AP and VD extent of the brain, but other landmarks visible in the MRI could be used as well. The more intensive, subject specific 3D atlas was constructed by warping 2D digitized brain atlas templates to the corresponding MRI slices using Edgewarp (Bookstein, 1990; Martin and Bowden, 2000). Edgewarp applied a nonlinear warping function to atlas templates based on manual landmark selection. When generating the warped atlas, we used the same landmarks listed above, as well as the borders of visible nuclei such as the caudate, putamen, globus pallidus, thalamus, and the optic tract. The warped atlas slices were then converted into 3D volumes as described above using Rhinoceros v3.0 (Fig. 1A).

The standard atlas currently contains more than twenty individual nuclei and structures. To improve visualization, Cicerone only displays nuclei selected by the user and provides variable opacity and color for each structure.

2.2 Cicerone Setup

Imaging data (MRI and CT) are used to customize the 3D brain atlas to the specific animal and to set up a reference coordinate system. In our two monkeys, a CT was acquired to visualize the skull and the external landmarks (ear canals and orbital ridges) so that the stereotactic reference frame could be registered with the internal brain structures. An MRI was acquired to customize the 3D atlas and align the CT with the atlas. The MRI and CT were co-registered in Analyze 6.0 (AnalyzeDirect, Lenexa, KS) using an intensity-based mutual information

algorithm (Viola and Wells, 1997). The co-registered imaging data was then imported into Cicerone as VTK volume files.

Cicerone's default coordinate system is based on the AC-PC plane. The center of the AC is defined as the origin and the horizontal plane is perpendicular to the vertical plane through the AC-PC line. Although in monkey stereotactic neurosurgery it is common to use a frame and an atlas referenced to the orbitomeatal plane. This is a plane defined by the interaural line (line between tips of the earbars) and the inferior orbital ridges. The origin is defined as the midpoint of the interaural line and the orbitomeatal plane (Frankfurt zero). In Cicerone, the user can position earbars and orbital ridge bars to define the orbitomeatal plane using the skull rendering extracted as a contour from the CT data (Fig. 1B). The user can then switch from the 'AC-PC coordinate system' to the 'stereotactic coordinate system' (i.e. orbitomeatal plane). When moving to the 'stereotactic coordinate system', the origin is shifted to Frankfurt zero and rotation around ML axis is performed so that the orbitomeatal plane is horizontal.

If a subject-specific atlas has not been previously created, the user can scale the brain atlas in Cicerone to fit the imaging data. All files (MRI, CT, brain atlas), user preferences, and transformations performed during the set-up are stored in a configuration file for easy upload during later sessions.

2.3. Stereotactic neurosurgical planning

Cicerone provides tools to interactively position up to three recording chambers on the animal's skull. This makes it possible to ensure, prior to surgery, that the electrodes can reach the desired target areas and that the chambers and electrode trajectories will not interfere with each other once the chambers are placed on the skull. The chambers can be positioned in any oblique plane, providing an advantage over currently used methods which allow only coronal or sagittal orientation as dictated by traditional 2D print atlases. Chambers of any size and shape can be integrated into Cicerone. Two chamber designs were used in this study. Both were cylindrical with 19.1 mm and 15.1 mm inner diameters, respectively. The user can also choose between several angles on the base of the chamber to find one that best fits the contour of the skull in a chosen location prior to surgery. The chamber coordinates provided by Cicerone can be applied directly to the stereotactic frame, after the frame is properly zeroed.

Cicerone's current algorithm for coordinate calculation is designed for use with the Kopf frame (model 1430 and electrode manipulator model 1460; David Kopf Instruments, Tujunga, CA). The coordinates of the recording site markers in 3D space (i.e. electrode tip location) are determined by constructing a transformation matrix based on the rotation angles and translation distances of the head chamber and electrode. The order of transformation operations is set so that the movement of virtual chamber/electrode reflects physical movement of the electrode manipulator on the stereotactic frame and the microdrive (i.e. the electrode manipulator sets the chamber position on the skull while the microdrive sets the electrode position within the chamber). The resulting matrix:

$$M = T_2 * R_{y1} * R_{x2} * T_1 * R_{x1},$$

where R_{x1} is -90 degree rotation around ML axis necessary because x and y axes are reversed in Rhinoceros and VTK (Rhinoceros is used to build the head chambers and electrodes which are then imported into Cicerone); T_1 is a translation matrix incorporating chamber movement along the ML and VD axes and electrode movement within the chamber along all three axes (the order of these translation coordinates depends on the orientation of the physical electrode manipulator since there are two possible orientations on the frame); R_{x2} is rotation around the ML axis set by the vertical dial of the electrode manipulator; R_{y1} is rotation around the VD

axis set by the horizontal dial of the electrode manipulator; and T_2 is translation set by the chamber AP movement.

After the chamber placement surgery it is advisable to perform another CT scan to verify the chamber position on the skull.

2.4 Neurophysiological data collection and visualization

During MER data collection the user can view the electrode position within the 3D anatomical space by entering microdrive coordinates into Cicerone (Fig. 1D). The microelectrode recording (or stimulation) locations are displayed as small markers. The marker color is set according to their likely anatomical location (based on neuronal firing properties) and the marker shape is determined based on the presence or absence of a sensorimotor response. The user has the option to translate or scale the 3D atlas nuclei to better match the MER data as well as individually adjust the depth of each recording track to compensate for improperly zeroed electrodes. The user can pan, zoom and rotate the view, adjust the display properties of the 3D atlas, and scroll through the MRI in three orthogonal planes. The anatomy and the site markers can also be viewed in 2D, one slice at a time, which is sometimes preferable to a 3D view. The MER data are saved to a text file and can be re-imported into the program at a later time or stored to a central database.

2.5 Theoretical DBS volume of activation

A novel feature of Cicerone is its ability to predict the volume of tissue activated (VTA) by a DBS electrode for a given electrode position and stimulation parameter setting (Fig. 1C). Currently, Cicerone contains a database of pre-calculated VTAs for a 4 contact cylindrical monkey DBS electrode (0.75 mm diameter; 0.5 mm contact height). Our methods of VTA calculation have been previously described (Butson and McIntyre, 2005;2006;Butson et al., 2006a,b;Miocinovic et al., 2006). Briefly, the volumes were created by calculating activation thresholds for multi-compartment cable models of straight myelinated axons (2 μ m in diameter) built in NEURON simulation environment (Hines and Carnevale 1997). More than a hundred axons were placed perpendicular to an axisymmetric finite element model (FEM) of the cylindrical monkey DBS electrode in a 6×6 mm grid and stimulated with extracellular voltages generated in the tissue medium by the electrode. Activation thresholds (DBS voltage necessary to produce a propagating action potential in the axon models) were calculated for each axon and the results were used to extract contours representing VTAs for stimuli of various amplitudes (1–5V). The same procedure was repeated for different electrode configurations (monopolar or bipolar), stimulation pulse widths (60–210 μ s), and frequencies (100–185 Hz). The VTAs do not vary based on the electrode location because the bulk tissue conductivity was assumed to be homogenous and isotropic for VTA calculation.

2.6 Chamber implantation and microelectrode mapping

Chamber placement, microelectrode mapping and DBS electrode implantation were performed on two female rhesus monkeys (*Macaca mulatta*; ID numbers 04-m-005 and 04-m-001) weighing 5.2 kg and 6.7 kg, respectively. All surgical and behavioral protocols were approved by the Institutional Animal Care and Use Committee and complied with United States Public Health Service policy on the humane care and use of laboratory animals.

Imaging—T2-weighted MRI scans were performed using a Siemens MAGNETOM Trio 3 Tesla scanner (Siemens Medical Systems, Iselin, NJ) with the monkeys under propofol anesthesia. Twenty 2 mm thick coronal slices and twenty 2 mm thick sagittal slices were imaged (256×256 pixels; 0.47×0.47 mm in plane resolution). The two volumes were co-registered and fused using Analyze 6.0 software (AnalyzeDirect, Lenexa, KS). CT scans were performed with a Siemens SOMATOM Sensation. CT images were acquired in the axial plane in 0.6 mm or

1 mm increments (135 slices at 512×512; 0.24×0.24 mm in plane resolution). The CT volume was co-registered with the fused MRI images in Analyze.

Surgical Procedures—A hemiparkinsonian syndrome was induced by unilateral intracarotid injection of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, 0.5 – 0.6 mg/kg over the course of ~15 minutes) during aseptic surgical procedure under isoflurane anesthesia. After a stable hemiparkinsonian state was achieved, craniotomies were trephined and recording chambers were implanted over the craniotomy in a subsequent aseptic procedure under isoflurane anesthesia with the head held in a primate stereotactic frame (Kopf frame model 1430). In each animal, two craniotomies were performed and two metal chambers were anchored over the right cerebral hemisphere. The chambers' stereotactic coordinates were determined using Cicerone as described in section 2.3.

In monkey 04-m-005, the chamber to be used to later implant the DBS electrode in the globus pallidus (GP) was placed in the coronal plane at 30 deg from the midline (lateral-to-medial), 12.5 mm anterior and 2.1 mm lateral (in frame coordinates). The DBS electrode was implanted in a separate procedure after several weeks of mapping during which electrophysiological data was collected through this chamber. The second recording chamber to be used for later microelectrode recording in the thalamus, was placed in the sagittal plane at 20 deg from the vertical axis (anterior-to-posterior), 6 mm anterior and 5.5 mm lateral (in frame coordinates). Postoperative CT indicated that the location of the GP DBS chamber was 0.2 mm more lateral and 0.7 mm more anterior than planned, and the location of the thalamic recording chamber was 0.3 mm more medial and 0.4 mm more posterior than planned. These discrepancies were due to inherent inaccuracies in the surgical procedure, such as minor head misalignment in the stereotaxic frame and head movement due to forces exerted during craniotomy.

In monkey 04-m-001, the chamber later to be used to implant the DBS electrode in the STN was placed at an oblique orientation so that the DBS electrode would be oriented along the long axis of the nucleus. The chamber was angled 30 deg from the vertical axis and 15 deg from the sagittal plane (lateral-to-medial), 5 mm lateral and 11.2 mm anterior (in frame coordinates). The second chamber to be used for later microelectrode recording in the thalamus was placed in the sagittal plane at 25 deg (posterior-to-anterior), 8.4 mm lateral and 23.2 mm anterior (in frame coordinates). Post-operative CT indicated that both chambers were misplaced by a five degree angle which was consistent with an unintentional head tilt during surgery due to an improperly positioned palate bar. Prior to starting microelectrode mapping the post-operative CT was imported into Cicerone and the virtual chambers were repositioned to reflect their actual positions on the skull.

Microelectrode mapping—Extracellular neuronal activity was recorded through the chambers targeting GP and STN, respectively, to find the optimal position for the DBS electrode implantation. Epoxylite-coated tungsten microelectrodes (0.5–1.0 MΩ) were positioned within the chamber using a microdrive (MO-95-lp, Narishige Scientific Instruments, Tokyo, Japan). Because the recording chambers were stereotactically positioned on the skull, the frame was not used for MER mapping or the subsequent DBS electrode implantation. During MER and DBS electrode implantations animals were sitting in primate chairs with their heads stabilized with a metal head post. Recording penetrations were made in coronal planes in monkey 04-m-005 and in oblique planes (15 degrees from sagittal plane) in monkey 04-m-001. The analog neuronal signal was amplified (A-M systems model 3000, Sequim, WA), bandpass filtered at 500–3000Hz (Krohn-Hite model 3380, Brockton, MA), digitally sampled at 25kHz and stored for later offline analysis (Power 1401 and Spike2 software, Cambridge Electronic Design, Cambridge, UK). Based on their discharge patterns together with other physiological features and anatomical landmarks, neurons were classified as being located in the striatum, globus pallidus externus (GPe), globus pallidus internus (GPi),

STN or unclassified. Neurons in the striatum exhibited typical low tonic firing rates and characteristic injury discharge in response to electrode movement (DeLong, 1971). Transition into the pallidum was characterized by increased background activity and high tonic firing rates (Turner and Anderson, 1997). The border between GPe and GPi was characterized by little or no single neuron activity as the electrode tip crossed the internal medullary lamina and by the presence of “border neurons” characterized by a more tonic and lower frequency discharge pattern. In addition, most GPe neurons could be characterized as either ‘pausers’ with high frequency discharge rates separated by long pauses, or ‘bursters’ with low mean discharge rates and periodic bursts. GPi neurons were more likely to fire continuously at high frequencies without long periods of silence (DeLong, 1971). The STN was recognized by a dramatic increase in background activity as the electrode exited the internal capsule and by single neuron or multiunit activity that often discharged in characteristic bursts (DeLong et al., 1985). The optic tract was identified by the change in baseline noise level of neural recordings in response to flashes from a strobe light (Photoc stimulator model PS33, Grass-Telefactor, Astro-Med, Inc., West Warwick, RI) in a darkened room. GPe, GPi and STN neurons were also tested for sensorimotor responses (change in discharge activity in response to passive or active movement or tactile stimulation).

Data Collection and analysis—We tested the accuracy of Cicerone by having one experimenter map the basal ganglia using the software while another experimenter simultaneously used the conventional method of plotting recording sites on graph paper. We evaluated how well the recording data matched Cicerone’s custom atlases (warped 3D atlas and linearly scaled 3D atlas) compared to two conventional atlases (standard 3D atlas and print atlas). For monkey 04-m-005 who had mapping data recorded in the coronal plane, we used the Paxinos et al. (1999) print atlas. For monkey 04-m-001 we used the sagittal Ilinsky et al. (2002) print atlas. The standard 3D atlas was built from the Martin and Bowden (2000) digital atlas as described above. Since their original 2D atlas was made from a longtailed macaque, we scaled it to better approximate rhesus brain using the average scaling factors from Martin and Bowden (2000) (1.08 in AP, 1.06 in DV and 1.0 in ML direction). As a result the standard 3D atlas was adapted to the rhesus species, but not customized to either animal.

For each atlas we calculated the number of recording sites that were within their proper nuclei, and for sites that were outside their nucleus we calculated the number of recording sites that were within 1 mm from the border of their proper nucleus. Recordings had to be aligned with each atlas, so all the atlases were shifted to properly match the GP recording data with the GP atlas structure in monkey 04-m-005, and the STN recording data with the STN atlas structure in monkey 04-m-001. The GP and STN recordings were selected in the respective animals because these were the target nuclei for DBS electrode implantation. Initially, the atlas AC was aligned with the MRI-defined AC. However to achieve the proper fit with the neurophysiologic data (GP or STN), the 3D atlases had to be shifted within the skull by varying amounts reported in the Results section (see also Discussion). Track depths were also adjusted in both monkeys. In monkey 04-m-001, 6 tracks (all recorded with the same electrode) were shifted by 2 mm, and in monkey 04-m-005, multiple shifts were performed averaging 0.6 mm. For print atlas accuracy analysis we selected the nearest available atlas slices that best fit the data (allowing for track depth adjustments), and overlaid the atlas transparencies with MER data plotted on the graph paper. We tested the differences in the total numbers of MER sites inside the nuclei, < 1 mm outside and > 1 mm outside between the four atlases using chi-square test with Bonferroni correction for multiple comparisons. A significant difference was accepted when p-value was < 0.01.

3. RESULTS

We used the Cicerone software system to plan the implantation of recording and stimulation chambers in two rhesus monkeys and to visualize MER data with respect to the surrounding basal ganglia neuroanatomy. The accuracy of Cicerone's subject-specific atlases (warped and linearly-scaled) and the standard atlases (3D and print) was evaluated by comparing how well neurophysiologically defined MER locations matched the atlas-defined nuclei. Data entry using Cicerone was performed at equal speed as taking paper notes and it did not slow down the mapping process. An additional advantage of Cicerone was that the data did not have to be recopied for entry into a computer database.

In monkey 04-m-005, 14 electrode penetrations were made (data from two tracks were discarded because they were deemed unreliable) in 5 coronal planes and a total of 1271 locations with extracellular unit activity were recorded. Of those, 579 were classified as belonging to striatum (63%), GPe (10%), GPi (10%), or optic tract (16%) (Fig. 2A-D). Figure 3A shows the percentage of classified recording sites and their position with respect to the atlas-defined nuclei. The total percentage of MER sites within their proper nuclei was 73% for Cicerone's warped 3D atlas, 72% for Cicerone's linearly-scaled 3D atlas, 63% for the standard 3D atlas and 61% for the print atlas. The warped atlas had to be moved an average of 0.5 mm to align the GP MER data with the atlas-defined structures (0.15 mm medial, 1.15 mm anterior and 0.1 mm ventral). For the linearly-scaled atlas the average was 1.2 mm (1.4 mm lateral, 0.85 mm anterior and 1.3 mm ventral) and 1.6 mm for the standard 3D atlas (2.9 mm lateral, 0.45 mm posterior and 1.45 mm ventral). The corresponding translations were difficult to estimate for the print atlas since the chambers were rotated and translated in a 3D space that cannot be captured with a 2D print atlas.

In monkey 04-m-001, there were 16 electrode tracks in 7 oblique planes and a total of 1480 recording sites, of which 417 were classified as striatum (54%), GPe (18%), GPi (7%), STN (17%), or optic tract (4%) (Fig. 2E-H). The total percentage of MER sites within their proper nuclei was 79%, 66%, 42% and 48% for Cicerone's warped 3D atlas, Cicerone's linearly-scaled 3D atlas, the standard 3D atlas and the print atlas, respectively (Fig. 3B). The warped atlas had to be moved an average of 0.8 mm to align the STN MER data with the atlas-defined structure (0.75 mm lateral, 1.6 mm anterior and 0.15 mm ventral). For the linearly-scaled atlas the average was 1.0 mm (1.1 mm lateral, 1.25 mm anterior and 0.7 mm ventral) and 1.2 mm for the standard 3D atlas (1.95 mm lateral, 0.65 mm anterior and 1.0 mm ventral).

The DBS electrodes were implanted in the motor regions of the posterior GP and lateral STN, respectively, as defined by the neurophysiologic data. Figure 1C shows the theoretical DBS VTA for monkey 04-m-001 which was used to assist in deciding the optimal electrode position. A post-implantation CT confirmed the electrode locations and preliminary results show therapeutic effects with stimulation suggesting that the electrodes were placed within the desired nuclei.

4. DISCUSSION

This study evaluated the utility of a 3D visualization and database software system for stereotactic neurosurgical planning and neurophysiological microelectrode recordings in non-human primate research. The purpose of the Cicerone system is to aid with head chamber positioning and provide visual feedback during the microelectrode recording procedures. Cicerone also provides guidance in DBS electrode implantation by estimating the volume of tissue activated for a given DBS electrode position and stimulation parameter setting. Further, the database components of the system simplify the neurophysiological record keeping process and allow for advanced data analysis and visualization. We demonstrated Cicerone's

capabilities by analyzing the match between subject-specific 3D atlases and MER data in two parkinsonian rhesus monkeys undergoing DBS implantation. 3D atlases customized to the MRIs of the individual animals, by either non-linear warping or linear scaling, provided a better match with MER data than either a standard 3D atlas or a print atlas. The limitations of various techniques integrated into the software system are discussed below.

The standard 3D atlas used in this study, created from a longtailed macaque (*M. fascicularis*) digital template atlas (Martin and Bowden, 2000), was adapted for use in rhesus macaque (*M. mulatta*). Analysis by Bowden and Dubach (Ch 5. in Martin and Bowden, 2000) using published atlases of various primate species concluded that with appropriate linear scaling the template atlas was suitable for stereotactic targeting in several other species of macaque (*M. mulatta*, *M. fuscata*) and baboon (*P. papio*, *P. cynocephalus*, *P. anubis*). Their analysis consisted of first calculating the mean linear ratios for each species by comparing positions of 13 brain landmarks in the template atlas and in the atlases of given species. The mean linear ratios for each species were then used to scale the template atlas. The discrepancy between the landmark positions in the scaled template atlas and the actual atlas for that species were calculated, and an absolute discrepancy of 0.5 mm or less was considered acceptable. It should be noted that this comparative analysis was not performed for cortex or the lower brain stem so the scaled template atlas would not necessarily be suitable for use in these brain areas.

While the appropriately scaled *M. fascicularis* template atlas can be used for similar species with reasonable accuracy, inter-individual variability is still a limiting factor for atlas based stereotactic targeting (Percheron and Lacourly, 1973; Francois et al., 1996; Deogaonkar et al., 2005; Martin and Bowden, 2000). Our results show that the standard 3D atlas (scaled using mean linear ratios for *M. mulatta*) provided limited agreement between the atlas and MER data. Customization of the 3D brain atlas to the MRI of the specific animal significantly improved the accuracy of the anatomical match of the model to the MER (Fig. 3). Interestingly, simple linear scaling of the 3D brain atlas to match the MRI generated an effectively equal match for our target nuclei, compared to the more difficult and time consuming non-linear warping.

Unlike human stereotactic neurosurgery, image guidance is less commonly employed in non-human primate procedures. Our experience suggests that the integration of pre-operative imaging and 3D visualization software can provide invaluable assistance in surgical planning and data collection in non-human primates. Our goal was to position two metal chambers (19 and 15 mm diameter) on small skulls, approximately 70 mm long and 50 mm wide, such that the chambers did not interfere with each other and that microelectrode recording from selected subcortical structures could occur without interference from the implanted DBS lead. After importing a CT scan and visualizing the skull, Cicerone's planning tools made it easy to move and rotate chambers in 3D virtual space and find the optimal positions prior to surgery. Cicerone can also be used with just an MRI where bony landmarks are defined using an MRI-compatible stereotactic frame. However, a CT provides much better skull visualization and it does not introduce artifacts from head chambers, skull screws and head posts implanted on the skull. In its simplest form Cicerone can also be used without any imaging data.

Additional problems that confront every neuronavigation system are imaging and registration accuracy. Imaging issues such as voxel size and image distortion have been extensively analyzed (Sumanaweera et al., 1994; Hardy and Barnett, 1998; Poggi et al., 2003). Animal procedures provide an additional challenge because two imaging modalities must be co-registered, an MRI used to create a subject-specific atlas and a CT used to visualize external bony landmarks that define the stereotactic frame space. This provides yet another potential source of error which can be partially compensated for by adjusting the CT/MRI fusion and/or position of the 3D atlas within the skull. Despite the list of issues that limit stereotactic

accuracy, the use of image-guided tools, such as Cicerone, provide substantial improvements over currently used techniques in non-human primate research.

MER data acquisition using Cicerone was simple, as fast as using paper records, and it allowed for immediate visualization of recording locations in the context of the 3D neuroanatomy. Two potential problems that can affect the accuracy of MER mapping are electrode deflection and brain shift (Finnis et al., 2003). Cicerone assumes that the electrode is following a perfectly straight virtual trajectory through the brain, when in fact deviations in the trajectory may occur. The degree of error is hard to estimate. The CT scans following the DBS electrode implantation in one of our monkeys showed a slight curving of the electrode trajectory throughout its length which resulted in a ~0.75 mm deflection at the tip. The DBS electrode is flexible and the curvature may have resulted from a number of factors. Another source of error that should be recognized is the microdrive guide tube. The relationship between the guide tube and the microdrive electrode attachment may not be perfectly aligned and result in an improper electrode penetration angle. In fact we noticed this problem with our equipment, prompting us to custom-design a metal stage for the microdrive for future studies.

Deformation of the brain during open-skull neurosurgical procedures can be a significant source of error for neuronavigation systems (Hastreiter et al., 2004). Brain shift in human surgeries increases with time, so MER trajectories obtained early in the procedure are more likely to align with atlases customized to the preoperative MRI/CT scans. This problem is likely even more pronounced in animal procedures where permanent craniotomies are used and microelectrode mapping occurs over a period of several days. It is unfortunately not useful to obtain an MRI following the craniotomy because of the large artifact caused by the metal chambers and skull screws, so we must rely on the image taken when the animal's skull was intact. In addition, during the MRI scan the animal is in the supine position, but during MER data collection the animal is sitting upright in the primate chair which might cause a slight anterior/posterior shift in the predicted electrode position (see below) (Rohlfing et al., 2003).

We found it necessary to provide Cicerone users with the option of repositioning the 3D atlas to better match the MER data. For the purpose of evaluating the system accuracy we moved the 3D nuclei using only the mapping data that defined our target structures, the GP in monkey 04-m-005 or STN in monkey 04-m-001. In practice, we reposition the 3D atlas after the first few recording tracks; thereby providing a better estimate for the subsequent recordings. In both animals we found that Cicerone's warped 3D atlas had to be shifted anteriorly by 1–1.5 mm and ~0.5 mm in the other two directions. These shifts attempt to provide correction for all potential errors (electrode deflection, brain shift, microdrive imprecision, size and shape of 3D nuclei, MRI/CT co-registration), but it is difficult to estimate how much each problem contributes to the overall inaccuracy. Nonetheless, the overall ~1 mm accuracy is within the range of clinical stereotactic systems (Maciunas et al., 1994). Another limitation that should be recognized is that several microelectrodes are typically used during the mapping procedure. It is difficult to zero each electrode to precisely the same location, which can cause the depths of the individual mapping tracks to be slightly offset from each other. Cicerone enables the user to move each mapping track independently to align them properly, usually using a well defined anatomical region present in the track.

A novel feature of Cicerone is the ability to visualize the estimated volume of tissue activated (VTA) by the DBS electrode. This offers a potential benefit to researchers who could anticipate stimulation effects before the actual electrode implantation, and plan the electrode trajectory to achieve the desired interaction between the VTA, MER data and 3D neuroanatomy. In this study we concentrated on stimulation models customized to the scaled clinical DBS system used in the Vitek laboratory (Hashimoto et al., 2003; Elder et al., 2005). However, future versions of Cicerone can be easily adapted to any given electrode design and stimulation

paradigm. The VTA predictions are based on theoretical models of the neural response to extracellular electric fields (Butson and McIntyre, 2005;2006;Butson et al., 2006a,b;Miocinovic et al., 2006). Our VTA predictions incorporate the latest advances in neural stimulation modeling such as explicit characterization of capacitance and impedance of the electrode-tissue interface and detailed multi-compartment biophysical models of myelinated axons. Assumptions used to make the VTA predictions in Cicerone are that all the axons were of the same diameter, axonal trajectories and orientations were uniform, and bulk tissue conductivity was homogeneous. While all these factors influence the spread of stimulation, certain trade offs were necessary to reduce the computational complexity and limit the memory requirements for the Cicerone software system.

Experimental validation of the VTA models is a difficult task. We are actively pursuing research studies that link our model predictions with electrophysiological recordings in humans and non-human primates (Miocinovic et al., 2006;Butson et al., 2006b). The results of these studies show that our models can accurately predict stimulation spread into the corticospinal tract during STN DBS. In turn, it is currently possible to use neurostimulation models to make quantitative, experimentally relevant, predictions. And, while the current VTA prediction functions in Cicerone are relatively simple, we believe that the synergistic evolution of our modeling technology and experimental analysis will allow for continuous improvement in their accuracy and validity.

Our results show that Cicerone has the potential to significantly increase the accuracy and precision of stereotactic implantation of neuromodulatory devices. But, it should be noted that the utility of Cicerone will depend on the users' skill in customizing the 3D atlas to animals' MRI/CT, experience in characterizing neuronal firing properties from the MER data, and precision in making atlas/track shift adjustments. Although the Cicerone system used in this study requires additional software components (Analyze, Edgewarp) to be fully functional, we are currently in the process of further refinements that will result in a stand-alone software system. Our intention is to provide Cicerone to the non-human primate scientific research community free of charge.

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Figure 1.

Cicerone display and graphical user interface. (A) A customized 3D atlas is aligned with the animal's MRI. (B) The skull contour is extracted from the CT data and co-registered with the MRI and 3D brain atlas. Head chambers are interactively positioned within the stereotactic space defined by ear bars and orbital bars. (C) Theoretical volume of tissue activated by the DBS electrode is displayed within the STN of monkey 04-m-001 for the given set of stimulation parameters (5V, 135 Hz, 90 μ s, bipolar stimulation). (D) The user interface provides functions for manipulating the imaging data, 3D brain atlas, chambers, electrodes and defining MER locations.

Figure 2.

Microelectrode recording data and Cicerone atlas from monkeys 04-m-004 (A-D) and 04-m-001 (E-H) (A) MER sites defined as GPe or GPi are displayed as green or blue spheres within the GPe and GPi structures of Cicerone's warped 3D atlas. (B) The chamber and MER data from four recording tracks are shown within a coronal slice of Cicerone's warped 3D atlas. (C). Zoomed in view of figure (B). (D) Same four tracks as in (B) and (C) displayed within the standard 3D atlas. MER sites from putamen (red), caudate (yellow), GPe (green), GPi (blue), STN (maroon), optic tract (pink), electrophysiologically quiet sites without a visual stimulation response (black), border cells (brown), and unclassified (white) are displayed with markers. The marker shape is determined by the presence (cylinder, cube or cone) or absence (sphere) of a sensorimotor response. Caudate and putamen sites are distinguished solely by their inferred anatomical location. (E) STN and GPi MER sites are shown within transparent nuclei of Cicerone's warped 3D atlas. (F) Five MER tracks in an oblique slice (15 degrees from sagittal plane) from Cicerone's warped 3D atlas. (G) Zoomed in view of figure (F). (H) Same five tracks as in (F) and (G) displayed within the standard 3D atlas. Each leg of 3D scale bars represents 3 mm (D = dorsal, P = posterior, M = medial).

Figure 3.

Cicerone atlas accuracy for monkey 04-m-005 (A) and 04-m-001 (B). Percentage of MER sites that were located within their atlas-defined nucleus, less than 1 mm from the nucleus border or more than 1 mm from the nucleus border for Cicerone's warped custom 3D atlas ('W'), Cicerone's linearly scaled custom 3D atlas ('L'), Cicerone's standard 3D atlas ('S') and 2D print atlas ('P'). Connecting lines indicate significant difference between the atlases. Total numbers in the three categories (inside nucleus, < 1 mm outside, > 1 mm outside) for each atlas were compared to the warped atlas totals using the chi-square test with Bonferroni correction ($p < 0.005$). Since striatum sites made up more than half of all MER sites and were therefore overrepresented in the totals, calculations for significance were repeated without the striatum sites (indicated with asterisks). Statistical analysis of the pooled data (totals for both monkeys added) yielded the same significant differences between the atlases as shown in B.