Localization of metal electrodes in the intact rat brain using registration of 3-D micro-computed tomography images to a magnetic resonance histology atlas

Metal electrode localization in intact rat brain

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Abstract:

Simultaneous neural recordings taken from multiple areas of the rodent brain are garnering growing interest due to the insight they can provide about spatially distributed neural circuitry. The promise of such recordings has inspired great progress in methods for surgically implanting large numbers of metal electrodes into intact rodent brains. However, methods for localizing the precise location of these electrodes have remained severely lacking. Traditional histological techniques that require slicing and staining of physical brain tissue are cumbersome, and become increasingly impractical as the number of implanted electrodes increases. Here we solve these problems by describing a method that registers 3-D computerized tomography (CT) images of intact rat brains implanted with metal electrode bundles to a Magnetic Resonance Imaging Histology (MRH) Atlas. Our method allows accurate visualization of each electrode bundle’s trajectory and location without removing the electrodes from the brain or surgically implanting external markers. In addition, unlike physical brain slices, once the 3D images of the electrode bundles and the MRH atlas are registered, it is possible to verify electrode placements from many angles by “re-slicing” the images along different planes of view. Further, our method can be fully automated and easily scaled to applications with large numbers of specimens. Our digital imaging approach to efficiently localizing metal electrodes offers a substantial addition to currently available methods, which, in turn, may help accelerate the rate at which insights are gleaned from rodent network neuroscience.
Significance Statement:

The digital imaging technique we present here allows unparalleled 3-D visualization of the anatomical location of large numbers of electrodes implanted deep in the rodent brain. The anatomical location of each electrode can be determined quickly without removing the electrodes from the brain, can be observed from many different angles of view, and can be automated. These features offer substantial improvements over currently available histological methods of electrode location verification.
Introduction:

A major goal of neuroscience is to understand how spatially distributed neural networks facilitate specific types of behavior (Lewis et al., 2015). Rodents are ideal species for this application, due to their complex behaviors and suitability for genetic and molecular tools. To maximize the utility of using rodents to study spatially distributed neural networks, continuous neural activity must be assessed in many brain regions of a rodent brain simultaneously.

Reflecting this notion, the United States’ BRAIN (Brain Research through Advancing Innovative Neurotechnologies) Initiative has labeled the measurement of multi-site electrical activity in awake, behaving rodents a “high priority research area” (Advisory Committee to the NIH Director Interim Report, Sept 16, 2013).

The predominant method for measuring neural activity in rodents is to record electrical signals from 15-50 μm-thick metal microwires (electrodes) implanted into the brain. Thus, many efforts to measure neural activity across spatially distributed networks in rodents have employed microwire multi-electrode arrays (MEAs) to maximize the number of electrodes that can be implanted in one brain at the same time. At present, recordings that use up to 64 microwires spread across up to five brain regions have been reported in rats (de Araujo et al., 2006; Headley et al., 2015; Igarashi et al., 2014; Nicolelis et al., 1997), and up to 64 microwires spread across up to eleven brain regions have been reported in mice (Dzirasa et al., 2011; Sigurdsson et al., 2010). In all of these cases, individual MEAs were implanted into spatially separated brain regions.

One of the methodological challenges of using MEAs is that although the techniques for implanting large numbers of electrodes have improved dramatically, the techniques for identifying the precise location of each electrode implanted in the brain have remained almost
identical to those used throughout the last century. The most common way to determine electrode placements is to stain harvested brain slices with a nucleic acid stain (“Nissl stain”; Einarson, 1932), and infer the location of electrodes from the absence of stained neurons in a linear shape that matches that of an electrode. Although this electrode verification approach is effective for traditional applications, it is a very cumbersome and time-intensive procedure with disadvantages that become unmanageable when large numbers of electrodes are placed in the brain simultaneously. Most notably, brain tissue can be severely damaged before electrodes can be localized, electrode tracks can be altered by the removal of electrode bundles, and brain slices can be lost during the process of cutting or staining (Fischer et al., 2008). The normal wear and tear of occasionally losing or damaging a brain slice is not a problem for most applications, but it can become extremely disruptive when only one brain slice contains evidence of a particular electrode, as happens when electrodes are very thin (15-25 μm). Frustratingly for the MEA field, such problems are only exacerbated when the number of implanted electrodes increases, because closely-packed electrode tracks decrease the integrity of brain tissue and make damage more likely. Since electrical recordings are rarely interpretable if their anatomical origins are unknown, these challenges of using histological stains to infer electrode placements have the potential to significantly limit the utility of multi-site recording.

Small animal brain imaging offers untapped strategies for solving the electrode location problems posed by multi-site recordings. In theory, the most straight-forward way to identify the exact location of implanted electrodes would be to image a brain while electrodes are still implanted. However, currently available imaging techniques, on their own, aren’t appropriate for this application. Magnetic Resonance Imaging (MRI) is fatally susceptible to metal artifacts, including those caused by tungsten (the metal most commonly used to make electrodes) and the
components in solder. MRI is also costly, and can require hours of scan time to produce an image with high enough resolution to identify very small brain regions. Computed tomography (CT) is an imaging modality that is less susceptible to metal artifacts, but that provides close to no tissue differentiation in the brain under standard conditions. Therefore, the ideal imaging solution would have the spatial resolution and tissue differentiation capability of MRI, the ability to visualize metal objects, and could be performed on intact animals with electrodes still implanted in their brains. Here we describe such a solution. Our method co-registers micro-CT images that depict the location of electrode bundles to a published high-resolution MR-atlas (Johnson et al., 2012). When completed, this method facilitates accurate anatomical localization of metal microwire bundles in a manner that is faster than any other method currently available.

Methods:

Animal Care and Use: All animals were housed in a humidity and temperature-controlled room, and water and food were provided ad libitum. Male Wistar Rats (N=7; http://www.criver.com/products-services/basic-research/find-a-model/wistar-rat) were pair-housed and were implanted with recording electrodes at 6-7 weeks of age. All studies were performed in accordance with Duke University’s Institutional Animal Care and Use Committee’s regulations.

Electrode Implantations: Rats were anesthetized with isoflurane, and placed in a stereotaxic frame. Stainless steel ground screws (Plastics One) were implanted above the anterior cranium and cerebellum; additional screws were implanted into the skull above the parietal lobe to improve stability. All screws were secured using C & B MetaBond (® Parkell, Inc.).
Craniotomies were made to allow implantation of the following S-isonel-coated tungsten arrays of 50 µm electrodes in the ten areas (five areas bilaterally) listed in Table 1.

Table 1: Location of implanted tungsten electrode arrays.

<table>
<thead>
<tr>
<th>Brain Region</th>
<th># of Electrodes</th>
<th>Coordinates (from Bregma and top of brain)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A/P</td>
</tr>
<tr>
<td>Bilateral Anterior Cingulate</td>
<td>2 per hemisphere</td>
<td>2.5</td>
</tr>
<tr>
<td>Bilateral Orbitofrontal Cortex</td>
<td>2 per hemisphere</td>
<td>3.7</td>
</tr>
<tr>
<td>Bilateral Anterior Insula</td>
<td>4 per hemisphere</td>
<td>2.2</td>
</tr>
<tr>
<td>Bilateral Basolateral Amygdala</td>
<td>4 per hemisphere</td>
<td>-3</td>
</tr>
<tr>
<td>Bilateral Olfactory Amygdala</td>
<td>3 per hemisphere</td>
<td>-1.4</td>
</tr>
</tbody>
</table>
The microwires in each array were separated by 250 µm. Each array was secured using additional C & B MetaBond. The tungsten electrode arrays (Figure 1A) were connected to a printed circuit board using silver paint (Figure 1B), and the circuit board was connected to a high density miniature connector (Omnetics) using flux-cored solder (Figure 1C; Kester SN63PB37 #66/44). The circuit board and connector were secured to the skull with dental cement after all electrode arrays were secured. All of the implant materials pictured in Figure 1 are commonly used in multi-electrode array preparations, but prevent the acquisition of MR images and cause artifacts in micro-CT images.

**Micro-CT scanning:** Rats (N=7) were anesthetized with pentobarbital (250 mg/kg) and perfused transcardially with 200 mL phosphate-buffered saline (PBS, pH 7.4) followed by 200 mL 4% paraformaldehyde in PBS. Intact heads were then detached from the body and refrigerated until scanning. Image data were acquired with a dual source micro-CT system. The system contains two G-297 x-ray tubes (Varian Medical Systems, Palo Alto, CA) with 0.3/0.8 mm focal spot size, two Epsilon High Frequency X-ray generators by EMD Technologies (Quebec, Canada) and two CCD based detectors with a Gd2O2S phosphor (XDI-VHR 2 Photonic Science, East Sussex, UK) with 22 µm pixels which are binned to 88 µm. The data acquisition was controlled by sequencing applications written in LabVIEW. Only one of the x-ray sources/detectors was used for these scans. 360 projections were acquired at 140 kVp, 50 mA, 5 ms per exposure with Cu filtration. Tomographic reconstruction was performed using the commercial software COBRA EXXIM (Exxim Computing Corporation, Pleasanton, CA, USA; http://www.exxim-cc.com/products_cobra). Metal causes beam hardening and scatter effects in CT images, which result in dark streaks between metal objects, and bright streaks in the surrounding area. COBRA
EXXIM provided a proprietary iterative Streak Artifact and Metal Artifact Reduction Algorithm (SAMARA) that is more tolerant to data imperfections than the standard Feldkamp method (Feldkamp et al., 1984). Artifacts are often handled by using an intensity cut-off to remove the pixels comprising the artifact, and replacing the missing voxels using interpolation of the surrounding voxels. As a consequence, voxels close to the object causing the artifact are often “smoothed out” and details are lost. The SAMARA algorithm removes artifacts while maintaining low and medium contrast around the artifact-causing object. The SAMARA parameters were set such that streak artifact reduction (SAR) was performed if

\[
\text{SAMARATAG\_HIGHCONTRASTLEVEL} = 1000 \text{ and } \\
\text{SAMARATAG\_LOWCONTRASTLEVEL} = 0. 
\]

Metal artifact reduction (MAR) was also performed if the parameter SAMARATAG\_HIDENSLEVEL=15000. The volumes were reconstructed in a $640^3$ matrix with a voxel size of 88 $\mu$m.

**Immunohistochemistry:** After CT scanning, the upper half of each rat’s skull was removed with the electrodes still attached, and the perfused brains were extracted from the rest of the skull. The extracted brains were then postfixed overnight in 4% paraformaldehyde at 4°C, put in 30% sucrose at 4°C until they sank (2-3 days), flash-frozen in isopentane, and stored at -80°C. Each brain was sectioned into 30 $\mu$m slices on a cryostat (Leica Microsystems) and mounted onto gelatin subbed slides. All sections were processed using a colorimetric Nissl stain. For the Nissl stain, mounted slides were placed in a 1:1 chloroform/alcohol mixture for one hour, gradually rehydrated through increasing concentrations of water:alcohol, stained with cresyl violet, cleared with an alcohol + glacial acetic acid, gradually dehydrated through decreasing concentrations of water:alcohol followed by xylene, and coverslipped using Permount Mounting Medium. Images of stained slices were taken with a Zeiss Axio Observer Z1 microscope at 10x magnification.
CT-to-MR Atlas registration: The overall goal of the registration process was to register CT images of individual rats implanted with electrodes to a previously published multidimensional magnetic resonance histology (MRH) atlas of the adult Wistar rat brain (Johnson et al., 2012).

This process differs from previously published CT-MR registration procedures that register CT images of an individual to MR images of that same individual (Azarion et al., 2014; Maintz et al., 1996; Mirzadeh et al., 2014; Princich et al., 2013; Vaquero et al., 2001), or registration procedures that register MR images of one individual to a canonical MR atlas (Sergejeva et al., 2015). Since metal artifacts prevented the acquisition of MR images from implanted rats, the registration process for the present application had to both cross modalities (CT to MR) and individual specimens (an individual CT scan to an atlas representing the average of 5 other specimens).

Procedures involved in image registration have been described and reviewed extensively elsewhere, and therefore will not be detailed here (Gholipour et al., 2007). At their core, these procedures aim to compare two image volumes iteratively until their “similarity” is maximized.

Registration procedures have three main components: transformation specifications (which define what kinds of degrees of freedom are allowed), a measure of similarity between the images being aligned, and an optimization framework (which determines how possible transformation parameters will be chosen). When registration procedures fail, they usually do so in one of two ways. The first kind of failure is when images are dramatically misaligned. This often occurs because the optimization strategy did not lead to an adequate sampling of the possible model parameters before converging on a solution, leading to transformation parameters that represent a local solution to the registration problem rather than a global solution. The second type of failure occurs when images are aligned approximately in the correct space, but
features of interest do not line up satisfactorily. This usually occurs because images were not
transformed in the correct dimensions or with enough degrees of freedom. The registration
procedure described below minimized both types of errors. The code used to implement these
procedures can be found at http://www.civm.duhs.duke.edu/eNeuro2015/.

Since soft tissue is poorly resolved in CT images, our registration approach was based on
the skull. We found that two general strategies improved the success of this skull-focused
approach. First, although non-rigid registration transformations were necessary to account for
the differences in sizes and shapes between individual rats and the rat atlas, non-rigid registration
was greatly improved when it was preceded by a rigid registration step to place the CT images
and MRH atlas in approximately the correct space. Second, registration was improved when as
much of the non-skull image as possible was masked out of the CT images and MR atlas.
Implementing these steps drastically reduces the likelihood of converging on transformation
parameters that represent non-global registration solutions. These strategies resulted in the
multi-step registration procedure described below and illustrated in Figure 2. Although the
general approach behind this procedure should work for most scanning set-ups, specific
parameters may need to be optimized for different CT scanners, scanning parameters, or
electrode implantation methods.

Step 1: The acquisition methods and processing technique used for the MRH atlas were
described in detail by the group that published the atlas (Johnson et al., 2012). The MRH atlas
was downsampled to match the 88 μm isotropic voxel size of the CT images. (If a CT system
with a different voxel size is used, the MRH atlas should be downsampled to match the size of
CT data or the CT images should be downsampled to match the 25 μm voxel-size of the T2*-weighted structural images of the MRH atlas. Take note that the diffusion-weighted image series
of the MRH atlas is published at a resolution of 50 μm.)

Step 2: The MRH atlas and CT images were pre-processed to prepare them for a rigid registration step that would arrange them into approximately the same space.

Step 2a: The goal of this step is to mask out brain tissue differentiation from the chosen MRH image. The MRH atlas consists of a number of different data sets, each of which emphasizes a different type of soft tissue contrast. All the data sets are registered to the same space, so registration of the CT data to one data set effectively registers the CT data to all of the MR atlas images. Due to the inclusion of a skull-stripped version of the image, we chose to register the CT data to the “b0 image”, which is a non-diffusion weighted spin echo image that provides the baseline for diffusion tensor data acquisition. To do so, we made a “brain mask” using the boundaries of the skull-stripped b0 image. This initial brain mask was eroded with a sphere 20 voxels in diameter to ensure that no skull would be masked out. Voxels falling within the brain mask were then replaced with the mode intensity value of the MRH image. This mask resulted in an MRH image with homogeneous intensity values within the skull.

Step 2b: The goal of this step is to mask the electrode implant (referring to the electrodes, circuit board, wires, and connector) out of the CT image, and replace the masked voxels with the mode intensity value of the CT image. The method we used to isolate the electrode implant from the CT images in our preparation was based on the observation that voxels representing the electrode implant had higher intensity values than anything else in the image (including the skull) and appeared with a very low probability. This feature of electrodes has been found in CT images of human deep brain stimulators as well (Azarion et al., 2014). Taking advantage of these features, the most straight-forward way to mask out the electrode implant was to estimate a probability
density function of the intensity values in each of the CT image volumes. The estimate of this function was computed using the \textit{ksdensity} function in Matlab R2013 (The Mathworks, Inc., Natick, MA, USA), which is based on a normal kernel function and is evaluated at 100 equally spaced points that cover the full range of the inputted data. All voxels that had intensity values greater than the mean intensity value and an estimated probability density of less than 0.00001 were part of the electrode implant. This electrode implant footprint was then dilated by a sphere 5-voxels in diameter to ensure complete coverage of the electrodes and implant, and all the voxels on an individual CT scan within this final mask were replaced with the mode intensity value of that individual CT scan. This mask resulted in a CT image with effectively no electrodes or head implants. Other strategies for masking out electrodes can be used as necessary for specific preparations; the critical part of Step 2a is that voxels representing the electrode implant are replaced with the mode intensity value of the CT image.

\textit{Step 2c}: the intensity values of the CT images were inverted so that skull would have very low intensity values (would look dark to the eye) compared to the rest of the image, similar to the skull on the MR b0 image.

\textit{Step 3}: The CT images were registered to the MRH b0 atlas using a rigid registration transformation calculated by the \textit{imregister} function in Matlab R2013 (The Mathworks, Inc., Natick, MA, USA). As stated earlier, the goal of this step was to place the CT and MR atlas images in approximately the same space to optimize subsequent non-rigid transformations performed in later steps. Mattes Mutual Information—a statistical measure from Information Theory indicating the amount of information one random variable (image) gives about another (Mattes et al., 2001)—was used as the similarity metric for this step, due to its established
success in multi-modal imaging applications (Pluim et al., 2003). The One Plus One Evolutionary optimizer works by generating random samples around the current position in parametric space (Styner et al., 2000); we found this optimizer to reduce the number of dramatic registration failures due to non-global transformation solutions. The final parameters were:

- initial radius = 1.01, growth factor = 0.00325, maximum iterations = 500, optimizer = OnePlusOneEvolutionary, metric = MattesMutualInformation.

**Step 4**: The MRH atlas and CT images were pre-processed to prepare them for affine (nonrigid) registration. To minimize registration interference from the brain tissue within the MRH b0 image, the brain mask from Step 2a was eroded with a sphere 5 voxels in diameter and applied to both the rigidly registered CT and the b0 image. In this case, we found registration to be best if voxels were fully excluded from the registration process rather than be replaced by mode intensity values.

**Step 5**: The rigidly registered CT images were registered to the MRH b0 atlas using a 9 parameter affine similarity registration transformation, allowing for translation, rotation, and scaling to improve the overall fit due to anatomical differences between the specimens and the MRH atlas.

**Step 5a**: The similarity registration transformation was calculated using the `imregister` function in Matlab (The Mathworks, Inc., Natick, MA, USA). As in Step 2c, Mattes Mutual Information was used as the similarity metric, the One Plus One Evolutionary was used as the optimizer. The final parameters were: initial radius = 1.01, growth factor = 0.00325, maximum iterations = 500, optimizer = OnePlusOneEvolutionary, metric = MattesMutualInformation.
Step 5b: The resulting rigid and affine transformation matrices were recorded and applied to the original, unmasked CT images.

Assessment of registration success: Perfect registration between CT images and the MRH atlas would lead to perfect alignment of all corresponding points in both images. Assessing the accuracy of this alignment would therefore require measuring the distance between a very dense set of landmarks present in both sets of images that were not used in the registration process. Like many digital imaging situations, such landmarks were not available. Surrogate measures have been proposed to help assess the accuracy of image registrations, including tissue label overlap scores and image similarity measures, but recent studies have shown these measures to be unreliable (Rohlfing, 2012). Thus, expert knowledge and evaluation is considered the gold standard of image registration evaluation (Akbarzadeh et al., 2013).

For the present study, this expert validation was provided in the form of three human rat anatomy experts who assessed through visual inspection 1) the overlap of skull features in the CT images and MRH images, and 2) the correspondence of electrode bundles in the registered CT overlaid on the MRH atlas with the physical electrode tracks observed in physical slices of the same brain. Examining the overlap of skull features is accomplished by overlaying the registered CT images and MRH images in an image visualization package such as the freely-available 3D Slicer (www.slicer.org; (Fedorov et al., 2012), and toggling through different transparency levels of the two image volumes. An anatomy expert familiar with the image visualization software can accomplish this type of visual inspection in about one minute.

The correspondence of electrodes in the registered CT with the physical electrode tracks observed in physical brain slices was examined by comparing CT images overlaid on the MRH atlas with collected histological brain slices viewed under 4x magnification. Distances were
measured on the CT overlaid on the MRH atlas using the ruler tool in 3D Slicer. Distances were measured on the histological slices using the ruler tool in the Nikon NIS-Elements Basic Research Microscope Imaging Software (version 3.2) calibrated to a Nikon Plan Fluor 4x/0.13 microscope lens. Experts chose fifteen electrodes and measured distances from an electrode to a landmark in the brain in both the registered CT overlaid on the MRH atlas and in the histological slice. The distances were then compared by subtracting the distance measurements from one another. The median of all the difference measurements was used to estimate the accuracy of the CT-to-MRH method. Each expert chose their own electrodes and their own landmarks.

Electrode configuration simulations: The goal of the electrode simulations was to determine whether the configuration of electrode bundles would affect the consistency and robustness of our registration procedure. To accomplish this, first, we acquired a CT scan of one non-implanted rat. The CT scan was registered to the MRH atlas as described above (except no masking of electrodes was required), and the rigid and affine transformation matrices were saved. Next, a set of 1000 fiducials were randomly generated and applied to the non-registered CT image of the non-implanted rat. These fiducials provided a dense set of landmarks present in both sets of images that could be used to evaluate the change in registration process caused by the addition of simulated electrodes. 23 fiducials falling outside of the skull (as determined by back-transforming the MRH brain mask to the native space of the CT image) were discarded, leaving a total of 977 fiducials. The coordinates of the 977 fiducials were then transformed using the saved rigid and affine registration transformation matrices, and these newly registered coordinates were used as the reference coordinates for all subsequent electrode simulations.

Third, we simulated 1000 implanted rat skulls. To simulate different electrode
configurations, the electrode bundles and rest of the head implant were isolated using voxel intensity values from the registered CT image of each implanted rat. For each simulated electrode configuration, one isolated head implant and ten electrode bundles were randomly chosen to be applied to the CT image of the non-implanted rat. The head implant was shifted by random amounts along the anterior-posterior and left-right axes. The electrode bundles were shifted by random amounts along the anterior-posterior, left-right, and dorsal-ventral axes. The amount of translation for each implant or bundle along each axis was determined by independent random draws from Gaussian distributions with a mean of 0 and a standard deviation of 5 voxels, with the following exception: to allow for even more variation in simulated wire placement, the amount of translation for the bundles along the anterior-posterior and left-right axes was drawn from a Gaussian distribution with a mean of 0 and a standard deviation of 10 voxels. To make sure simulated electrode bundles did not extend outside of the skull, all electrodes that extended outside of the MRH brain mask were removed. The resulting simulated head implant and collection of electrode bundles were then back-transformed to the native space of the non-implanted CT, and placed on the non-implanted CT image by replacing the CT values in those voxels with the intensity values of the original head implant or electrode bundle.

Each simulated CT image was registered using the procedures described for the CT images of the truly implanted rats (all stated parameters were the same, except the brain mask for the rigid registration was eroded with a 10-voxel sphere instead of 20-voxel sphere). For each of the 1000 simulations, the new set of registration transformation matrices acquired through the registration process were applied to the unregistered set of 977 fiducials. The coordinates of these fiducials were compared to the coordinates recorded from the registration of the non-implanted rat. The Euclidean distance between each fiducial and its corresponding fiducial on the
reference list was calculated after the rigid registration step and again after the non-rigid registration step. For each simulation, the mean, standard deviation and median of the distance (or “movement”) between the simulated and reference coordinates across all 977 fiducials was recorded. Estimates of the registration error caused by the head implant and electrodes were assessed by analyzing these distance metrics of the 977 fiducials in each of the 1000 simulations (977,000 fiducials in total).

Statistics used to evaluate electrode configuration simulations:

When statistical tests are discussed in the main manuscript, they will be followed by a superscript lowercase letter. This letter corresponds to the lower case letter preceding each row of the “Goal” column in Table 2.

Table 2: Goal and Characteristics of Statistical Tests Used to Evaluate Electrode Configuration Simulations.

<table>
<thead>
<tr>
<th>Goal</th>
<th>Distribution of Data</th>
<th>Type of Test</th>
<th>Power of Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Test differences in fiducial movement between brain segments after rigid registration only”</td>
<td>Highly skewed right</td>
<td>Kruskal-Wallis</td>
<td>Chi-square = 3.00e+05</td>
</tr>
<tr>
<td>“Test differences in fiducial movement between brain segments after rigid registration followed by non-rigid registration”</td>
<td>Highly skewed right</td>
<td>Kruskal-Wallis</td>
<td>Chi-square = 2.33e+05</td>
</tr>
<tr>
<td>“Test differences in fiducial movement after rigid registration and rigid registration followed by non-rigid registration”</td>
<td>Highly skewed right</td>
<td>Mann-Whitney U</td>
<td>Z value = 30.4165 Rank Sum = 1.01e+12</td>
</tr>
</tbody>
</table>
Results:

The CT scans successfully allowed visualization of both the skull and the implanted electrode bundles (Figure 3A). Although the metal in the skull screws and head implant did cause considerable image artifacts, artifact reduction procedures significantly improved the homogeneity of the images (Figure 3B). The 88-μm voxel size of our micro-CT system could not always clearly differentiate between individual microwires within a correctly implanted bundle (Figure 3C, left panel; differentiation between microwires in the insula bundle on the left side of the brain is less clear, differentiation between microwires in each midline anterior cingulate bundle is more clear), but it did allow visualization of individual severely misplaced or bent microwires (Figure 3C, right panel), as well as bundles separated by more than 250 µm (Figure 3C, right panel: anterior cingulate bundles at midline are separated by 500 µm). As expected, no brain tissue could be differentiated from CT scans.

Our multi-step registration procedure based on the skull yielded convincing registration between individual CT scans and the MRH atlas. Three anatomy experts reported excellent correspondence (less than 0.5 mm in all locations) between the boundaries of the CT skull and those of the MRH atlas skull in all seven rats (Figure 4). Confirming this correspondence, the histology performed on brain slices after CT scanning indicated that the co-registration procedure yielded very accurate results in all animals (examples shown in Figure 5).

Highlighting one of the advantages of using a 3-D MRH atlas, when the physical brain slices used for histology were not cut perfectly perpendicular to the anterior-posterior axis, the registered CT overlaid on the MRH could be “resliced” to match the plane of the histology slice without losing any image resolution. The anatomy experts estimated a median difference of 32
µm between the location of the electrodes in the registered CT and the location of electrode tracks in stained brain slices.

Simulation experiments illustrated that head implants and electrode bundles did affect the registration process (Figure 6A-6D). The median change in fiducial coordinates when implants and electrodes were present compared to when they were absent was approximately 0.2-0.4 mm, depending on the brain location (Figure 6C). Fiducials in anterior parts of the brain were more affected by the presence of electrodes than fiducials in posterior parts of the brain (Figure 6C; significant differences between brain segments after rigid registration, as well as after rigid plus non-rigid registration, p<.001\(^{a,b}\), Kruskal-Wallis test). The latter result is likely because the orientation of the olfactory bulb and most anterior parts of the frontal cortex relative to surrounding bone structures are extremely variable from animal to animal, whereas the orientation of the center of the brain is much more consistent. Of note, the non-rigid registration step did not dramatically change fiducial placements within these simulation experiments (Figure 6D). When it did change the fiducial placements, however, on average it placed them closer to the coordinates retrieved from registration without simulated implants and electrodes more often than it placed them farther away. Applying non-rigid registration after rigid registration resulted in a median change of 0.26 mm from the coordinates retrieved from registration without simulated implants, whereas applying rigid registration alone resulted in a median change of 0.27 mm from the coordinates retrieved from registration without simulated implants (p<.001\(^{c}\), Mann-Whitney U test). Still, sometimes the non-rigid registration step did make the registration worse as assessed by fiducials landing farther away from the coordinates retrieved from registration without simulated implants after rigid and non-rigid registration was applied than when only rigid registration was applied (Figure 6D). These detrimental effects, when they occurred, were
more likely to manifest in posterior parts of the brain than anterior parts of the brain (pie charts in Figure 6D), but were usually small in magnitude (less than 0.1 mm, shown by the red bars in the histograms of Figure 6D). Occasionally registration failed dramatically before or after the non-rigid registration step during these simulations (as reflected by the tails of the histograms in Figure 6D), due to the selection of transformation parameters that optimized local solutions rather than global solutions. When these failures occurred, they were visually obvious and resulted in significant lack of overlap between the CT image volume and the MRH atlas, so they could easily be detected. Once detected, we found that these failures could be corrected by tuning the mask and registration parameters.

Discussion:

Measuring electrical activity from electrodes implanted across multiple brain areas simultaneously in awake, behaving rodents holds great potential for uncovering the mechanisms underlying distributed neural networks. Multi-site recording technology has made significant strides. The next step is to implement electrode localization methods that permit these exciting recording technologies to reach their full potential.

Here, we have described a technique that combines micro-CT images with a high resolution MRH atlas to identify the anatomical location of metal electrode bundles in an intact rodent brain. The 3D view of all implanted electrodes provided by our technique provides an unparalleled opportunity to visualize the true trajectory of each electrode bundle and measure the spatial relationship between multiple independent bundles in vivo. Our technique does not
require removing the electrodes from the brain, nor does it require the surgical implantation of
precisely placed external markers. Further, unlike physical brain slices, the 3D images of the
electrode bundles and the MRH atlas they are registered into can be “resliced” to view any
desired plane of tissue without extra manual work or expensive reconstruction software
(Markovitz et al., 2012). This allows for the verification of the location of electrode tips from
many different visual angles with ease. In addition, the MRH atlas contains eight different
interchangeable reference images that use unique contrasts to highlight specific aspects of soft
tissue architecture; having access to the entire collection of images improves opportunities to
visualize the precise anatomical location of the end of an electrode. Although the preparation
used in the current study employed perfused animals, this technique could easily be extended to
live anesthetized animals as well.

The primary alternative to using our digital imaging approach for localizing electrodes is
to use standard histological (Nissl) techniques. Histological techniques have severe limitations
that become increasingly problematic as the number of electrodes implanted in the brain grows.
The first limitation is that histological techniques require a significant time investment. After a
brain is removed from a skull, it must be post-fixed, cryoprotected in a sucrose solution
overnight, and flash frozen before it is ready to slice. Then, very thin slices are painstaking cut
one by one, and mounted onto slides for staining and eventually coverslipping (Gerfen, 2003).
Overall, the entire electrode localization procedure takes days to weeks to complete if multiple
brains must be processed sequentially. In comparison, our imaging technique takes 15-20
minutes after the brain scans are acquired. This improvement in speed is one of the greatest
advantages of our digital imaging approach.

A more problematic, but less ubiquitous, limitation with histological techniques are that
slice preparation and Nissl stain protocols can severely damage critical brain tissue before electrodes can be localized. Especially when strong adhesives and multiple screws are needed to secure electrical connectors to the skull of an animal, the process of removing electrode bundles from a brain can bend the electrodes, alter the electrode paths, or cause extra tissue damage. Cutting brain slices is also a notoriously finicky process that can result in the loss of brain slices. Further, traditional Nissl stains require many dehydration and hydration steps that may warp and damage brain tissue. Even if the tissue processing is completely perfectly, it is often difficult to identify the precise location of the tip of very thin electrodes, especially if brain slices are not cut in the exact same geometric plane as the electrode tracks. All of these limitations can be overcome or completely avoided using our CT-to-MRH atlas technique.

One of the greatest benefits of our CT-to-MRH atlas technique that would be difficult and potentially cost prohibitive to accomplish using histology alone is that localizing metal electrodes can be fully automated once CT images are acquired. As a consequence, metal electrodes can be localized in large numbers of specimens without much manual labor. Further, as atlases become increasingly detailed the utility and programmability of the method will increase. In particular, it will become possible to automate the final verdict of whether a particular electrode bundle landed in an intended anatomical location. Figure 7 illustrates how a currently available segmentation atlas makes it possible to determine whether bundles in the present study landed in the amygdala; once a sufficiently segmented rat atlas becomes available with the boundaries of all brain regions demarcated and labeled, it will be possible to determine whether electrodes landed in even smaller brain structures or specific nuclei.

Another feature of our approach is that the success of any registration can be confirmed
by visual inspection that assesses whether the overlap between the skull of the CT image and the skull of the MRH image is satisfactory. An automatic algorithm can be used to detect dramatic registration errors, but we recommend making specific efforts to visually confirm registration results when multiple electrodes are implanted in the most anterior parts of the brain, as our simulation experiments indicated such electrode placements are most likely to interfere with registration. Such visual inspections should take no more than a few minutes per rat. If a particular registration is found to be suboptimal either by an automatic algorithm or visual inspection, users can tune the mask and registration parameters until the overlap between the CT and MRH skulls is acceptable. When tuning is required, we recommend varying the initial radius and growth factor registration parameters rather than changing the optimizer or similarity metric.

In future applications, CT systems using smaller voxel sizes can be used to aid in visualizing very thin electrodes or localizing electrodes to small nuclei. The custom-built system used in the present study was optimized for other in vivo micro-CT applications where the resolution of 88 µm is adequate. This level of resolution is informative for localizing 50 µm electrodes to larger structures, such as the amygdala, that are at least 1000 µm in width and height. When higher resolution localization is necessary, commercial systems with resolutions as small as 4.5 µm can be employed (see, for example, the Quantum GX microCT Imaging System by PerkinElmer), although the radiation dose required for this level of resolution necessitates terminal or post-mortem studies. Importantly, the general procedure for registering CT images collected at < 80 µm resolution to the MRH atlas would be the same as those described here (with the possible addition of an extra down-sampling step if the CT resolution is smaller than that of the MRH atlas, followed by a transformation step to the CT images at their
original resolution). Also useful to remember, no matter what resolution the CT images are collected at, they will be overlaid on the MRH atlas that has a resolution of 25 µm.

The present paper focuses on applying the CT-to-MRH atlas electrode localization technique to microwire multi-electrode arrays (MEAs) because they often contain more metal than other types of multi-electrode arrays and are therefore particularly challenging to visualize using metal-sensitive imaging techniques such as MRI. Another interesting future direction for the work presented here, perhaps in combination with using CT imaging protocols that allow higher resolution, is to extend the CT-to-MRH atlas to other types of multi-electrode arrays, such as multi-site silicon probes. Silicon probes are MEAs with multiple flat metallic recording sites spaced along non-metallic probes (in contrast with the microwire MEAs described in the present study that are completely metal and that have recording sites restricted to the end of the electrodes). It is now possible to use multishank, high-density recording silicon probes to record from up to 512 channels in awake behaving rodents (Berényi et al., 2014). Just as with multiwire MEAs, precise anatomical localization of each recording site on a silicon probe is important for interpreting acquired neurophysiological data.

Based on the results we describe here, the CT-to-MRH atlas electrode localization technique is a promising option for localizing silicon probe recording sites efficiently and cost-effectively. To assess this option, the first step would be to determine whether either the outline of the silicon probe implants or the recording sites themselves could be visualized using CT imaging. If the proportion of x-rays that pass through the material used to make the silicon probe differ from the proportion of x-rays that passes through brain tissue, the outline of the silicone probe should be visible using CT. The likelihood of successfully visualizing individual recording sites will depend on the type of metal used, but given that the wire MEAs used in the
present study contained more metal than most silicon probe preparations, we do not anticipate that metallic artifacts caused by silicon probes will be debilitating. Assuming that either the probe outline or the recording sites can be visualized using CT, the next step would be to tailor the technique to the specific preparations and goals of each experiment. Silicon probe designs vary widely (see http://neuronexus.com/products/neural-probes and http://www.cambridgeneurotech.com/silicon%20probes_array%20types.htm for commercial examples), but the probes are typically 15 μm thick, and have heterogeneous widths that often begin with a sharp tapered tip and grow to around 100 μm at the uppermost recording site. The metal recording sites, themselves, may range in area from 150-300 μm², and are spaced anywhere from 20-200 μm apart, depending on the application. As an example of how individual probe designs may affect how the CT-to-MRH atlas would be applied, if recording sites are spaced 20 μm apart and the study goals require each recording site to be visualized individually, electrode localization steps will likely need to be implemented post-mortem with a CT system that can accommodate small voxel sizes of less than 10 μm. However, if the recording sites are spaced 200 μm apart, larger voxel sizes compatible with non-lethal radiation doses will likely be sufficient. Further, since the distance and geometric configuration of recording sites in silicon probes is known with machine-implemented precision, the anatomical location of each recording site can be determined as long as the boundaries and/or axis of the probe are known, and larger voxel sizes may be sufficient to visualize these properties of the probe. More research needs to be done to determine how to best optimize CT imaging of silicon probes, but extending the CT-to-MRH atlas method to other types of MEAs will be a fruitful area for future development.

The CT-to-MRH atlas approach to efficiently localizing metal electrodes we describe
Here offers a substantial addition to currently available histological electrode placement strategies in multiple domains. Our general registration strategy can be easily adapted to new CT imaging and electrode implant procedures. The field of neuroscience is in a very exciting time where it is now possible to measure electrical activity in multiple areas of the rodent brain at the same time. Taking advantage of the power of digital imaging to advance efficient electrode localization strategies will, in turn, accelerate the rate at which these new multi-site recording techniques will help to decipher the mechanisms and functions of spatially distributed neural networks.

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Advisory Committee to the NIH Director Interim Report, Sept 16, 2013


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Figure 1: Sources of metal in electrode implants. A. Tungsten electrodes. B. Silver paint to connect tungsten wires to gold pads on the circuit board. C. Solder to connect the Omnetics connector to the circuit board.

Figure 2: Schematic of micro-CT to MRH registration procedure.

Figure 3: Micro-CT images of implanted rat brains. A. 3-D reconstructions illustrate 3-D structure of electrode bundles and head implants. B. Examples of CT cross-sections without (left) and with (right) artifact reduction applied. C. 88 μm voxels do not permit clear differentiation of individual wires within electrode bundles (2-4 individual wires are contained in each pictured bundle, despite their appearance as singular objects), but separate bundles are easily visualized (left), as are individual wires that are severely misplaced or bent (right).

Figure 4: Example of registration between individual CT scans and the MRH atlas. A. Coronal slice of MRH atlas. B. Corresponding slice in un-registered CT. C. Corresponding slice in registered CT.

Figure 5: Histological validation of CT to MRH atlas registration. Column 1 = CT image. Column 2 = registered electrode mask from CT overlaid on MRH atlas. Column 3 = corresponding brain slice stained with cresyl violet. Row A = orbitofrontal cortex bundle and top of insula bundle (both in correct location) overlaid on the b0 image from the MRH atlas. Row B = anterior insula bundle (correct location) overlaid on the b0 image from the MRH atlas. Row C = olfactory amygdala bundle (landed on the very lateral edge of intended area) overlaid on the gradient recalled echo (GRE) image from the MRH atlas. In each row, the atlas contrast image that best highlighted the soft tissue architecture of the brain area around the electrode was chosen. For the examples illustrated in Row A and C, the physical brain slices used for histology were not cut perfectly perpendicular to the anterior-posterior axis. Highlighting the advantages of 3-D image volumes, the registered CT overlaid on the MRH were resliced to match the plane of the histology slice shown in Column 3 (as illustrated).

Figure 6: Results of electrode and head implant simulations. A. Schematic of simulations. B. Illustration of 977 fiducials on a glass brain separated into four labeled segments. C. Box plots of the mean and standard deviation of Euclidean distance between all fiducials on the registered (after both rigid and non-rigid registration) non-implanted brain and registered simulated implanted brains across all 1000 simulations. Red line = median of all 1000 simulations. Box limits represent the 1st and 3rd quartiles. Whiskers represent the 5th and 95th percentiles. D. Percentage of 977,000 fiducials (the 977 fiducials that landed within the confines of the brain across 1000 simulations) whose Euclidean distance between the registered simulated implanted brain and non-implanted brain was reduced, increased, or unchanged by the non-rigid registration step compared to rigid registration alone (pie charts). Histograms of the absolute
value of the change in error (in Euclidean distance) caused by the non-rigid registration step
(stacked bar charts; unchanged fiducials excluded) in the 977,000 fiducials across all
imputations. Note that bin sizes increase after the dashed line.

Figure 7: Electrode bundles overlaid on segmentation atlas. A. Coronal brain slice of two
amygdala bundles overlaid on the segmentation atlas and GRE atlas image. The colors of the
segmentation atlas are as follows: orange = amygdala, purple = hippocampus, yellow =
caudate/putamen, and blue = corpus callosum. B. 3D rendering of all bundles overlaid on a glass
brain with the amygdala (orange) and hippocampus (purple) from the segmentation atlas shown.