Can combined CT and TOF-MRI assist in neuro-anatomical surgery planning in small animal models?

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Aims
Deep brain stimulation (DBS) for neurological and psychiatric disorders like Parkinson's disease or major depression disorder requires the implantation of electrodes for the application of electrical pulses in deep brain anatomical locations. For such procedures, pre-operative or intra-operative multi-modality brain magnetic resonance imaging (MRI) or computed tomography angiography (CTA) images of the individual patient are extensively investigated to define the optimal trajectory for electrode insertion to avoid vasculature and functionally important brain areas. Unlike DBS in humans, planning of brain interventions in preclinical rodent models is typically restricted to defining the target and entry points in a generalized anatomical small animal brain atlas1 and transforming these onto the individual animal using a stereotactic reference frame. As current atlases provide limited or no blood vessel information, the outcome of neurosurgical small animal model experiments could be deleteriously influenced when a sub-optimal electrode trajectory ruptures the cerebral vasculature resulting in severe systemic effects. However, the feasibility of individual pre-operative imaging-based surgical path planning in animal studies is limited. Therefore, we aim to build a stereotactic (probabilistic) atlas based on anatomical (CT, MRI) and cerebral vasculature (TOF-MRI, CTA) information that can be used for neurosurgical planning (e.g. electrode implantation), without requiring the acquisition of vasculature and anatomical reference images for each individual animal. Here, we validate vasculature information from TOF-MRI with CT(A) and assess the intra-strain variability in skull reference points and cerebral vasculature for neurosurgery planning and subsequent (probabilistic) atlas building. Using this atlas, we aim to evaluate the risk of a user defined electrode trajectory damaging a blood vessel on its path. The use of such a method will be readily applicable to DBS in small animal models and also to a wide range of stereotactic surgeries like targeted injection of viral vectors, contrast agents, cells for the creation of neural disease models and in situ cell labeling applications.

Method
In vivo 3D anatomical MR brain images and 2D multi-slice MR angiography (MRA) time-of-flight cerebral angiography images (FLASH-TOF, isotropic resolution of 195 µm) were acquired for 10 male Wistar rats in a 9.4T Bruker small animal MRI scanner. In vivo and ex vivo CT images of the full rat head were acquired on a SkyScan1076 small animal CT scanner. In vivo CT images of the full rat skull were acquired from isoflurane gas-anesthetized rats (3% for induction, 1.8 % for maintenance), positioned with bregma in the center of the FOV, using the following parameters: 35 µm isotropic resolution, 49 kV source voltage, 200 µA source current, 0.5 mm Al filter, 180 ms exposure time, 0.8° rotation step, 2 averages, 2 connected scans to cover the complete rat skull length (this is important to include enough landmarks for coregistration such as lambda, bregma, nasal suture,...). After the last in vivo imaging time point, rats were sacrificed by administration of an overdose of anaesthetics (i.e. nembutal, to which 20% heparine was added to avoid blood clotting) and transcardially perfused, first with heparinized saline to flush the blood away, than with
paraformaldehyde (4% in PBS) to fix the tissue, followed by a saline flush and in a last step with 30% BaSO₄ (in 2% gelatine) as a blood pool contrast agent for ex vivo CTA. We are currently evaluating a second perfusion protocol that would allow us to visualize the vessel tree on histological tissue sections. To this end, the BaSO₄ in the final perfusion step is mixed with liquid latex and waterproof black drawing ink. Ex vivo CTA images were acquired from the packed specimen with the following parameters: 35 µm isotropic resolution, 100 kV source voltage, 100 µA source current, 1 mm Al filter, 220 ms integration time, 0.7° rotation step, 3 averages, 2 connected scans to cover the complete rat skull length.

We used an in-house developed image analysis pipeline²,³ for image pre-processing (e.g. RF intensity inhomogeneity correction) and spatial normalization of MR and CT anatomical images, MRA and CTA vasculature images and reference atlas template images. A vasculature average image was constructed in atlas space (figure 1). We use ex vivo CTA for validation of the MRA-TOF. For the planning of stereotactic surgery, the targets are visualized in the Paxinos-MR template along with the multi-modality information of MR/CT anatomical and MRA images normalized to atlas space. The risk of the electrode damaging the vasculature is computed by representing vessels and electrodes in terms of Euclidean distance maps. A 2D automatic ray casting approach with potential trajectories radiating from the target point towards the skull is presented with associated information on the vasculature along each path. The coordinates of entry point, the angle of entry and the depth of incision along with the associated risk (maximum intensity and averaged sum-of-pixel intensities along trajectory) are presented to the user (figure 1, E).

Results
The information from multi-modality (MRI, CT and atlas) images of anatomy, vasculature and stereotactic coordinates was combined to realize an optimal 3D planning for stereotactic neurosurgery in rodents (figure 1).

Larger vessels are consistently visualized in all the TOF-MRI images that were processed and their geometric location is nearly identical. Depending on minor modifications in the animal position within the MRI scanner, some sections of the vessel tree were less visible for some animals compared to the others.

An average vasculature template has been constructed from the MRA-TOF images in the atlas space. The CT data serves as ground truth for validation of this vasculature atlas (ex vivo CTA) and for evaluation of the variability of bregma (in vivo and ex vivo CT). Visual comparison of the MRA-TOF vasculature (major vessels) in individual animals indicates minimal variability. This could indicate the feasibility to use the vasculature template as a representative of the population for more precise planning of stereotactic surgeries like the DBS application. To validate this objective, we used the multi-modality information to plan stereotactic surgery using a potential risk path and an estimated safe trajectory (figure 1, E). From these experiments we know that for optimal planning of stereotactic surgery (e.g. electrode implantation), coregistration of MRI/MRA images with CT data on bregma and lambda reference points on the skull is highly important. Therefore, information (CT) on the variability of bregma will be combined with the vasculature data in the atlas.
Figure 1: (A) Coregistration between MRI (top), MRA (middle) and MRI-MRA (bottom, red overlay). (B) 3D volume rendering of MRA images of the same 2 animals. Arrows indicate regions of similarity (pink) and variability (blue). (C) CT images providing information on bregma and lambda reference points and vasculature for validation of MRA. (D) Surgery planning in 2D (Paxinos) atlas space with possible trajectories at different angles. (E, a-c) Average vasculature images in atlas (Paxinos) space: the maximum-intensity MRA-TOF vasculature information constructed from 4 consecutive slices at the same location, with * marking the green target region. (d) Risk assessment: the averaged sum-of-pixel intensities along each of the trajectories determine the associated risk of the electrode traversing through hyperintense pixels (=vasculature in TOF-MRI). Based on the averaged sum-of-pixel intensities along each of the trajectories, the paths are color-coded: high (red) – medium (cyan) – lower risk (green) (e).

Conclusion
For pre-clinical stereotactic surgeries, optimal trajectory planning is valuable input to avoid injuring vasculature. We address this issue by investigating the intra-strain variability in cerebral vasculature combined with the position of bregma for the Wistar rat strain. Provided that the intra-strain variability is small, a probabilistic vasculature atlas for the given strain could form a reference for brain surgery planning. We use image registration to spatially align multi-modal anatomical and vasculature information from age and weight matched animals from the same strain to the common reference frame in a standard atlas space. Excluding the acquisition effects, we observe consistency with vasculature for the test group. We demonstrate the methodology for building a vasculature template and subsequent use for planning neuromodulation experiments. We are currently extending this study with more animals, where validation of the proposed safe and risk paths through stereotactic surgeries,
together with validation with CTA using contrast agents, are pursued not only for electrode insertion but also for the injection of cells and viral vectors.
References: