ASSESSMENT OF VARIABILITY WITHIN SMALL ANIMAL STEREOTACTIC NEUROSURGERY AND INCLUSION OF VASCULATURE INFORMATION FOR PLANNING NEURO-ANATOMICAL SURGERY IN THE RODENT BRAIN

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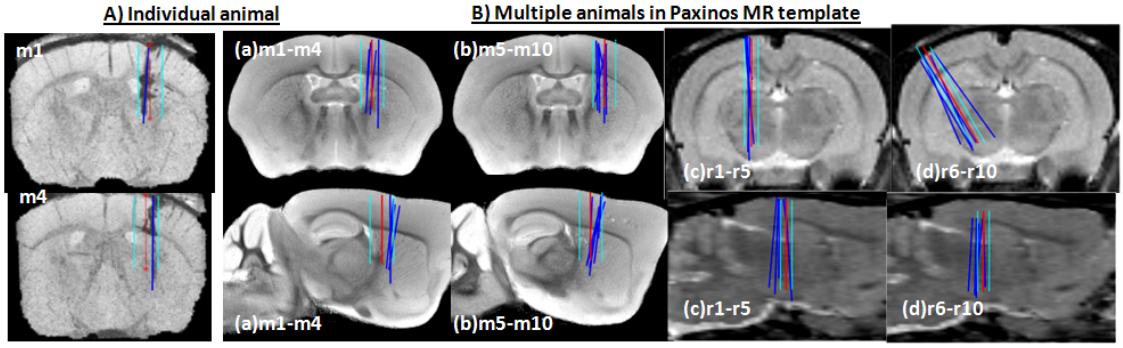
INTRODUCTION: Background: Neuro-degeneration research using small animal models, often involve stereotactic intervention to deliver cells or contrast agents (1) to specific anatomical targets. Likewise, injection of neuro toxins to induce neuro-degeneration and deep brain stimulation therapy (DBS) of neuro anatomical targets (2,3) in neuromodulation experiments demands stereotactic neuro-anatomical surgery. Such needle or electrode insertions are typically done by hand with help of stereotactic positioning device and 2D anatomical atlases to help locate the target injection location with reference to anatomical landmarks (e.g. bregma). The accuracy of needle tip to reach pre-defined anatomical target determines the success of the neuro-surgical interventions. When in-accurate, the animal experiments can be inconclusive or misleading, thus increasing the number of animals, the cost, and the duration of the study (4). Still, the use of image and robotic guidance for achieving higher accuracy remains limited. Problem: With only a simple representative 2D anatomical information of rodent brain, in-accuracies in targeting continue to occur either during the stereotactic surgery (due to limitations of stereotactic device, operator variability, errors in locating bregma reference, inter-and intra-strain variability), or while identifying optimal trajectory (e.g. missing vasculature information). In such cases, only ex vivo 2D histology can reveal offsets in the needle reaching the target site. Moreover, precise targeting is necessary to avoid morbidity of the animal due to systemic deleterious effects like injury to vasculature. Solution: Post-operative image based follow-up may help identify such confounding effects at a earlier stage, much before the animals are subjected to behavioral studies, cell tracking etc. With high resolution in vivo small animal imaging, and dedicated image analysis methods, we may not only be able to identify major in-accuracies within interventions, but also help identify optimal needle trajectory with minimum deleterious effects (avoiding injury to blood vessels). In this regard, based on *in vivo* MR images we first record the existing variability in stereotactic surgery within cell labeling and neuromodulation applications. Multi-modal imaging (micro-MRI, micro-CT), and advanced image analysis routines for spatial normalization to Paxinos atlas enables 3D assessment of both variability in injection trajectories and the evaluation of injury induced by the needle trajectories.

METHODS: 10 C57BL/6 mice (m1-m10) and 10 female wistar rats (r1-r10) were used in this study. Mice underwent stereotactic surgery for labeling stem cells niche through stereotactic viral vector injections (lenti-viral (LV), or adeno-associate vector (AAV) or PBS) in the right and/or left striata or sub-ventricular zone (SVZ). The Wistar rats underwent unilateral stereotactic sham injection of PBS into the substantia nigra (SNc), the potential region of interest in Parkinson's model. Based on previous studies, coordinates of the above anatomical targets (i.e. SVZ, striatum, SNc) were defined using corresponding Paxinos atlases (4). Our animals were age and weight matched compared to Paxinos atlas. For both the mice and rat animal groups, T_2*-w 3D MRI images were acquired on a 9.4T small animal scanner (Bruker Biospin, Ettlingen, Germany). T_2*-w images reveal hypo intense contrast along the needle trajectory in both the vector injected mice and the sham injected rats. In addition, for the sub-group of rats pre-operative micro-CT images (35 µm isotropic in a SkyScan1076 *in vivo* µCT scanner) were acquired for visualizing the bregma-lambda sutures. Also, to assess the risk of injury to vasculature along the needle trajectory, 2D multi-slice MR angiography (MRA) time-of-flight images were also acquired for the same group. The hypo intense injection tracts with MR images are first manually segmented, and the longitudinal axis is determined by principal component analysis (PCA). Comparison of similar anatomical target sites, injection trajectories across animals is facilitated by spatial normalization of individual study images to common-reference template. Using the image analysis routines described in (3), the multi-modal information (brain, skull, vasculature) are spatially normalized (rigid) to respective standard MR anatomical templates in Paxinos space (7,8), for assessing the variability in stereotactic surgery. Needle trajectories of different animals (in blue) as obtained from PCA are over laid on the MR templates, along with planned trajectory (in red) with offset at ±0.5 mm (in cyan).



RESULTS: Fig. A illustrates the coronal view of representative animals, with the hypointense needle tract and the corresponding trajectory obtained from PCA (in blue), along with intended trajectory (in red) and offset (in cyan). Similar trajectories in atlas space along coronal and sagital planes for animals with different anatomical targets are shown in Fig. B (a) SVZ (90º dorso-ventral from target, m1-m4) (b) Striatum (90º, m5-m10) (c) SNc (90º r1-r5) (d) (30º r6-r10). In all cases, although the needle tip remains offset from the intended target site (red), the medio-lateral offset (i.e at entry point) appear lower in comparison to anteroposteriror offsets (angle). Variability in the dorso-ventral directtion could be attributed to difficult in accurately detecting the end of needle tip. But the systematic error in medio-lateral direction resulting in oblique trajectories, may partially be explained by either the subtle head movement during the surgery or variation in brain size in comparison to Paxinos. To be specific, we are investigating the offsets in bregma landmark identified from the CT, alongside that of the paxinos atlas (data not shown). By overlaying the post-operative anatomical image with the needle trajectory and the segmentation of post-operative hemorrhage illustrates the deleterious injury to vasculature (not shown). Considering the above variability and the necessity to include the vasculature information for planning, we are extending this work further to assess the risk associated with a injection trajectory, and possibly identify optimal trajectory with due validation using CT angiography and histology.

CONCLUSIONS:. Errors in 2D representative atlas based small animal stereotactic surgery is investigated. By using multi-modal information of brain, skull and vasculature we identify the source of variability and as well the deleterious effect of in-accurate stereotactic surgery. Although the results are preliminary, the findings has gained interest among neuro-scientists who see the opportunity to include the vasculature information for planning stereotactic investigations in small animal models. In future, optimal planning and image-based follow up of stereotactic surgeries in small animals may help exclude outlier animals at an much earlier stage, and possibly may reduce the number of animals used for such experiments.

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