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Population based MRI and DTI templates of the adult ferret brain and tools for voxelwise analysis[☆]

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ABSTRACT

Non-invasive imaging has the potential to play a crucial role in the characterization and translation of experimental animal models to investigate human brain development and disorders, especially when employed to study animal models that more accurately represent features of human neuroanatomy. The purpose of this study was to build and make available MRI and DTI templates and analysis tools for the ferret brain as the ferret is a well-suited species for pre-clinical MRI studies with folded cortical surface, relatively high white matter volume and body dimensions that allow imaging with pre-clinical MRI scanners. Four ferret brain templates were built in this study – *in-vivo* MRI and DTI and *ex-vivo* MRI and DTI – using brain images across many ferrets and region of interest (ROI) masks corresponding to established ferret neuroanatomy were generated by semi-automatic and manual segmentation. The templates and ROI masks were used to create a web-based ferret brain viewing software for browsing the MRI and DTI volumes with annotations based on the ROI masks. A second objective of this study was to provide a careful description of the imaging methods used for acquisition, processing, registration and template building and to demonstrate several voxelwise analysis methods including Jacobian analysis of morphometry differences between the female and male brain and bias-free identification of DTI abnormalities in an injured ferret brain. The templates, tools and methodological optimization presented in this study are intended to advance non-invasive imaging approaches for human-similar animal species that will enable the use of pre-clinical MRI studies for understanding and treating brain disorders.

Introduction

Non-invasive brain imaging and especially MRI has become increasingly employed to characterize brain development and disorders in animal models with the goal to identify common markers and target features of experimental models that overlap with imaging findings in humans. Furthermore, improvements to the quality of pre-clinical image acquisition and the evolution of sophisticated image processing, modeling and analysis tools are promising for providing outcome measures in basic brain research and in the development of therapies. An additional avenue to improve the effectiveness of pre-clinical studies is the extension of experimental models to species with brains that more closely resemble the human brain and may offer a more relevant system for understanding features of the normal and disordered brain. This is especially evident for approaches that are sensitive to tissue features that are absent in rodent models such as a folded cortical geometry or complex white matter systems. The combination of appropriate animal models with outcome measures that also apply to human subjects and patients has the potential to identify and target the features most relevant for understanding and treating human disorders.

The ferret, of the mustelid genus, has been identified as a potentially informative model system in neuroscience research as the ferret brain cortex is folded, or gyrencephalic, and contains a relatively greater white mater volume than rodent species (Fox and Marini, 2014). The primary brain research application for the ferret has been for the study of cortical development as the ferret is altricial and born before gyrification of the cortex allowing for postnatal investigation of

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the mechanisms that underlie cortical folding (Empie et al., 2015; Jackson et al., 1989; Noctor et al., 1997; Poluch and Juliano, 2015). A number of studies of acquired neurologic disorders including cortical dysplasia (Poluch and Juliano, 2015; Poluch et al., 2008; Abbah et al., 2014), epilepsy (Youngblood et al., 2015) and traumatic brain injury (Hutchinson et al., 2016; Schwerin et al., 2014) have taken advantage of the distinct neuroanatomical features of the ferret brain. Several basic neuroscience research approaches have also successfully employed the ferret brain to make important advances such as understanding cortical neurophysiology by slice recordings (Shu et al., 2003; Sanchez-Vives and McCormick, 2000) and mapping of cortical neural activity by in-vivo optical imaging (Schwartz and Bonhoeffer, 2001). Efforts toward mapping the ferret genome (Peng et al., 2014) and the development genetic tools in the ferret (Kou et al., 2015) demonstrate the potential for generating meaningful experimental models that are highly relevant to human neurologic disorders.

MRI methods are well suited to the study of the ferret brain not only for their non-invasive characterization of the whole brain and in-vivo applications, but also for the rich foundation of quantitative tools that have been developed to study the anatomy, morphology and microstructure of the human brain. These may be readily adapted for use in the ferret, which is one of the only gyrencephalic animals with body dimensions that allow imaging in specialized pre-clinical scanners. Several MRI studies of the ferret brain have been performed to characterize the anatomical changes during developmental gyrification by conventional MRI approaches (Neal et al., 2007) as well as more advanced quantitative morphological analysis (Knutsen et al., 2010; Knutsen et al., 2013) and diffusion tensor imaging (DTI) (Barnette et al., 2009; Kroenke et al., 2009; Jespersen et al., 2012). These studies have provided an important basis to study the effects of developmental injury or disorder and several initial studies of enucleation (Bock et al., 2010; Bock et al., 2012) and chronic hypoxia (Tao et al., 2012) demonstrate the utility of quantitative MRI for characterizing the effects of disruptions in normal brain development. MRI studies in the adult ferret may also be beneficial for disorders that are influenced by brain anatomy and white matter content such as traumatic brain injury, for which several T2 and DTI abnormalities have been identified acutely following injury (Hutchinson et al., 2016).

Increasingly, advanced MRI analysis approaches have made use of voxelwise techniques that depend on the accurate warping of multiple individual brains into a common template space and subsequent statistical analysis at each voxel as is common for fMRI and morphometry studies. Advances in registration algorithms for structural MRI (Avants et al., 2008) have improved reliable warping of neuroanatomical regions across brains into a common space and provided template building methods to generate average brain volumes that preserve anatomical boundaries and edges. Recently, the ability of correcting EPI distortions in diffusion weighted images and tensor-based approaches for registration of DTI data (Irfanoglu et al., 2016; Zhang et al., 2006) have made possible template generation techniques for DTI that are morphologically faithful and high quality. This in turn has allowed improved voxelwise analysis and morphometric studies to be performed in DTI data taking advantage of the intrinsic anatomical landmarks that DTI is able to detect. Migration of advanced registration and voxelwise analysis methods into pre-clinical MRI studies provides a useful methodological basis for quantitative analysis methods that is high-throughput, unbiased and systematic. In light of these advantages, brain templates have been generated for several species including rodents (Aggarwal et al., 2009; Dorr et al., 2008; Papp et al., 2014; Johnson et al., 2010), nonhuman primates (Black et al., 2001; Black et al., 2001; Frey et al., 2011; McLaren et al., 2009; Calabrese et al., 2015; Hikishima et al., 2011), sheep (Nitzsche et al., 2015) and zebrafish (Ullmann et al., 2010) among others. These normative templates have been used for the consistent registration of MRI and DTI data to a common space, which allows anatomical localization of structures, quantitative analysis of morphological features and voxelwise statistical analysis of quantitative metrics. As the quality of pre-clinical MRI data improves and the ability of increasingly sophisticated registration algorithms to faithfully coregister image data is realized, voxelwise MRI tools will provide a new perspective to understand neuroanatomical, physiological and structural features of brain tissue in animal model studies.

The objectives of this study were to generate and make available high quality population based templates for in-vivo and ex-vivo structural and diffusion tensor MRI of the ferret brain and to optimize MRI processing pipelines, registration tools and voxelwise analysis for ferret brain images. Diffeomorphic scalar and tensor based registration techniques were implemented to warp multiple structural and DTI images of each modality to a common space resulting in four templates: *in-vivo* MRI, *in-vivo* DTI, *ex-vivo* MRI and *ex-vivo* DTI. Based on these and with reference to known ferret neuroanatomy (Fox and Marini, 2014), the brain volume was segmented into 48 regions of interest (ROIs). The resulting template and ROI masks were then incorporated into a web-based image viewing software for visualization and annotated with neuroanatomical information using the ROI masks. In addition to providing templates, ROI tools and a visualization software, this study applied these tools to optimize and demonstrate several template based analysis approaches. First, the variability of DTI and morphometry metrics across normal ferret brains were evaluated using ROI analysis in template space. Next, as an example of potential applications of the use of the template for single subject analysis, voxelwise morphometric analysis was used to show differences between a single female ferret brain and the male ferret template. Finally, tensor-based registration and warping of an injured ferret brain to the DTI template was used for voxelwise detection of post-traumatic DTI abnormalities. The primary goal of this work is to provide a methodological framework for building a set of high quality *in-vivo* and *ex-vivo* MRI templates the ferret brain. We hope that the use of these templates in conjunction with the ROI and voxelwise analysis techniques we propose will facilitate research that will advance our understanding of brain development and brain disorders for which the ferret is an ideal model.

Methods

MRI and DTI acquisition

All animals were housed and treated in accordance with national guidelines (i.e. the NIH guide) and adhering to an animal study protocol that was approved by the Uniformed Services University of Health Sciences institutional animal care and use committee. It should be noted that a subset of the raw data included in this study (n=10 in-vivo MRI scans) were previously used in a separate study of brain injury to provide baseline and normative MRI and DTI values (Hutchinson et al., 2016). Adult male ferrets (*Mustela putorius furo*) were used in this study with an age range of 5–10 months and weight range of 1.3–2.4 kg.

In-vivo MRI and DTI acquisition

Ferrets underwent in-vivo MRI scanning to obtain structural images (n=26) and DTI data sets (n=12). During each MRI session the ferret was anesthetized with inhaled isoflurane (5.0% induction, 1–3% maintenance) and warmed by a circulating water heating pad. Anesthesia level and water pad temperature were adjusted according to physiological monitoring of temperature and respiration rate.

In-vivo ferret imaging was conducted using a horizontal bore Bruker 7T MRI system with either a 6 cm (Doty, Columbia, SC) or 8.6 cm (Bruker, Billerica, MA) quadrature volume coil for transmit and receive and ParaVision software to acquire the structural T2 weighted MRI (versions 5.1, n=12 and 6.0, n=14) and DTI (Paravision version 6.0 only) as described below:

T2 weighted MRI. A multi-echo Rapid Acquisition with Relaxation