Automated Atlas-Driven ROI Specification in the Rat with Application to phMRI

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Introduction: Interpretation and analysis of rat brain functional and pharmacological MR studies is often performed with reference to a standard anatomical atlas, such as that of Paxinos and Watson [1]. However, ROI analyses typically rely on operator-dependent hand-drawn contours. This approach is compromised by the fact that MRI functional slices are often thicker (1-2mm) than those in the atlas and thus cover several atlas slices. Also, larger anatomical structures can span several MRI slices and present complicated 3D forms. Recently, digitised versions of selected rat brain structures have been created and applied to Mn^{2+} tract tracing and PET imaging [2,3]. Here, we report a 3-D digital representation of rat brain anatomical structures from the Paxinos atlas, to which a probabilistic MRI template set is spatially co-registered, enabling MRI rat brain studies to be spatially normalised into atlas stereotactic coordinates. The full 3D extent of the atlas structures is then directly available to the MRI data, enabling rapid and unbiased (operator-independent) anatomical hypothesis-based analyses.

Methods: ***Digitised atlas and MRI template:*** External (brain contour) and internal contours were extracted from electronic versions of the 78 coronal slices in ref. [1] and reconstructed as 3-D images. The more detailed right-hand side structures were used and reflected about the midline to provide a symmetric database while maintaining ipsilateral and contralateral distinction to allow interrogation of left/right differences. Images were rebinned to matrix size 256x256x214 to enforce an equal inter-slice spacing of 0.1mm. A rat brain MRI template set (created from T_2w RARE anatomical images of *N=97* Sprague-Dawley rats, encompassing co-registered anatomical composite and probabilistic tissue class distribution maps) was co-registered with the digitised atlas by spatially normalising the MRI brain tissue class map to a smoothed version of the external contour atlas image; the transformation parameters were then applied to the other images in the template set. ***Evaluation and use:*** Positions of easily identifiable internal and external pointlike structures were examined to confirm correspondence between atlas and MRI template images. For automated ROI extraction, selected atlas structures define weighted 3-D image masks enabling mean time courses to be exported. (At the user's discretion, MRI pixels residing entirely within the 3-D structure can be retained by thresholding the mask at 100%, or a "partial volume" pixel weighting can be used by retaining the full fractional mask). We also defined larger, composite, ROIs as unions of smaller nuclei for a number of brain areas for which the individual component nuclei may be too small to be reliably measured at typical f/phMRI acquisition resolutions. These included mPFC, hypothalamus, Amygdala and several thalamic areas. This automated ROI approach was applied to several well-characterized pharmacological MRI (phMRI) data sets including selective dopamine D_3 antagonist modulation of amphetamine response (*N=16*) [4].

Results: Co-registration of the MRI template to the atlas is illustrated in Fig. 1(a). Overall, registration was good, although with some slight differences remaining at the ventral brain edge, reflecting slight MRI signal dropout near the ear canals ($\sim z_{bregma}$ - 6 mm) and deformations between *ex vivo* and *in vivo* brains. CSF also occupied more space in the MRI images compared with the atlas slices. Subjects from the evaluation phMRI studies were normalized into the digital atlas space via the stereotactic MRI template. ROIs were then selected as a list of atlas structures, and mean time courses exported from the same set of pixels for each animal. Individual exported ROI time courses were then processed by a General Linear Model analysis and were also available for visualization. Fig. 1(b,c) illustrate image overlays reconstructed from the ROI analysis for visual presentation analogous to standard pixel-wise statistical overlays. It is important to note that the intensity of each 3-D ROI reflects the average of the whole structure and is now independent of the spatial position at which the 3-D data set is sliced for display.

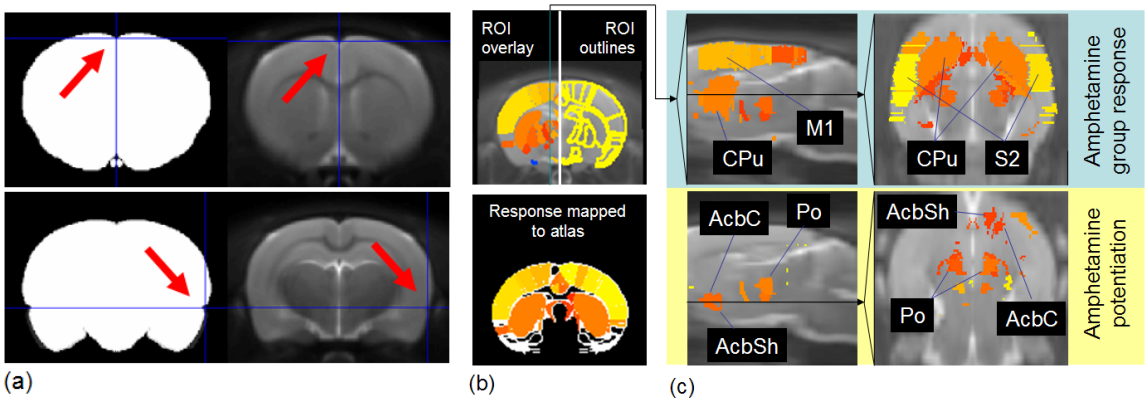

Figure 1*: (a) Co-registration of atlas external brain contours with MRI template. Corresponding points at the mediodorsal boundary and rhinal fissure are indicated. (b) Atlas-based analysis of phMRI studies can be visualised as anatomical structure response overlays on anatomical images (top), or just as coloured atlas structures (bottom), here illustrated for amphetamine. (c) Sagittal and horizontal slices through thresholded group amphetamine response (top) or modulation by D_3 antagonist (bottom). In (b) and (c) the colour of each "patch" represents the mean group rCBV response in the corresponding atlas structure.*

Discussion: The approach presented here facilitates ROI analysis of rat brain data, particularly in phMRI studies where activation is often distributed through the brain and hypotheses related to the involvement specific brain structures are tested. Normalisation to a common template itself provides the advantages of using the same ROI (however defined) for each subject and the extraction of stereotactic co-ordinates for features of interest in the MRI data (cf. ref. [5]). The ability to use atlas structures as ROIs brings the advantages of speed and facility with which ROI time courses or summary statistics can be selected and extracted for further analysis avoiding subjective operator-defined limits. Moreover, the full 3-D extent of the structure is retained and symmetric right/left ROIs are available for laterality tests. Alternatively, a structure of interest can be used to focus a pixel-wise analysis in a "small volume" approach analogous to fMRI [6]. However, the accuracy and validity of the ROI assignments is limited by residual differences between the MRI template and the atlas (mostly along the ventral edge and around ventricles) as well as by the MRI acquisition resolution, residual inter-animal spatial variability (for group studies) and the spatial normalisation algorithms. As such, performance is most reliable with judicious selection of structures of an appropriate spatial scale – small nuclei may be below the intrinsic resolution of the MRI acquisition and here our composite ROIs provide a useful option. Also, any heterogeneous functional responses in larger structures (such as the CPu) are no longer resolvable if the structure is used to define an ROI. As functional divisions in such structures become established [7] they could provide a useful extension to the current work. Finally, current registration and normalisation algorithms have tended to be optimised for human data, and evaluation for rat studies is ongoing.

References: [1] Paxinos G and Watson C (1998) *The Rat Brain in Stereotactic coordinates*, 4th edn (Elsevier). [2] Leergaard TB *et al.* (2003) *NeuroImage* **20** 1591. [3] Rubins DJ *et al.* (2003) *NeuroImage* **20** 2100. [4] Schwarz AJ *et al.* (2004) *Synapse* **54**(1) 1. [5] Schweinhardt P *et al.* 2003 *J. Neurosci. Meth.* **129**(2) 105 [6] Maldjian JA *et al.* (2003) *NeuroImage* **19** 1233. [7] Voorn P *et al.* (2004) Trends Neurosci. **27**(8) 468.