Training induced volume changes seen by structural MRI correlate with neuronal process remodelling

J. P. Lerch[1], A. P. Yiu[2], A. Martinez-Cabal[2], T. Pekar[2], V. D. Bohbot[3], P. Frankland[2], R. M. Henkelman[1], S. A. Josselyn[2], and J. G. Sled[1]
[1]Mouse Imaging Centre, Hospital for Sick Children, Toronto, Ontario, Canada, [2]Program in Neuroscience and Mental Health, Hospital for Sick Children, Toronto, Ontario, Canada, [3]Douglas, Department of Psychiatry, McGill University, Montreal, Quebec, Canada

Introduction. Several studies have recently shown training induced brain shape changes detectable by structural MRI [1-2]. Previously, we showed that five days of training using a maze is sufficient to cause region specific local volume changes in mice detectable by high-resolution MRI with the aid of image registration software [3]. Here we repeated our first experiment [3] and further correlated the MRI derived volume changes with immunohistochemical stains in order to account for the training induced volume differences. We examined four possible cellular hypotheses to account for the volume changes: (1) alterations in neuron numbers/sizes; (2) alterations in astrocyte numbers/sizes; (3) increased neurogenesis/survival of new neurons; and (4) remodelling of neuronal processes. Slices through the hippocampus and striatum were thus stained for NeuN, GFAP, double-cortin, and GAP-43 respectively.

Mice and training. Thirty-six 2.5 month old C57Bl6/129Sv mice were divided into four groups of 9 mice each. 3 groups were trained for five days on differing variants of the Morris Water Maze (MWM); the *spatial MWM* group learnt to find a hidden platform using distal spatial landmarks; the *non-spatial cued MWM* group learnt to find a flagged platform without the aid of distal landmarks; and the *spatial cued MWM* group learnt to find a flagged platform but also had access to distal spatial landmarks. A fourth group of *control* mice were not trained on the MWM.

Imaging and analysis. Ten days after training the mice were sacrificed, anaesthetized (Ketamine, 100 mg/kg and Rompun, 20 mg/kg, ip), perfused through the heart with phosphate buffered saline (PBS, 30 ml, pH 7.4, 25˚C) followed by paraformaldehyde (4% PFA; 30 mL, iced) plus 2mM ProHance in PBS. Bodies, along with the skin, lower jaw, ears and the cartilaginous nose tip were removed. The remaining skull structures containing the brain were allowed to postfix in 4% PFA plus 2 mM ProHance at 4˚C for 12 hours. The skulls are then transferred to solution containing 1X PBS + 0.02% sodium azide + 2mM ProHance for 4 days at 15 °C. A multi-channel 7.0 Tesla MRI scanner (Varian Inc., Palo Alto, CA) with a 6 cm inner bore diameter insert gradient was used to acquire anatomical images of brains within skulls. Prior to imaging, the samples were removed from the contrast agent solution, blotted and placed into plastic tubes (13 mm diam) filled with a proton-free susceptibility-matching fluid (Fluorinert FC-77, 3M Corp., St. Paul, MN). Three custom-built, solenoid coils (14 mm diam, 18.3 cm in length) with over wound ends were used to image three brains in parallel. Parameters used in the scans were optimized for grey/white matter contrast: a T2-weighted, 3D fast spin-echo sequence with 6 echoes, with TR/TE= 325/32 ms, four averages, field-of-view 14 x 14 x 25 mm^3 and matrix size = 432 x 432 x 780 giving an image with 32 µm isotropic voxels. Total imaging time was 11.3 hours. The resulting images underwent a non-linear deformation algorithm to bring all brains into exact correspondence, and the Jacobian determinants of the deformation fields were used to measure local volume change [4].

Immunohistochemistry. Following MR imaging, the 36 fixed brains were removed from the skull and paraffin embedded. 4-8 consecutive 5µm coronal slices per stain were taken through the hippocampus/dentate gyrus as well as the striatum. Sections were stained with either mouse anti-NeuN (1:100, Chemicon), rabbit anti-GAP-43 (1:100, Abcam), or rabbit anit-GFAP (1:100, SIGNET). A further 10 5µm coronal slices were acquired through the dentate gyrus and stained for DCX (1:1000, Cell Signalling). For NeuN, GFAP, and GAP-43 quantification proceeded by first digitizing the slides using a slide scanner at 40X resolution. The dentate gyrus, CA1 and CA3 regions of the hippocampus, and striatum were then manually outlined on the digitized slides while blinded to their provenance. A neural net classifier [5] was then trained to discriminate between active stain (NeuN, GFAP, or GAP-43), counter-stain (DAPI), and background, and the same classification rules applied to every single slide of the same stain henceforth to ensure completely unbiased, quantitative results. The proportion of pixels stained by the active stain was the measure used for subsequent analyses. For the DCX stain the total number of immunoreactive cells per slice was quantified by a blind observer and the total surface of the granular cell layer of the hippocampus measured, therefore reporting the density of cells per tissue area.

Results. The MRI results replicated our previous study and showed relative enlargements in the hippocampus for the *spatial MWM* group whereas the *non-spatial cued MWM* group featured growth in the striatum. We detected a significant positive correlation between GAP-43 and structure volume ($p=0.0002$), but found no correlation between MR volume and any other IHC measure (double-cortin: $p=0.4$; NeuN: $p=0.89$; GFAP: $p=0.59$;).

Discussion. GAP-43 has been previously implicated in memory storage, and by binding to actin and fodrin is believed to cause morphological change at presynaptic terminals through protein-protein interactions [6]. We can thus conclude that, among the hypotheses tested, the largest explanatory factor for learning induced MRI detectable volume changes is the remodelling of neuronal processes.

References. [1] Scholz et al., Nat. Neurosci, 2009. [2] Draganski et al., Nature, vol 427 (6972), 2004. [3] Lerch et al., ISMRM, 2008. [4] Spring et al., NeuroImage, vol 35 (4), 2007. [5] Zijdenbos et al., IEEE TMI, vol 21 (10), 2002. [6] Routtenberg et al., PNAS, vol 97 (13), 2000.