Longitudinal Effects of Irradiation and Voluntary Exercise on Hippocampal Gray Matter Loss

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Introduction
From studies in humans and rodents it is known that voluntary exercise is related to an increase of hippocampal gray matter assessed with MRI techniques [1, 2]. It is however not clear whether these findings result from the known hippocampal plasticity in the dentate gyrus through voluntary exercise [3, 4]. In an attempt to approach the underlying mechanisms of exercise induced gray matter increase, we performed hippocampal irradiation - which effectively inhibits hippocampal neurogenesis [5] - in one half of afterwards voluntary wheel-running mice. Structural profiles of the whole brain before and after the exercise period were assessed using in vivo Voxel-Based Morphometry (VBM) on a 9.4T animal scanner equipped with a cryogenic mouse-brain-coil before and after the exercise period.

Methods
45 C57BL/6N male mice were assigned to one of the treatment groups: hippocampal irradiation (IR) with 10 Gray in total or sham irradiation (SR) applied during a ketamine anesthesia. All mice were single-housed in Macrolon Type III cages, on a 12-h light-dark cycle. At the age of 9 weeks, 10 IR (IR-runner = IRR) and 10 SR (SR-runner = SRR) mice were given free access to a running wheel (diameter 11.5 cm), 10 IR (IR-sedentary = IRS) and 10 SR (SR-sedentary = SRS) had access to a blocked running wheel. After 6 to 8 weeks of voluntary wheel running (~9-10 km/night) all mice were investigated with structural MRI. Five animals of each group were additionally scanned before the period of exercise. 

T2-weighted high resolution 3D-morphometric data were acquired using a RARE-Sequence with a resolution of 78x78x156 μm at TE = 50 ms. The tissue segmentation of the MR images into gray matter (GM), white matter and CSF was performed in several steps, including brain extraction and group-specific a priori template creation with DARTEL using a 2-step subsequential individual segmentation with SPM8 described elsewhere [2] (Fig. 1). The segmented and normalized-modified tissue class images were smoothed with 0.4 mm Gaussian kernel and analyzed voxelwise with second level models over the whole brain (cross-sectional: two-sample t-tests; longitudinal: full factorial model with repeated measurements) [6].

Results
Cross-sectional comparison of the VBM whole brain results after the treatment period revealed a significant cluster (p<0.001 uncorrected, min. 10 voxels) of increased hippocampal gray matter (GM) in SRR compared to SRS (Fig. 2A). Hippocampal GM was as well higher in SRR compared to IRR (Fig. 2B). In SRS the GM volume was higher compared to IRS. No significant differences were seen comparing IRR and IRS. The longitudinal comparison showed an age related GM loss in the hippocampal area over all groups (Fig. 3D). This age related GM loss was significantly reduced in the SRR mice (Fig. 3B) but not in the IRR mice (Fig. 3E).

Discussion
These results corroborated our earlier findings [2] and findings in human MR studies [7, 8], where increased hippocampal volume was associated with voluntary exercise. We can now specify this increase of hippocampal volume as a decelerated gray matter loss over time in voluntary exercising mice. We found no increase of hippocampal volume in voluntary running mice after hippocampal irradiation, an intervention which effectively inhibits hippocampal neurogenesis [5]. Moreover, non-exercising mice had higher hippocampal GM compared to irradiated non-exercising mice. Running had no effect on hippocampal GM in irradiated mice. These findings suggest an association of the plastic changes through voluntary wheel running and VBM-detected gray matter volume, which seem to be inhibited through irradiation.

References

Fig. 1: Dartel group apriori template for gray matter (yellow), white matter (red) and cerebrospinal fluid (white).

Fig. 2: Cross-sectional gray matter differences between non-radiated runners (SRR) and sedentary (SRS) (A) and SRR and radiated runners (IRR) (B) after exercise (p < 0.001 uncor.)

Fig. 3: Longitudinal gray matter (GM)-changes showing the GM-loss in non-radiated sedentary (SRS) (A), non-radiated runner (SRR) (B), Higher GM loss in SRS compared to SRR (C). Longitudinal loss of GM in all mice (D). GM loss in irradiated runner (IRR) (E) and higher GM loss in IRR compared to SRR (F).