Structural brain changes after rotarod training in mice

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Target Audience

This study is targeted at Neuroscientists interested in experience-dependent structural brain plasticity.

Purpose

This study investigates experience-related structural plasticity in response to a standard motor training task in adult mice. To cover a wider array of structural changes we test for volume changes based on T2-weighted images and microstructural changes based on maps of fractional anisotropy (FA) derived from diffusion-weighted MRI. Firstly, this study aims to establish a standard motor skill task that reliably elicits structural changes in adult mice. Here we chose the well-tested rotarod task [1]. Secondly, this study aims to identify a mouse strain that might serve as a background for genetic modification necessary to get at the cause of the cellular changes. Here we use C57BL/B6 mice, the most widely used background strain for genetically modified mice.

Methods

Behavioural Protocol: A total of 48 mice (C57BL/B6, 27 female), age 16 weeks participated. 25 mice (11 female) were trained on the accelerating rotarod (4 to 40 rpm over 5 min) ten times a day for eight days (80 trials). Mice were placed on a rotating drum and the time was measured until they fell off or the maximum trial length was reached. Controls were handled 2 min a day. *Image acquisition*: Brains were fixated and scanned within skulls on a 7 T, 40 cm diameter bore magnet (Varian Inc. Palo Alto, CA). A T2-weighted sequence was used to obtain images for deformation based morphometry (3DFSE, TR=2000 ms, echo train length=6, TEeff=42 ms, FOV=25×28×14 mm, matrix size=450×504×250, time=12 h) and a diffusion-weighted FSE sequence was used to obtain FA maps (30 directions, b=1917 s/mm², 5 b0-images, TR = 325 ms, echo train length=6, first TE=30 ms, TE=6 ms for remaining 5 echoes, 2 averages, FOV=25×14×14 mm, matrix size=192×108×108, time=14 h). Brains were distortion corrected and aligned using nonlinear image registration with iterative template refinement [2,3]. All brains were rigidly registered towards a pre-existing mouse brain average. To test specifically for bilateral changes the left-right flipped version of each rigidly aligned brain was included in the following (non-linear) registration steps. FA maps obtained with FSL's dtifit were registered separately. *Statistics*: Group differences between trained and control mice and correlations with average latency within trained mice were estimated using linear mixed effects modelling at each voxel with gender as a co-variate. Hemisphere was included as a random effect to test for bilateral changes/correlations. Clusters were localize using a number of atlases [4,5,6].

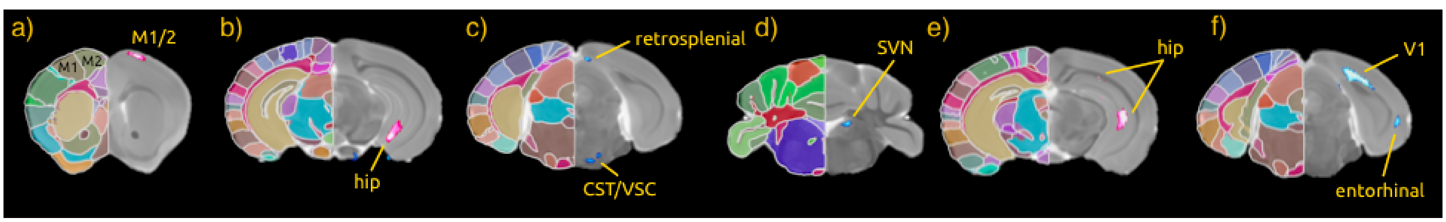

Fig 1: Regions where volume or FA either correlated with rotarod performance or changed after rotarod training. Note, all clusters are bilateral as per analysis, and can thus be displayed on one hemisphere. (increases in volume/FA and pos. corr with latency are red)

Results

Behaviour: When comparing rotarod latency between the first and last day, mice improved significantly ($p<0.001$). Mice reached their maximum performance on average at trial 64 (±12). *MRI*: The voxel-wise tests for differences between trained mice and controls and correlations with average latency revealed three distinct sets of brain regions. Firstly, we found motor and balance-related regions. M1/M2 volume correlates with behaviour (a). The cerebellum (not shown), the vestibular nuclei (SVN/MVN) (d), and the cortico-spinal/spino-cerebellar tracts (CST,VSCT) (c) change in volume after training. FA in the visual cortex correlates with behaviour and might be related to balancing (f). Secondly, we found learning related regions, not specific to motor learning such as the hippocampus. Here we found changes in volume (b) and FA (not shown) and FA-behaviour correlations (e). Thirdly, we found regions possibly related to anxiety and stress experienced by mice suspended on top of the rotarod, such as thalamic nuclei and the hypothalamus (not shown).

Conclusion

The rotarod is a standard test that taxes motor coordination and balance. By using two MR-derived measures, volume and FA, we were able to show a number of motor and vestibular regions related to training and task performance. Interestingly, the hippocampus, a neuronaly active region, that is not directly related to motor skill learning is both changing and behaviourally relevant. Finally, changes in (hypo)thalamic areas might indicate stress/anxiety related aspects of the rotarod task. In conclusion, by using complementary measures of microstructure and volume this study reveals the substantial structural reorganization of the adult mouse brain following only a relatively brief period of motor training. This study paves the way for studies of mice that are genetically engineered to allow the investigation of the cause of these structural changes.

References

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