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Rotarod training in mice is associated with changes in brain structure observable with multimodal MRI

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ABSTRACT

The brain has been shown to remain structurally plastic even throughout adulthood. However, little is known how motor-skill training affects different MRI modalities in the adult mouse brain. The aim of this study is to investigate whether rotarod training, a simple motor training task taken from the standard test battery, is associated with structural plasticity observable with different MRI modalities in adult C57BL/6 mice. The rotarod is a standard test that taxes motor coordination and balance. We use T_2-weighted MRI followed by deformation-based morphometry to assess local volume and fractional anisotropy (FA) derived from diffusion MRI to assess microstructure ex-vivo. Using deformation-based morphometry we found that the hippocampus, frontal cortex and amygdala are larger in rotarod-trained mice compared to untrained controls. Surprisingly, the cerebellum and white matter in the corpus callosum underlying the primary motor cortex are smaller after training. We also found that the volume of the motor cortex is positively correlated with better rotarod performance. Diffusion imaging indicates group differences and behavioral correlations with FA, a measure of microstructure. Trained mice have higher FA in the hippocampus. Better rotarod performance is associated with higher FA in the hippocampus and lower FA in the primary visual cortex. This is the first study to reveal the substantial structural reorganization of the adult mouse brain following only a relatively brief period of motor-skill training by using complementary measures of microstructure and volume.

Introduction

Behavioral change depends on the reorganization of neural circuitry. This reorganization might be achieved by the modification of already existing synapses through protein synthesis (Schacher et al., 1988). In rodents more persistent behavioral changes are associated with more dramatic structural changes, such as neurogenesis, dendritic/axonal arborization, myelinogenesis, and gliogenesis (Demerens et al., 1996; Gross, 2000; Hickmott and Steen, 2005; Loseva et al., 2009). These changes, albeit small, might occur in sufficient number or density to potentially alter the tissue structure to a degree that is observable with magnetic resonance imaging (MRI). The aim of this study is to investigate whether motor skill training can elicit marked structural plasticity observable with multimodal MRI in adult mice of a common background strain.

Of particular scientific interest is the question whether the adult brain still exhibits marked plasticity. Being able to modulate this structural plasticity might help behavioral recovery in humans suffering from neurological diseases. Recent human MRI studies have suggested that both gray and white matter continue to be malleable throughout adulthood in response to training a motor skill (Bezzola et al., 2011; Draganski et al., 2004; Jäncke et al., 2009; Scholz et al., 2009; Taubert et al., 2010).

Animal studies have been able to reveal the cellular changes associated with changes in experience. In adult rodents increases in cell number/proliferation have been found in response to spatial navigation tasks (Drapeau et al., 2003), environmental enrichment (Kempermann et al., 1997, 1998), and exercise (van Praag et al., 1999). These previous studies have relied on histology to quantify the structural changes and have thus focused on a region of interest, usually the hippocampal formation, which is known to be structurally plastic throughout adulthood. Thus, relatively little is known about the effect of experience on the whole brain. Further, while the association between cellular changes and behavior is informative, the cause of the cellular changes remains largely unknown.

A small number of unimodal studies have found experience-related changes using MRI in adult mice, thus allowing detection of structural changes all over the brain. We found volumetric changes in mice trained

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on the Morris water maze for five days (Lerch et al., 2011b). Different brain areas increased in size (2–4%) depending on the strategy employed by the mice. The volume changes were related to GAP-43 staining, an axonal growth cone marker, but not to markers linked to number and size of neurons and astrocytes. This suggests an increase in neuronal processes in specific training-related brain areas.

Local volume is not the only useful MRI measure of structural plasticity. Ding et al. (2013) found changes in fractional anisotropy (FA) after only 1 h of fear conditioning, most of which seemed to return to baseline after one day. Fractional anisotropy is a measure of tissue organization derived from diffusion-weighted MRI. It is high in well-aligned tissue such as white matter tracts and lower in gray matter where dendritic trees point in various orientations. The authors hypothesize that the observed changes in amygdala, hippocampus and cingulum might be due to changes in dendritic branching or glial remodeling.

Further experience-related structural changes have been reported in response to Morris water maze training in rats using diffusion MRI (Blumenfeld-Katzir et al., 2011). Changes in apparent diffusion coefficient (ADC), a measure related to overall diffusivity, were found in a number of regions, such as the dentate gyrus and piriform cortex. Here ADC was decreased in learners compared to exercise and home cage controls. Interestingly, ADC seemed to be decreased in exercise controls in S1/S2 compared to the two other groups. This suggests that while the majority of the changes were learning-related some microstructural changes might have been specific due to exercise (i.e. swimming). A recent rat study found myelin-related FA increases in the corpus callosum following reaching training but restricted the analysis to white matter tracts (Sampaio-Baptista et al., 2013).

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, which is part of a battery of tasks used to assess rodent motor function (Brooks and Dunnett, 2009; Shiotsuki et al., 2010). Briefly, mice are placed on a rotating drum and the time is measured until they fall off or the maximum trial length is reached. The rotation speed is increased smoothly to raise difficulty over time. Secondly, this study aims to identify a mouse strain that might serve as a background for genetic modification necessary to determine the cause of the cellular changes. Here we use C57BL/6 mice, the most widely used background strain for genetically modified mice.

This study will thus establish the structurally modulating brain regions and the size and direction of the changes using ex vivo MR imaging. We test for volume changes based on T_2-weighted images and microstructural changes based on FA maps derived from diffusion-weighted MRI to cover a wider variety of underlying cellular changes. Multimodal whole-brain imaging will potentially give us access to a wider array of underlying cellular changes. Although the rotarod task is widely-used for motor assessment in mice its effects on whole brain structure are unknown. Importantly, this work will therefore pave the way for studies in mice genetically engineered to allow the investigation of the cause of these structural changes utilizing a task similar to the motor training paradigms commonly used in human imaging plasticity studies.

The rotarod task trains the motor system symmetrically as mice align orthogonally to the axis of the rotarod drum. We therefore expect bilateral brain changes both in volume and microstructure, especially in brain areas involved in motor execution, balance, and learning. This hypothesis allows us to specifically test for bilateral group differences, thus increasing statistical power by exploiting the symmetry of the expected structural changes. Finally, we hypothesize that if structural alterations are behaviorally relevant they would correlate with overall latency.

Methods

Mice

A total of 48 C57BL/B6 mice, aged 16 weeks, were used in this experiment and housed in cages of 3–5 separated according to sex (21 male). All animal experiments were approved by the animal ethics committee of the Hospital for Sick Children.

Behavior

Out of the experimental mice 25 (14 male) were trained on the rotarod (Med Associates Inc.) with a rod diameter of 32 mm and an accelerating speed of 4 to 40 rpm over 5 min. Four mice were tested simultaneously on the rotarod, ten times a day for eight days in a row resulting in 80 trials per animal. Latency was measured as the time from the beginning of the trial (start of the accelerated rod rotation) until the mouse falls off and onto the lever that stops the timer. The maximum trial length was 300 s. The remaining mice functioned as controls with no behavioral testing. Controls were handled 2 min a day for 5 consecutive days. Mice were perfused one day after the last training day.

Behavioral analysis

Behavioral analysis of the latencies measured at each trial was carried out using the R software package. Average latency per animal across trials and days was estimated using a Bayesian model that accounted for the 'censoring', i.e. the end of the trial after a maximum of 300 s. The resulting parameter estimates are thus more a measure of skill than performance, i.e. the model takes into account that an animal could potentially perform past the artificially imposed performance limit.

In the following we will use the notation from Gelman (2007). Briefly, the basic model assumes average latencies μ for each mouse j came from a common normal distribution $\mu_j \sim N(\overline{\mu}, \overline{\tau})$. The likelihood function for latencies y read $y_i \sim N(y_{j[i]}, \tau)$, where $j[i]$ refers to subject $j = 1..25$ and measurement $i = 1..2000$. We used a minimally informative normal prior on the mean of the distribution of average performances $\overline{\mu} \sim N\left(0, 10^{-5}\right)$ and a uniform prior on the precisions $\tau, \overline{\tau} \sim U(0, 100)^{-2}$. The precision is defined as the reciprocal of the variance. Posteriors were estimated using Gibbs sampling with 30,000 samples after burn-in. Latencies of more than 300 s were drawn from the likelihood function.

Similarly, a quadratic model was used to estimate the number of trials till peak performance was achieved for each mouse j. Latency y at each measurement i was modeled as a function of trial number $x = 1..80$ and three parameters: $\hat{y}_i = \beta_{0j[i]} + \beta_{1j[i]}x_i + \beta_{2j[i]}x_i^2$. Data as well as betas were assumed to be normally distributed: $y_i \sim N(\hat{y}_{j[i]}, \tau)$, $\beta_{nj} \sim N\left(y_{\beta_n}, \tau_{\beta_n}\right)$, for $n = 0..2$. This model seemed to have a reasonable fit (Pearson's r = 0.84, see Fig. 1C) and captured the main trends in the data. The peak trial was defined as the trial that contained the maximum of the quadratic fit. We adopted a significance threshold of $p < 0.001$ for tests on behavioral measures. Standard deviations are reported in parenthesis following means.

Image acquisition

Mice were perfused and brains were subsequently immersion fixated (Cahill et al., 2012). Brains were left within skulls for scanning on a multi-channel 7.0 T, 40 cm diameter bore magnet (Varian Inc. Palo Alto, CA) to avoid deformations and damage during removal. A T_2-weighted sequence was used to obtain images for deformation based morphometry. A custom-built 16-coil solenoid array was used to image 16 samples concurrently (Lerch et al., 2011b) at 56 μm isotropic voxel resolution. The 3D fast spin-echo sequence had the following parameters: TR = 2000 ms, echo train length = 6, TEeff = 42 ms, field-of-view (FOV) = 25 × 28 × 14 mm and matrix size = 450 × 504 × 250. Total imaging time was 11.7 h.

Additionally, brains were scanned with a custom built 3-coil solenoid array in an insert gradient using a 3D diffusion-weighted

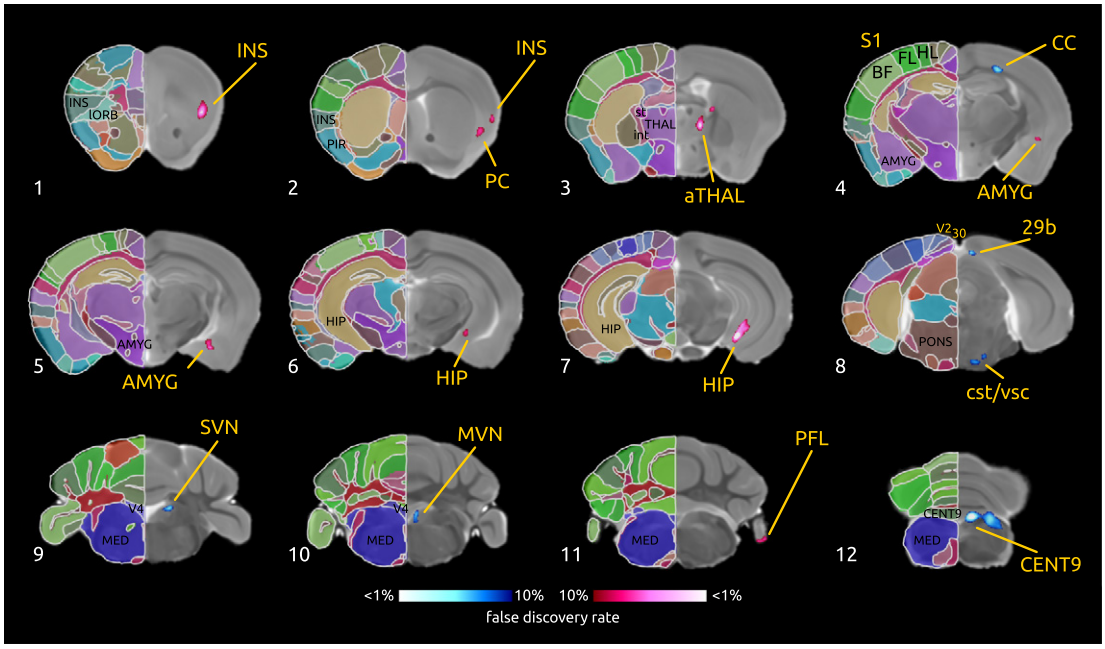

Fig. 1. Volumetric group differences. Differences between rotarod trained mice and controls are estimated across both hemispheres as bilateral effects. Significant clusters are depicted only on the right hemisphere of the average as they are symmetric. Labels from a 291-structure atlas are displayed on the left hemisphere for orientation. (10% FDR corrected; rotarod trained > controls, red; controls > rotarod trained, blue; Abbreviations: 29b/30, retrosplenial cortex; AMYG, amygdala; aTHAL, anterior thalamus; BF, barrel field; CC, corpus callosum; CENT9, cerebellar central lobule 9; cst, corticospinal tract; FL, primary somatosensory, forelimb region; HIP, hippocampus; HL, primary somatosensory, hindlimb region; INS, insular cortex; int, internal capsule; lORB, lateral orbital cortex; MED, medualla; MVN, medial vestibular nucleus; PC, piriform cortex; PFL, paraflocculus; S1, primary somatosensory cortex; st, stria terminalis; SVN, superior vestibular nucleus; V2, secondary visual cortex; V4, 4th ventricle; vsc, ventral spinocerebellar tract.)

fast-spin echo sequence (30 directions with b = 1917 smm^{-2}, 5 b0-images, TR = 325 ms, echo train length = 6, first TE = 30 ms, TE = 6 ms for the remaining 5 echoes, two averages, FOV = 25 mm × 14 mm × 14 mm, matrix size = 192 × 108 × 108, yielding 130 μm isotropic voxels, total imaging time = 13.5 h).

Image analysis

High-resolution anatomical images were corrected for geometric distortions based on phantom data prior to image registration. Briefly, for each coil we estimated the displacement field to correct for the deformations by aligning scans of machine-milled plastic phantoms with known surface geometry to their actual geometry. Brains were aligned using nonlinear image registration with iterative template refinement encapsulated into a custom pipeline (Kovacević et al., 2005; Lerch et al., 2011a). Non-linear image registration relied on the diffeomorphic registration algorithm which is part of the Advanced Normalization Tools (ANTS) (Avants et al., 2011). First, all brains were rigidly registered towards a pre-existing mouse brain average. The left-right flipped version of each rigidly aligned brain was included in all following registration steps, to test for bilateral changes. Thus, 48 original brains plus another 48 brains flipped across the midline were passed to the next registration step, which consisted of estimating the affine transformation between each pair of brains. The pair-wise transformations were then averaged per brain to obtain the final affine registration used to align each brain and create an average. This affine template functioned as the target for the first non-linear registration step, yielding the next refined template (Avants et al., 2011). At each non-linear registration step all brains and their flipped versions were aligned to the template. Three nonlinear targets with progressively finer non-linear registration parameters were generated. For the high resolution anatomical images the log Jacobian determinants of the deformation fields produced by aligning each individual image to the final target were then smoothed (0.2 mm FWHM) and fed into statistical analysis to test for volumetric changes. All individual Jacobian determinants had linear components removed to account for differences in overall brain size, i.e. estimated expansions and contractions were relative to total brain volume.

Dtifit of the FSL software package (Jenkinson et al., 2012) was used to fit tensors to the diffusion data and create maps of fractional anisotropy (FA), axial diffusivity (AD), and radial diffusivity (RD). Similarly to the high-resolution anatomical data, FA maps were non-linearly registered using iterative template refinement. After registration FA maps were smoothed (0.2 mm FWHM) and fed into statistical analysis to test for differences in FA.

Image statistics

Group differences between trained and control mice and correlations with behavior within trained mice were estimated with linear mixed effects modeling at each voxel using the RMINC tools. We tested for increases and decreases (two-tailed). We included group as a fixed effect to test for group differences and average latency to test for behavioral correlations. Mouse ID and brain hemisphere were included as random effects. Gender was included as a co-variate to account for potential sex differences. FA averaged across the brain was included as a covariate in the analysis of the diffusion data to account for a slowly varying (global) bias field. The resulting p-values were corrected for multiple comparisons with false discovery rate (FDR) (Genovese et al., 2002).

A q-value of 10% was chosen as a threshold of significance based on previous studies and simulations (Lerch et al., 2008; van Eede et al., 2013). To localize the significant clusters anatomical labels were aligned to the final average. An atlas with 62 structures was used for rough localization (Dorr et al., 2008) and a more elaborate atlas with 291 structures for more precise localization within the cortex (Ullmann et al., 2013; Steadman et al., 2013). Additionally, we adopted explorative thresholding for the FA maps of $p < 0.005$, uncorrected. To reduce the risk of false positives we employed an additional arbitrary cluster-size threshold of 0.01 mm^{-3}.

For each significant cluster, the size, center of gravity (CoG), and anatomical structure are reported. For the statistically most significant voxel within each cluster we report the percentage difference between

control mice and rotarod-trained mice for both volume and FA. The behavioral correlations are reported in percent volume (or FA) change e per 10 s average latency change.

Results

Behavior

Mice received ten rotarod trials on each of eight successive days. Mice were allowed to stay on the rotarod for a maximum of 300 s per trial. Latencies increased significantly by 100 s (±14) when comparing performance on the first day to the last day ($p < 0.001$, Fig. 2C). Males had overall lower latency ($p = 0.002$), but there was no interaction between sex and day ($p = 0.45$), suggesting that both sexes learned similarly well. According to a quadratic fit to the latency data mice reached their maximum performance on average at trial 64 (±12). There was no sex difference in number of trials till peak performance was achieved ($p = 0.65$).

Volumetry

Brains of rotarod-trained mice did not differ in total brain volume from controls ($p = 0.47$), nor was there a significant sex difference ($p = 0.14$).

Testing for voxel-wise differences revealed a distinct pattern of changes of rotarod-trained mice relative to controls. In general, volume increases were found in the gray matter of the forebrain and decreases in the gray matter of the hindbrain and the white matter of the forebrain (Fig. 1, SI Table 1). In the following we will briefly localize all significant clusters (10% FDR corrected).

Volume was increased in rotarod-trained mice in two clusters in the area of the insular, piriform and orbitofrontal cortices (Fig. 1, slices 1, 2).

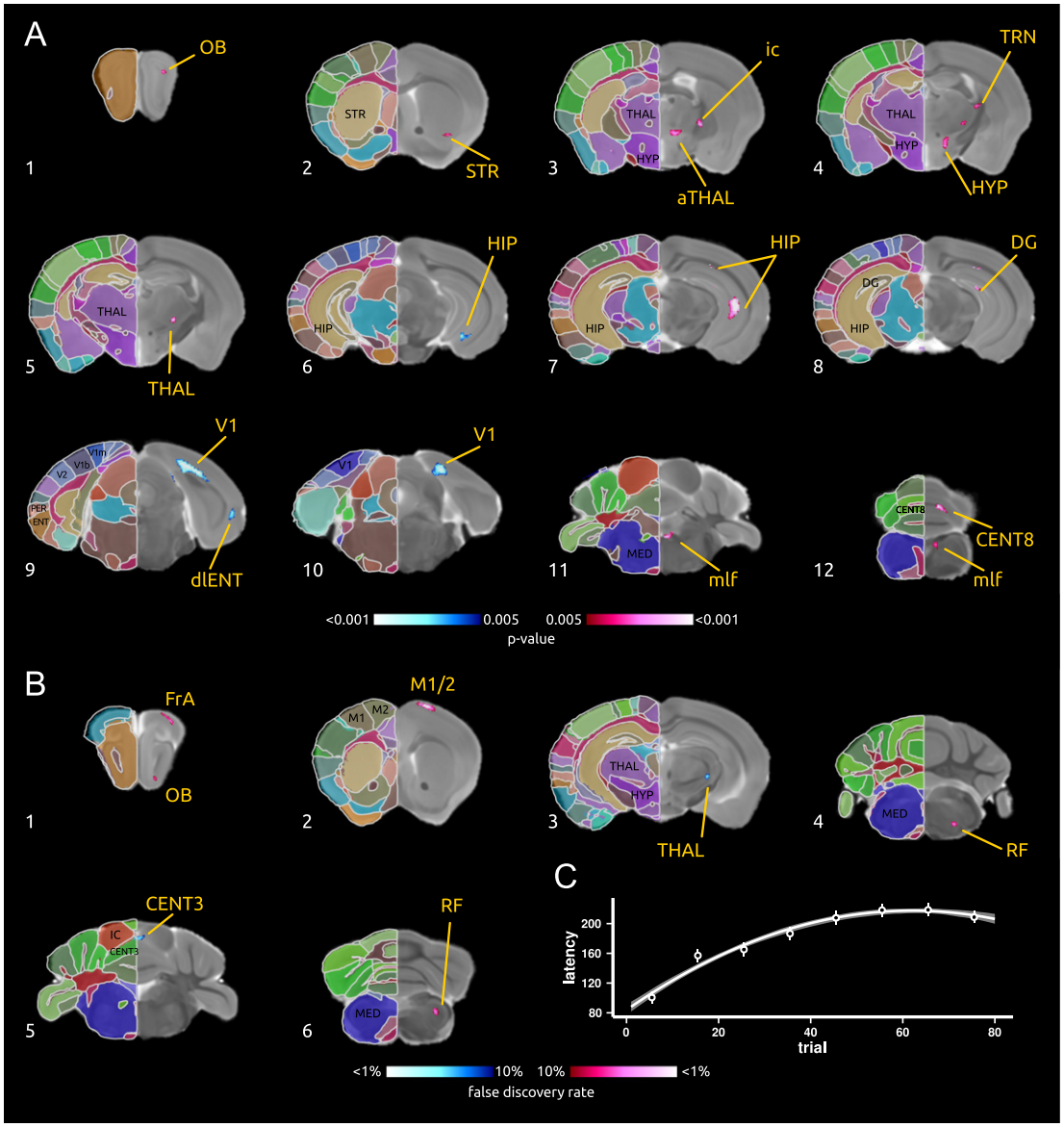

Fig. 2. Behavioral correlations with MR measures. (A) Regions where FA correlates with the average rotarod latency of the whole training period. (B) Regions where local volume correlates with average latency ($p < 0.005$, uncorrected; longer latency correlated with higher FA or larger volume, red; shorter latency correlated with lower FA or smaller volume, blue). Correlations were estimated across both hemispheres as bilateral effects. Significant clusters are depicted only on the right hemisphere of the average as they are symmetric. (C) Quadratic fit to latency (s) averaged across trials within day (whiskers represent 90% confidence intervals). Labels from a 291-structure atlas are displayed on the left hemisphere for orientation. (Abbreviations: aTHAL, anterior thalamus; CENT3/8, cerebellar central lobule 3/8; DG, dentate gyrus; dlENT, dorsolateral entorhinal cortex; FrA, frontal association cortex; HIP, hippocampus; HYP, hypothalamus; IC, inferior colliculus; ic, internal capsule; lem, mendial lemniscus; M1/2, primary/secondary motor cortex; MED, medualla; mlf, medial longitudinal fasciculus; OB, olfactory bulb; RF, reticular formation; STR, striatum; THAL, thalamus; TRN, thalamic reticular nucleus; V1, primary visual cortex.)

Another large cluster of increased volume was found around the molecular layer of the hippocampus (slice 7). Further increases were found in the anterior thalamus just dorsally of the mammilothalamic tract (slice 3), the amygdala (slices 4, 5), and the paraflocculus of the cerebellum (slices 11). Several white matter tracts contained regions of decreased volume, such as the corpus callosum underlying the primary somatosensory cortex (slice 4), the cortico spinal tract and the ventral spinocerebellar tracts (slice 8). Finally, gray matter decreases were found in the central lobule 9 of the cerebellum (slice 12), the medial/superior vestibular nuclei (slices 9, 10), and the retrosplenial cortex (slice 8).

Testing for voxel-wise differences revealed several significant clusters where average latency correlated with local volume (10% FDR corrected, Fig. 2B, SI Table 2). In the rotarod-trained mice longer average latency was associated with larger volume of an extensive region of the primary/secondary motor cortex (Fig. 2B, slice 2). There were several smaller clusters with the same direction of correlation, one in the olfactory bulb (slice 1) and two in the reticular formation of the medulla (4,6), and one in the frontal association cortex (slice 1). There were negative correlations in the reticular nucleus of the thalamus (slice 3), and in the central lobule 3 of the cerebellum (slice 5).

Fractional anisotropy

Voxel-wise FA differences and behavioral correlations did not survive whole-brain correction for multiple comparisons. Although overall FA contrast was well preserved in all samples, it is possible that the fixation process might have caused cellular alterations through protein cross-linking which normalized the diffusion characteristics, thus decreasing the chance of detecting brain differences (Shepherd et al., 2009).

Therefore, in a second explorative analysis we tested for increases in FA at an explorative statistical threshold. FA was increased in the hippocampus of rotarod-trained mice compared to controls ($p < 0.005$, uncorrected, Fig. 3, SI Table 3).

The behavioral correlation revealed that higher average latency was associated with higher FA in two hippocampal regions immediately adjacent to the area where FA differed between groups ($p < 0.005$, uncorrected, Fig. 2A, slice 7, SI Table 4). Further positive correlations were found in the hypothalamus (slice 4), the thalamus (3,4,5), the medial longitudinal fasciculus (slice 12), lobule 8 of the cerebellum (slice 11), the striatum (slice 2), and the olfactory bulbs (slice 1). An extended area where lower latency was correlated with higher FA was found in the visual cortex and entorhinal cortex (9,10).

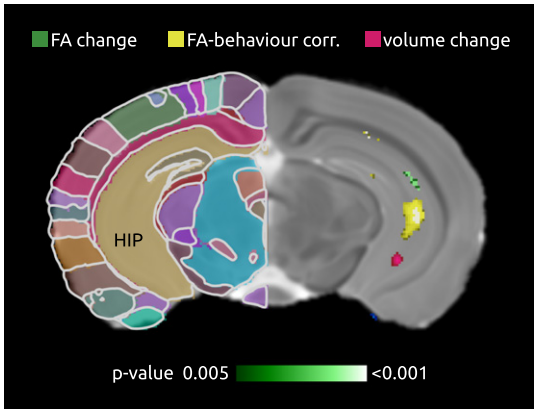

Fig. 3. Volumetric changes, FA changes and FA-behavior correlations can be found in the hippocampus. Hippocampal clusters from previous figures are added to the FA changes to illustrate spatial proximity. Volume is increased in the dorsal hippocampus (10% FDR corrected; rotarod trained > controls). FA is increased in the dorsal hippocampus and correlates positively with longer average latencies ($p < 0.005$, uncorrected; rotarod trained > controls, green; longer latency correlated with higher FA, yellow; abbreviations: HIP, hippocampus).

Discussion

The aim of this study was to investigate whether a simple motor training task taken from the standard test battery could elicit marked structural plasticity observable with MRI in adult mice of a common background strain. Our analysis took into account that rotarod running taxes the motor system and by extension the brain symmetrically, i.e. the rotarod could be described as a bilateral movement training task (Nakagawa et al., 2013). We found that eight days of training was associated with volume increases in the prefrontal cortex, the hippocampus, the thalamus, and the amygdala. Interestingly, volume decreases were found in the cerebellum, the retrosplenial cortex, and the vestibular nuclei of the brainstem. Further, volume decreases were found in white matter, such as the corpus callosum underlying the primary somatosensory cortex, the cortico spinal tract, and the ventral spinocerebellar tract. Among rotarod-trained mice, longer latency was associated with increased volume across a large cluster in the primary/secondary motor cortex.

An explorative analysis showed tentative increases in FA in rotarod-trained mice in the hippocampus. Behavioral correlations were more wide-spread and included the hippocampus where longer rotarod time was associated with higher FA. Further areas of positive correlations were found in the cerebellum, the striatum, the thalamus, and the hypothalamus. The relationship was reversed in the primary visual cortex and the dorsolateral entorhinal cortex.

Behavior

The behavioral improvement followed a time course similar to the one reported in previous studies of mice and rats (Buitrago et al., 2004; Cendelín et al., 2008). Most mice improved most quickly at the beginning of the training period and reached peak performance after about seven days of training. This indicates that most mice had reached a performance plateau by the end of the eight-day training period. Thus, the training elicited maximal behavioral change while not substantially overtraining the mice. Analysis of gait patterns has shown that the rotarod task trains motor skills and goes beyond locomotor ability and fitness (Buitrago et al., 2004). Rats learned to change from 'slow stepping' to 'running' at lower speeds during the course of the training, thus adopting an advanced and more fluid movement sequence. This suggests that the rotarod task has a substantial skill training component and that the resulting behavioral and structural brain changes are not purely the results of exercise.

Structural changes

The structural differences between rotarod-trained mice and controls formed a distinct pattern that could have only been revealed with a whole-brain technique such as MRI. The brain regions implicated in these changes and behavioral correlations fall into three broad categories: brain regions which could be considered to be directly related to rotarod running/balancing, brain regions which are associated with learning in general, and brain regions which seem to be neither directly nor indirectly related to rotarod running/balancing.

The motor cortex (M1/M2), the cerebellum, the striatum, and connected fiber tracts can be subsumed under regions that are to a certain degree directly related to motor-learning. They span almost the entire network of motor-related brain areas.

It has been shown that the transcription factor c-Fos, an indirect marker of neuronal activity, is upregulated in the cerebellum, the motor cortex, the cingulate cortex, and dorsal striatum of rotarod-trained mice (Bureau et al., 2010). The same study found that extracellular signal-regulated kinase, marker of long-term memory

stabilization, is activated in the cerebellum, the anterior cortex and the hippocampus specifically during the first day of training. This suggests that the brain regions observed in this study might be regions of increased neuronal activity and synaptic plasticity after rotarod training.

Two-photon imaging in the motor cortex of rotarod-trained mice has shown a transient increase in spine formation about two days after training (Yang et al., 2009). However, due to the concomitant increase in spine elimination the total gain in spine number was negligible. The number of persistent spines formed during the first two days of training correlated with rotarod performance. Similarly, spine elimination over the entire seven-day training period correlated with performance. These observations fit with our volumetric findings in the motor cortex where we did not find a volume increase in trained mice compared to controls; but we found an association between better performance and larger volume.

Apart from these directly motor-related regions, we found changes in the vestibular nuclei (MVN/SVN). These nuclei are involved in the vestibulo-ocular reflex (VOR), which stabilizes the retinal image during head motion by transforming vestibular input into oculomotor output (Beraneck and Cullen, 2007; Sekirnjak and du Lac, 2006). It is conceivable that a mouse which is otherwise living on the even surface of a shoe box-like container will have to learn how to adjust head and compensatory eye movements to the constantly shifting ground of a rotating rotarod. Vestibular system plasticity continues throughout life (Gittis and du Lac, 2006). It has been shown that in the short term modulation of the VOR depends on the cerebellar flocculus, an area where we also found volume increases. Consolidation of the motor memories is then assumed to happen in the vestibular nuclei (Boyden et al., 2004; Nagao and Kitazawa, 2003; Shutoh et al., 2006).

The visual cortex might be directly related to the balancing component of the rotarod task, although it is not part of the motor network. We found a large area in the deep layers of the primary visual cortex close to the white matter of the external capsule where longer latencies were associated with lower FA. These microstructural differences might be the result of differences in development and could be related to how efficiently the visual cortex processes visual cues which support estimation of head and body orientation.

In addition to these gray matter regions, we also found a number of white matter tracts that might be directly related to rotarod training. The corticospinal tract (CST) connects to spinal motor neurons that innervate the muscles necessary to accomplish the rotarod task. The ventral spinocerebellar tract (VSC) transmits information towards the cerebellum. VSC neurons are excited by reticulospinal, vestibulospinal, rubrospinal and especially corticospinal neurons which might provide early feedback about motor neuron activation in the spine (Hammar et al., 2011; Shrestha et al., 2012). Finally, we found volume decreases that could be either attributed to the deep layers of primary somatosensory cortex or the underlying corpus callosum. The direction of the change fits better with the direction of changes in the above described white matter tracts and suggests that the volume decrease might be related to white matter. In general, it is not immediately obvious why white matter tracts should have decreased volume compared to controls. A simple hypothesis might posit that increased activity is related to increased myelination (Demerens et al., 1996), which would most likely lead to an increase in volume due to the membranes added by oligodendrocytes. The fact that the volume decreases are focal and localized underneath the primary somatosensory cortex could indicate that the volume decrease is more related to axons innervating the corpus callosum at this particular location than to axons traveling along the corpus callosum. However, we did not find significant changes in FA in this region, which would support these changes in microstructure. The absence of FA differences in a white matter region of volume change is puzzling, but could be explained by the fact that fibers underlying the cortex make sharp turns to enter the cortex. In this scenario newly growing fibers or increases in myelin could modulate both radial and axial diffusivity similarly in a voxel containing fibers tangential and perpendicular to the cortical surface, yielding no substantial change in FA. Alternatively, a decrease in tract volume might also increase partial-voluming in the lower resolution diffusion data. An FA increase in the underlying white matter tract could thus be obscured by an increased proportion of less anisotropic gray matter within the same voxel.

In summary these changes and behavioral correlations suggest that a whole network of brain regions associated with sensorimotor, vestibular, and visual signal processing is recruited and adapts during rotarod training.

A number of brain regions, such as the hippocampal formation and the entorhinal cortex are probably not directly related to the motor and vestibular components of the rotarod task. The hippocampus does not seem to be necessary during acquisition of the motor learning task; it is rather involved in spatial and recognition memory (Gould et al., 2002). In addition, the hippocampal formation is an exceptionally plastic region with ongoing neurogenesis in the dentate gyrus of the hippocampus (Kempermann et al., 1997). Therefore the hippocampus might be considered a prime candidate within the adult brain to produce bulk cellular changes observable with MRI. Indeed it has been found to change structurally in a number of studies using MRI in learning rodents (Blumenfeld-Katzir et al., 2011; Lerch et al., 2011b; Shiotsuki et al., 2010).

Longer latencies were associated with higher FA in the hippocampus. The largest cluster was located dorsally in the hippocampal region with increased volume and around the molecular layer of CA1 in the intermediate hippocampus between the areas of volume and FA increases. While the association between FA and latency suggests behavioral relevancy, it should be noted that physical exercise (30 days of access to a running wheel) in young and aged mice has been shown to increase cell proliferation and neurogenesis in the hippocampus (Madroñal et al., 2010; van Praag et al., 2005). In addition, changes in vasculature have been observed in hippocampus following exercise (Van der Borght et al., 2009). These previous findings might indicate that the volume and FA changes we observed could come from different underlying cellular changes.

The anterior thalamus could be added to this second group of regions on the basis that it is heavily interconnected with the hippocampal formation, but not directly related to motor training (Shibata, 1993). Apart from direct connections between the anterior thalamic nuclei and the hippocampal formation there are also indirect projections via the retrosplenial cortex where we found volume decreases (Van Groen and Wyss, 1995). Specifically, we found volume increases in the ventral hippocampus spanning the layers between dentate gyrus and the molecular layer of CA1 and FA increases dorsally in this region between the molecular layer and the pyramidal layer of CA1. This suggests that the changes might be related to the apical dendrites of the pyramidal neurons of layer CA1 or interneurons residing in these layers.

In concert, these findings indicate that the hippocampus is the structurally most malleable in response to rotarod training. More importantly it also seems to be the only brain region that is both subject to change and behaviorally relevant in terms of volume and microstructure.

Finally, we found a third set of brain regions which seem neither directly nor indirectly related to motor skill training. Of these regions the amygdala could show up due to the stress and anxiety that the mice experience when they are suspended over ground on top of a rotating barrel. Dendritic complexity has been shown to increase in the amygdala after fear conditioning (Heinrichs et al., 2013).

Both insular and orbitofrontal cortices are part of a network of regions that have been related to interoception, i.e. they represent and process body temperature, pain, muscular, and visceral sensations (Christianson et al., 2008; Craig, 2002). These sensations are related to homeostatic needs and might be associated with behavioral motivations. In addition, the insula shares bi-directional connectivity with the ventral CA1/subiculum close to where we found volume increases (Fanselow and Dong, 2010; Moraga-Amaro and Stehberg, 2012). The

anterior hypothalamus has been related to temperature regulation and panting/sweating, autonomous responses that might be associated with the exercise component of the rotarod training.

Why small clusters within the olfactory bulb change volume or microstructure after rotarod training is less obvious. The olfactory bulb is in addition to the hippocampus, the only brain region that stays neuronally plastic throughout life. New neurons continue to migrate from the subventricular zone to the olfactory bulbs via the rostral migratory stream in the mature brain (Ghashghaei et al., 2007). Any form of experience, such as exercise, might have a net structural effect, by altering neuronal proliferation and survival globally, even if the stimulus is not olfactory in nature. However, current evidence favors specific olfaction-related stimuli as causes of neurogenesis in the olfactory bulb (Ma et al., 2009).

In summary, it seems that most of the regions in this category are associated with emotional, visceral, and interoceptive processes. Although it is interesting to see that these processes might be related to structural plasticity, this experiment aimed at the motor skill aspect of the rotarod task. Future experiments could potentially control for emotional/visceral differences by inducing similar levels of anxiety and exercise in the control group.

It is important to realize that the reported clusters can result from very different cellular processes depending on whether they are training or behavior-related and depending on whether they were detected using volumetry or diffusion imaging derived fractional anisotropy.

Due to the cross-sectional nature of this study it is not possible to disambiguate pre-existing differences from experience-related differences. For example, we found that mice with larger motor cortices are better at the rotarod task. This difference could be due to differences in development i.e. better performing mice had larger motor cortices before the start of the experiment. However, it is also possible that mice that spent more time on the rotarod had larger cortices at the end of the experiment as a result of the more intense training.

The differences in MRI-derived volume and fractional anisotropy cannot be unambiguously related to differences in underlying cellular structure and morphology. Hypotheses that have been proposed include difference in number and size of neurons and glial cells and their processes (Lerch et al., 2011b). Volume might be predominantly related to the volume taken up by the neuropil as well as extra and intra-cellular water content. Volume is measured across multiple voxels and thus requires (MR) contrast to estimate a shift in tissue boundaries. Fractional anisotropy is a intra-voxel measure linked to myelination, fiber density, and fiber coherence in white matter (Beaulieu, 2002). In gray matter fractional anisotropy might be related to the orientational distribution of anisotropic structures such as dendrites and axons.

Conclusion

Multiple human imaging studies have shown structural plasticity with motor learning (Bezzola et al., 2011; Draganski et al., 2004; Jäncke et al., 2009; Scholz et al., 2009; Taubert et al., 2010). However, it is often difficult to tightly control the training regime in human studies nor can the mechanisms underlying the structural brain changes be pursued. Here we show that, similar to previous studies of spatial memory in rodents, motor-skill learning can alter the brain at a scale detectable by MRI (Blumenfeld-Katzir et al., 2011; Lerch et al., 2011b). The three categories of directly task-related, learning-related and emotional/visceral regions show a distinct pattern of structural change and behavioral relevance across the mouse brain. Regions associated with sensorimotor and balance processing together with the generally learning-related and structurally plastic hippocampus dominate the emerging pattern. We also find evidence for regions linked to anxiety, stress, and visceral processing. The results of this study suggest that the standard rotarod training task is associated with changes in a whole network of motor learning-related and task-associated brain regions, detectable with multimodal MRI. It also seems to be the case that volume and microstructure are differentially sensitive to underlying tissue changes and complement each other. The fact that the results were obtained in the common background strain of C57BL/6 mice should encourage further studies using genetically-modified mice to investigate the underlying cellular process of experience-related structural changes in the adult brain.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.neuroimage.2014.12.003.

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