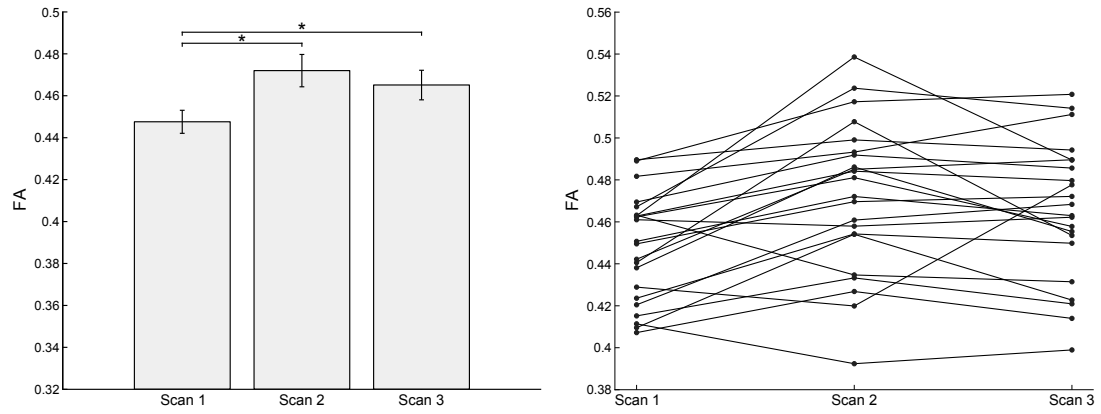
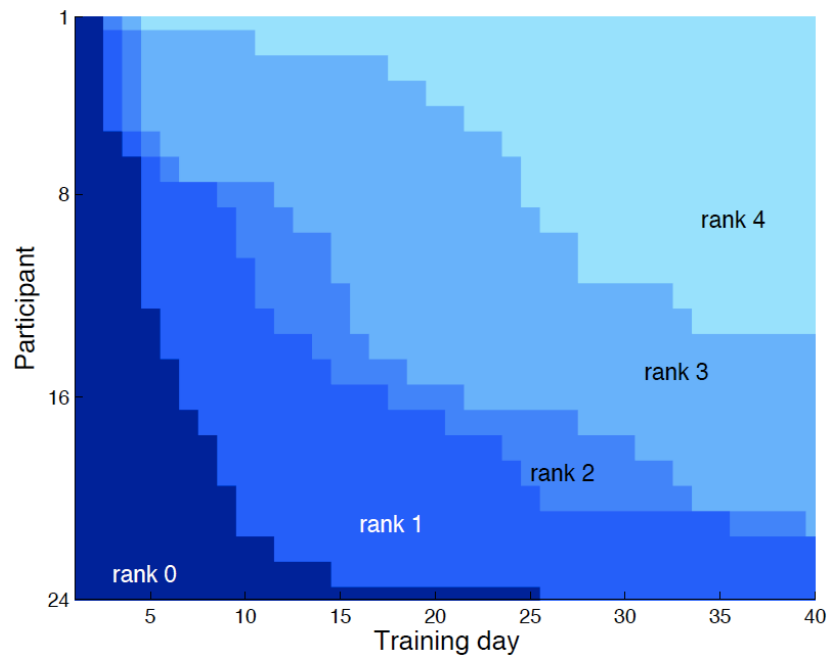


Supplementary Materials for Training induces changes in white matter architecture

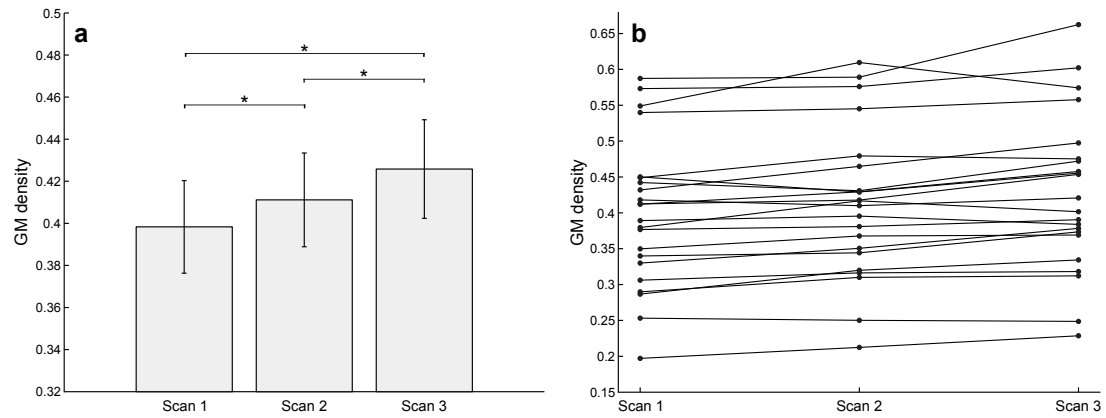
Jan Scholz, Miriam C. Klein, Timothy E.J. Behrens, Heidi Johansen-Berg



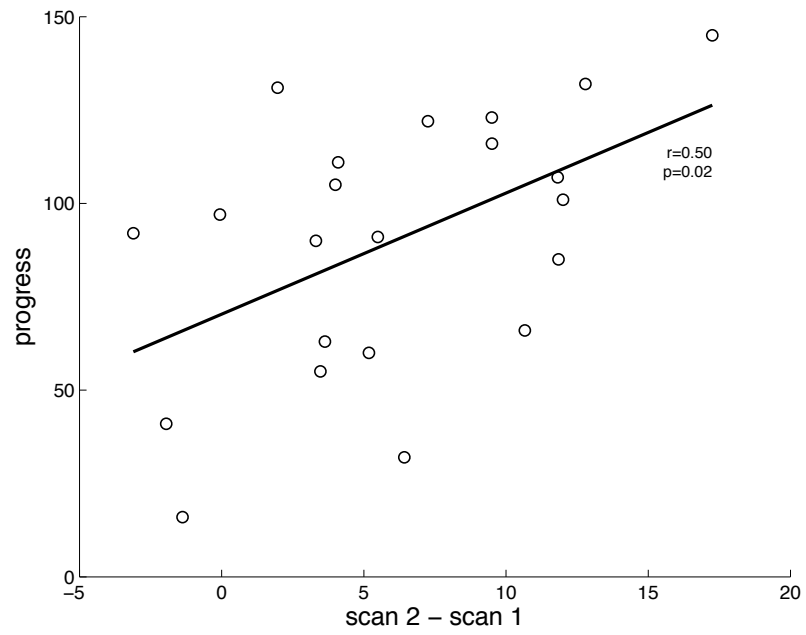
Supplementary Figure 1: Effects of training on FA. (a) Mean FA values at each time point. Error bars represent standard errors. * indicates significant differences ($p < 0.05$) between time points. (b) FA values are shown for each participant and each time point. Data points from the same individual are connected with a line.



Supplementary Figure 2: Behavioural data. Maximum performance score reached by each participant on each day during the training period. Rows correspond to participants, columns to days of training. Participants are ranked by performance score with the best performing subject on the top (rank 0: two-ball pattern; rank 1: 1 cycle with three balls; rank 2: 2 cycles; rank 3: 3 cycles; rank 4: 60 s endurance juggling).

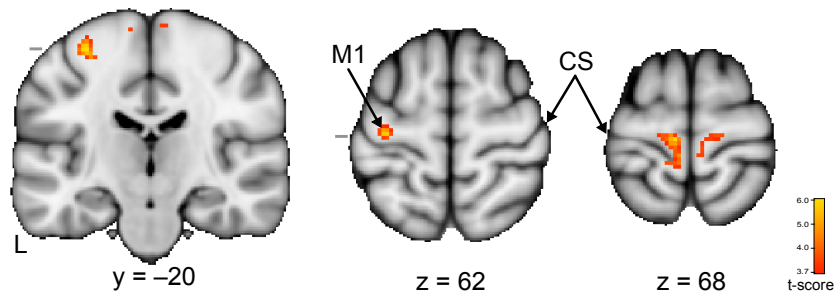


Supplementary Figure 3: Effects of training on gray matter density. (a) Mean gray matter density values for each time point. Error bars represent standard errors. * indicates significant differences ($p < 0.05$) between time points. (b) Gray matter density values are shown for each participant and each time point. Data points from the same individual are connected with a line.



Supplementary Figure 4: Correlation between gray matter change and performance.

Gray matter density change at the peak voxel within the cluster in the left parieto-occipital sulcus ($x=-10, y=-70, z=36$) correlated positively with juggling 'progress' score ($p=0.02$).



Supplementary Figure 5: Changes in primary motor cortex. Gray matter changes in primary motor cortex following the juggling training period ($p < 0.001$, uncorrected, cluster size > 20 voxels). Although these changes were not detected according to the original statistical criteria of this study, they were revealed using the uncorrected thresholds employed in previous studies.

Region	Side	t_{max}	Peak coordinates			Functional region
			x	y	z	
Medial parietal/POS ¹	l	6.22	-10	-70	36	-
Dorsomedial occipital	l	5.78	-6	-82	20	V3a
TOS	l	5.08	-16	-82	24	V7
Dorsomedial occipital	l	4.88	-12	-86	16	V3
Dorsomedial occipital/POS	l	4.62	-12	-82	32	-
Postero-medial IPS	l	4.60	-20	-70	34	IPS1/2
Postero-medial IPS	r	4.70	22	-80	28	IPS1/2
TOS	r	4.35	16	-82	26	V7
TOS	r	4.01	18	-90	26	V7
Dorsomedial occipital	r	3.80	6	-82	30	V3
Dorsomedial occipital	r	3.73	6	-76	14	V3/V2
Dorsomedial occipital	r	3.57	8	-96	16	V2/V3
Dorso-medial PrCG [†] (86 vox)	l	5.99	-10	-36	72	M1/S1 trunk area
PrCG [†] (49 vox)	l	5.72	-32	-20	60	M1 hand area
PrCG [†] (27 vox)	r	4.34	6	-32	66	M1 trunk area

Supplementary Table 1: Gray matter increases. Local statistical maxima of significant gray matter density increases during the juggling period ($p < 0.05$, corrected). Additional regions of gray matter change, found when using an exploratory analysis with the statistical criteria applied in previous studies of gray matter change ($\dagger p < 0.001$, uncorrected, > 20 voxels) are also given. Putative functional areas are indicated. (¹significant correlation with progress; POS=parieto-occipital sulcus, PrCG=precentral gyrus, TOS=transverse occipital sulcus.)

Supplementary methods

Volunteers

48 healthy volunteers (age range 18-33 years; mean age 25.02 years, standard deviation 3.34 years; 22 female) gave written informed consent and participated in this study. The Central Oxfordshire Research Ethics Committee approved the study.

24 of the volunteers (age range 21-32, mean age 24.92 years, standard deviation 2.89 years; 12 female) with no previous experience in juggling were assigned to a 'Training Group'. These volunteers were provided with three bean bags and written instructions on how to learn a basic three-ball juggling pattern, the '3-ball cascade'. Before commencement of training, volunteers received a baseline scan. Then they were instructed to practice the '3-ball cascade' for half an hour per day, five days per week, for six weeks. Volunteers reported their training progress daily via a web site. Progress was also monitored through group practice sessions that volunteers were invited to attend. Volunteers who mastered the '3-ball cascade' before the end of the training period were encouraged to practice more advanced juggling patterns. After six weeks of training, volunteers underwent a second scan. This was followed by a period of four weeks during which volunteers did not juggle, and then by a third scan. Two participants were unable to participate in the final scan.

24 of the volunteers (age range 18-33 years, mean age 25.13 years, standard deviation 3.79 years, 10 female) served as controls and received only two scans, six weeks apart, with no intervening juggling training.

Behavior

The volunteers reported training progress daily via a website on which they recorded whether they could sustain the following juggling patterns: 0: 2-ball pattern; 1: 1 cycle of 3-ball cascade; 2: 2 cycles; 3: 3 cycles; 4: 60 s 3-ball cascade. In addition, we videoed juggling performance (Canon HG10) on the day of the second and third scan

for evaluation by the experimenters according to the same criteria. We calculated two performance measures from these data: ‘progress’ and ‘level’. We defined progress as the cumulative sum of scores derived from the volunteers’ self-report on each of 40 training days. The second performance measure ‘level’ refers to the absolute level of performance reached after six weeks of juggling practice as judged by the experimenters. For ‘level’, we applied a more fine-grained scoring scheme from 1 to 9 (1: 1 or 2 cycles of ‘3-ball cascade’; 2: 3-5 cycles; 3: 5-10 s; 4: 10-20 s; 5: 20-30 s; 6: >30s; 7: >60 s; 8: >60 s and at least one other pattern for <60 s; 9: >60 s and at least one other pattern for >60 s).

MRI Data Acquisition

We acquired the data on a 3 Tesla Trio scanner (Siemens, Erlangen, Germany) with a 12-channel head coil. We used the same protocol for each volunteer and each scanning session. We acquired two sets of whole brain diffusion weighted volumes (60 directions; $b = 1000 \text{ smm}^{-2}$; 65 slices; voxel size $2 \times 2 \times 2 \text{ mm}^3$; repetition time (TR) = 9.3 s; echo time (TE) = 94 ms) plus six volumes without diffusion weighting ($b = 0 \text{ smm}^{-2}$). In addition, we acquired a field map matching the characteristics of the diffusion protocol ($TE_1 = 5.19 \text{ ms}$; $TE_2 = 7.65$). We acquired one T1-weighted anatomical image using a FLASH sequence (TR = 11.2 ms; TE = 4.7 ms; flip angle = 8° , voxel size = $1 \times 1 \times 1 \text{ mm}^3$).

Preprocessing and Statistical Analysis

We carried out analyses with the FSL package, version 4.1 (www.fmrib.ox.ac.uk/fsl)¹.

Diffusion data

We corrected diffusion data for eddy-currents and head motion using affine registration to the average of the non-diffusion-weighted volumes. We obtained FA maps by fitting a tensor model to the corrected diffusion data at each voxel. Mechanical vibration induced by the diffusion gradients affected the diffusion data,

resulting in localized signal attenuation for diffusion directions with a strong x-component. We minimized effects of this by modeling this signal attenuation and including it as a co-regressor in the diffusion tensor fit. Specifically, the co-regressor is a function of the x-component of the diffusion vector and represents the extent to which the artifact is present in the voxel under examination. We derived the co-regressor empirically from the data by fitting a tensor to the data from directions with low x-components to obtain an estimate of the tensor unaffected by artifact. We then used the residuals from applying this tensor to the full dataset to construct a function of the artifact dependence on the diffusion-weighting along x. We used a smooth approximation to this empirical dependence for the correction step. The empirically-derived co-regressor seemed to differ only in scaling across subjects. The co-regressor corrects for the erroneously low diffusion signal in the small number of directions affected. The correction removes erroneous FA values and corrects the direction. The resulting FA maps did not differ from FA maps acquired on a different system that was not affected by vibration in the same way (Siemens Sonata, 1.5 T).

We performed voxel-wise analyses of FA maps across subjects and time points using tract-based spatial statistics (TBSS)² on data from the first two scans. First, for each subject, we registered FA maps to a space mid-way between the space of scan one and scan two using an affine transformation, and averaged the two registered maps to generate a subject-wise mid-space template. We subsequently non-linearly aligned these templates to FSL's standard FA template (derived from 58 subjects) and averaged them to generate a study specific mean FA map. We then 'thinned' this mean image and thresholded it at an FA value of 0.2 to generate a white matter tract skeleton representing the center of the tracts common to all subjects. We accounted for residual variations in alignment by projecting local tract centers onto the skeleton for each subject. We then used FA data projected onto these skeletons in voxel-wise statistical comparisons. To retrieve corresponding FA values for the third time point, we registered FA maps of the third scan via the subject templates to the standard FA

template and projected FA values for the third scan onto the skeleton. We applied general linear models (GLMs) using permutation-based non-parametric testing. We formed clusters at $t > 2$ and tested for significance at $p < 0.05$, corrected for multiple comparisons across space. We first tested whether significant increases or decreases in structural measures had occurred after the training period (scan 1 versus scan 2). We then calculated the mean FA from within the resulting clusters in order to perform a series of post-hoc tests to test for effects of age and gender, interactions between time and group, interactions between hemisphere and time, and correlations with behavioural measures.

We de-projected any significant clusters identified by these analyses back onto each individual subject's FA map for each scanning session. This allowed us to confirm, by eye, that voxels showing significant effects were indeed located within the white matter in each individual.

T1-weighted data

We analyzed T1-weighted anatomical images using FSL's implementation of voxel based morphometry. First, for each subject, we registered structural images to mid-space using an affine transformation and averaged them to generate a subject-wise mid-space template. We ran brain-extraction³ on the mid-space average and back-projected the resulting mask onto the original images. We then carried out a tissue type segmentation using FAST⁴ and nonlinearly registered the resulting gray matter partial volume estimate (PVE) maps to Montreal Neurological Institute (MNI) 152 standard space to generate a study-specific template. We modulated the registered PVE maps using the warp field of the transformation to correct for local geometric expansions or contractions. Finally, we smoothed the PVE maps with an isotropic Gaussian kernel roughly matching cortical thickness (4.7 mm FWHM) and fed these smoothed maps into voxel-wise statistical analysis. We applied GLMs using

permutation-based non-parametric testing. We used a threshold of $t > 3$ to form clusters and tested for significance at $p < 0.05$, corrected for multiple comparisons across space.

We first tested for changes between scan one and scan two. We then calculated the mean gray matter density from within the resulting clusters in order to perform a series of post-hoc tests to test for effects of age and gender, interactions between time and group, interactions between hemisphere and time, and correlations with behavioural measures. Finally, we tested for changes between the first and the second scan by replicating the statistical criteria and smoothing of previous studies ($p < 0.001$, uncorrected, cluster size > 20 voxels and $p < 0.001$, uncorrected, unrestricted cluster size, smoothing kernel 10 mm FWHM).

Supplementary Results

White matter changes

Mean FA within the white matter cluster showing an increase in FA between scan 1 and scan 2 increased in the majority of individual subjects (Supplementary Fig. 1). Also, it is important to note that mean FA values for this region were greater than 0.39 for all subjects (range 0.39 to 0.54), well above typical values for gray matter (< 0.2) (Supplementary Fig. 1). This provides reassurance that the voxels under consideration were not subject to partial volume effects, consistent with the aims of the TBSS method².

The white matter cluster showing an increase in FA from scan 1 to scan 2 consisted of a lateral and a medial branch. The lateral branch covered the lateral wall of the IPS starting at the fundus of the IPS and ending near the dorsal surface of the inferior parietal lobule. The medial branch was underlying the medial wall of the IPS as well as the fundus of the parieto-occipital sulcus (POS). White matter tracts

running through this region are likely to connect to posterior parietal regions both medial and lateral to the IPS and to dorsal occipital lobe areas.

We ran a series of post hoc tests to probe the FA change in this region further. First, we found that the size of the FA increase did not correlate with age ($r=0.32, p=0.13$) and did not differ between males and females ($t=0.44, df=22, p=0.67$). Next, we compared FA increases over time between trained subjects and untrained controls. The control group did not show any FA change in this region ($t=-0.153, df=23, p=0.9$), resulting in a significant interaction between group and time ($F=9.66, df=(1,46), p=0.003$), confirming that the increase in FA was specific to the trained group (see Fig. 1, main manuscript). Finally, we compared FA values after four weeks without juggling (at scan 3) to values at scans 1 and 2. Although mean FA in this parietal white matter region showed some reduction following 4 weeks without juggling, FA at scan 3 was not significantly different from scan 2 ($t=1.39, df=21, p=0.18$), and was still significantly higher than scan 1 ($t=-4.0, df=21, p=0.001$) (Supplementary Fig. 1). The data therefore suggest that FA increases due to training persist to some degree even in the absence of continued practice. However, the hint of a possible reduction in FA between scans 2 and 3 should be explored by future studies with greater power to determine the timecourse of change.

Although the amount of practice was kept constant across subjects, there was considerable variation in the final level of juggling performance attained (Supplementary Fig. 2). Some of this variation was gender-dependent, with males reaching a higher final performance level than females ($t=2.31, df=22, p=0.03$), but showing no significant difference in the measure of juggling progress ($t=1.24, df=22, p=0.23$). Age showed no correlation with either performance measure (level: $r=-0.30, p=0.15$; progress: $r=-0.33, p=0.12$). To test the hypothesis that the FA increases reflect the efficacy of training, we tested for correlations between performance measures and FA increases in the cluster showing significant FA change. Neither

progress nor final performance level were significantly correlated with FA changes in the IPS cluster. Similarly, FA before the start of the juggling training did not correlate with progress or final performance level after the training period. Only a subset of the trained subjects (8/24) were able to juggle continuously for 60 seconds or more when their performance was videoed at the end of training although a larger subset (13/24) reported that they had achieved this level in their web report feedback provided during training. We found no significant difference in the degree of FA change, or in baseline FA in the right IPS white matter region, between either of these two subgroups and the remainder of the trained subjects (self-report: $t=0.42$, $df=22$, $p=0.68$; videoed: $t=-0.66$, $df=22$, $p=0.52$).

The control group of 24 subjects who received no training were scanned on 2 occasions, 6 weeks apart. This group showed no significant changes (or trends for change) in white matter microstructure over time.

Grey matter changes

Mean gray matter density values from within the gray matter cluster showing an increase in gray matter density from scan 1 to scan 2 increased in the majority of individual subjects (Supplementary Fig. 3).

Both hemispheres showed gray matter increases in the medial occipital and parietal lobes. In the right hemisphere the gray matter increase extended anteriorly as far as the transverse occipital sulcus (TOS) and the adjacent postero-medial IPS. These gray matter changes were adjacent to the medial branch of the white matter cluster that increased in FA during the juggling training period (Fig. 2d, main paper). In the left hemisphere the area of gray matter increase started more caudally near the calcarine sulcus and reached rostrally into and beyond the POS up to the medial parietal cortex. The occipital regions are likely to correspond to functional visual

areas V3A and area V7. The areas within the medial IPS are likely to include the complex of regions sometimes referred to as IPS1 and IPS2.

We ran a series of post hoc tests to probe the gray matter change further. First, we found that the magnitude of the gray matter increase in this region showed a trend to be greater in younger subjects for both the left ($r=-0.38$, $p=0.067$) and the right ($r=-0.37$, $p=0.07$) hemisphere but did not depend on gender (left: $t=1.09$, $df=22$, $p=0.29$; right: $t=1.26$, $df=22$, $p=0.22$). Next, we compared gray matter increases over time between trained subjects and untrained controls. The control group did not show any change in gray matter density in this region (left: $t=0.22$, $df=22$, $p=0.83$; right: $t=0.57$, $df=23$, $p=0.58$), resulting in a significant interaction between group and time ($F=7.49$, $df=(1,46)$, $p=0.009$), confirming that the increase in gray matter was specific to the trained group (see Fig. 2, main manuscript). Finally, we compared gray matter values in this region after four weeks without juggling (at scan 3) to values at scans 1 and 2. Gray matter density in the regions showing a training-related increase did not decline during the four week training abstinence period, in contrast to previous reports of declining gray matter density following a three month period of juggling abstinence⁵. Rather, in the current study, gray matter density in these regions continued to increase, so that gray matter density values within these regions at scan 3 were significantly greater than both scan 2 (left: $t=-4.62$, $df=22$, $p<0.001$; right: $t=-3.01$, $df=22$, $p=0.003$) and scan 1 (left: $t=-7.24$, $df=22$, $p<0.001$; right: $t=-6.23$, $df=22$, $p<0.001$) (Supplementary Fig. 3).

To test whether the gray matter changes we observed have behavioral relevance, we correlated the gray matter increases with measures of training progress and final performance level. While cluster-averaged increases in gray matter density did not correlate with behavioral scores (left hemisphere: level: $r=0.076$, $p=0.7$; progress: $r=0.28$; $p=0.19$; right hemisphere: level: $r=0.064$, $p=0.8$; progress: $r=0.18$, $p=0.40$), we found that gray matter change at the voxel with the most significant gray matter

increase across subjects (Table 1) correlated significantly with ‘progress’, the cumulative measure of the volunteers’ improvement (Supplementary Fig. 4). Gray matter density before the start of the juggling training did not correlate with progress (left: $r=0.25$, $p=0.24$; right: $r=0.25$; $p=0.24$) or final performance level (left: $r=0.25$, $p=0.23$; right: $r=0.22$, $p=0.31$) after the training period. When testing for differences in gray matter density changes between the subgroups of subjects who could juggle continuously for 60 seconds, we found no significant difference in the degree of gray matter density change, or in baseline gray matter density in the medial parietal cortex, between either of these two subgroups and the remainder of the trained subjects (self-report: left: $t=-0.40$, $df=22$, $p=0.70$; right: $t=-0.62$, $df=22$, $p=0.54$; videoed: left: $t=-0.51$, $df=22$, $p=0.64$; right: $t=-0.47$, $df=22$, $p=0.64$).

The control group of 24 subjects who received no training were scanned on 2 occasions, 6 weeks apart. This group showed no significant changes (or trends for change) in gray matter density over time.

Finally, to test whether additional areas of gray matter change could be detected in the training group using an exploratory analysis, we tested for changes between the first and the second scan by replicating the statistical criteria and smoothing of previous studies of gray matter change with training ($p<0.001$, uncorrected, cluster size >20 voxels and $p<0.001$, uncorrected, unrestricted cluster size, smoothing kernel 10 mm FWHM). In addition to parieto-occipital regions that emerged from our primary analysis, three clusters in primary motor areas (M1) emerged using an uncorrected threshold of $p<0.001$ (Supplementary Fig. 5). The clusters covered medial and dorso-medial aspects of bilateral M1. We found a highly specific cluster within the hand area of the left M1, as defined by the omega shape of the precentral gyrus ($t_{\max}=5.72$, $x=-32$, $y=-20$, $z=60$). We detected no other clusters of gray matter increase at this uncorrected threshold. We also tested for changes in the subgroup of trained subjects who mastered ‘endurance juggling’ (i.e., greater than 60 seconds

continuous juggling), as previous studies used these behavioural criteria. Again, we found evidence for gray matter increase in parietal cortex ($t_{\max}=6.52$, $x=-10$, $y=-68$, $z=38$, $p<0.05$ corrected) and motor cortex (left: $t_{\max}=5.47$, $x=-8$, $y=-28$, $z=72$; right: $t_{\max}=5.64$, $x=4$, $y=-28$, $z=72$, both trends at $p<0.1$, corrected) but no other regions.

Supplementary Discussion

Functional properties of altered brain regions

Juggling requires complex visuo-motor integration and reaching and grasping movements. The POS and medial IPS are implicated in visually guided movements of the eye, hand, and in attentional focusing and visuo-motor coordinate transformation⁶⁻¹⁰. In the present study, we also found changes lateral to the IPS and tissue adjacent to this region is associated with the disengagement and re-orientation of the attention^{11,12}. Activity in dorsomedial parietal cortex is associated with reaction time reductions during repeated performance of serial reaction time task¹³ and the gray matter changes seen in this region may reflect the learning of a repeated motor pattern during the course of learning to juggle. Dorsomedial occipital activity is also implicated in visuo-spatial imagery¹⁴ and gray matter changes found in this region may reflect visualization of the movements and ball trajectories involved in juggling.

Because juggling is a complex motor skill, we also expected changes in motor areas. Indeed, when using a more lenient statistical threshold, we detected changes after juggling training in primary motor cortex (M1) corresponding to the proximal upper limb, trunk and left hand area. Structural M1 changes might indicate functional adaptations that facilitate increased speed and accuracy of throwing and catching movements. The laterality of the findings conforms to the view of left hemisphere dominance for movement selection¹⁵.

Interpreting gray matter density changes

It is currently unclear which cellular processes underlie changes in gray matter observed with MRI. In animals a multitude of experience-related gray matter changes are observed (reviewed in^{16,17}). Synaptogenesis, dendritic branching and dendritic spine density are elevated in rats raised in enriched environmental conditions

compared to rats raised in impoverished conditions¹⁸⁻²⁰, even in adults^{21,22}. Because the synaptic and dendritic changes are relatively subtle, they would probably have to occur on a massive scale to have a detectable effect on the MRI signal.

Neurogenesis is another potential contributor to gray matter plasticity¹⁶. Although the number of new neurons added is small relative to the total population, increased neurogenesis in combination with increased survival might have a net-effect detectable by MRI.

Finally, the number of glial cells added to the adult brain is much larger than the number of new neurons¹⁷. Glial cells play an important role in inducing structural changes in both gray matter and white matter. Astrocytes enhance synaptic functioning²³ and oligodendrocytes wrap nerve fibers with insulating myelin sheaths. This raises the possibility that both gray matter and white matter changes might initially depend on similar glial-based mechanisms that precede the functionally relevant structural changes.

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