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# Learning in the Fast Lane: New Insights into Neuroplasticity

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## SUMMARY

**The timescale of structural remodeling that accompanies functional neuroplasticity is largely unknown. Although structural remodeling of human brain tissue is known to occur following long-term (weeks) acquisition of a new skill, little is known as to what happens structurally when the brain needs to adopt new sequences of procedural rules or memorize a cascade of events within minutes or hours. Using diffusion tensor imaging (DTI), an MRI-based framework, we examined subjects before and after a spatial learning and memory task. Microstructural changes (as reflected by DTI measures) of limbic system structures (hippocampus and parahippocampus) were significant after only 2 hr of training. This observation was also found in a supporting rat study. We conclude that cellular rearrangement of neural tissue can be detected by DTI, and that this modality may allow neuroplasticity to be localized over short timescales.**

## INTRODUCTION

Neuroplasticity, the capacity of the nervous system to modify its organization, involves a complex, multistep process that includes numerous time-dependent events occurring at the molecular, synaptic, electrophysiological, and structural organization levels. The scope of neuroplasticity is wide, ranging from short-term weakening and strengthening of existing synapses, through induction of long-term potentiation (LTP), to formation of long-lasting new neuronal connections. These modifications include subtle changes at the synaptic level (e.g., long-term changes in neurotransmitter release) and formation of new cellular structures (Bruel-Jungerman et al., 2007a, 2007b; Butz et al., 2009; Holtmaat and Svoboda, 2009; Muller et al., 2002; Theodosis et al., 2008). Functional changes at the synaptic level are thought to be more frequent and rapid than the formation of new cellular components (structural plasticity) (Bruel-Jungerman et al., 2007a). The timescale of structural plasticity is largely unknown; however, whereas neurogenesis and gliogenesis seem to happen within days, local morphological changes (formation of new synapses and dendrites on existing neurons) are thought to occur on shorter timescales (Bruel-Jungerman et al., 2007a; Butz et al., 2009; Holtmaat and Svoboda, 2009; Lamprecht and LeDoux, 2004; Matsuzaki et al., 2004; Muller et al., 2002; Theodosis et al., 2008). Neuronal implementation of a new long-lasting cognitive skill acquired over a long period (weeks or months) will necessarily induce such structural changes. Little is known, however, about the magnitude of these changes on a short timescale of learning (minutes to hours). Although invasive microscopy procedures were able to detect regional structural changes following short-term neuroplasticity (Xu et al., 2009; Yang et al., 2009), these effects were not detectable so far by noninvasive techniques such as magnetic resonance imaging (MRI) and for the whole brain.

Structural plasticity, which accompanies the neurophysiological aspects of neuroplasticity, is traditionally studied using postmortem histological procedures (Lamprecht and LeDoux, 2004; Theodosis et al., 2008). An alternative to histology is the use of in vivo structural imaging, a field that is becoming more popular in studies of the dynamic characteristics of neuroplasticity (Holtmaat et al., 2009; Holtmaat and Svoboda, 2009; Lamprecht and LeDoux, 2004; Muller et al., 2002). Although single components of neural tissue can be followed up by two-photon microscopy, a more comprehensive and regional characterization of neuroplasticity can be obtained with MRI. In previous MRI studies on structural plasticity induced by cognitive experience, the focus was on long-term training (weeks to months) (Blumenfeld-Katzir et al., 2011; Boyke et al., 2008; Draganski et al., 2004; Lee et al., 2010; Lerch et al., 2011; Münte et al., 2002; Scholz et al., 2009). Those studies raised new questions about neuroplasticity and its characteristics. What, for example, is the relationship between the gross MRI changes and histological observations? Can structural changes at the synaptic level account for the significant regional volumetric changes disclosed by MRI? And can MRI detect structural tissue remodeling over short timescales? With these questions in mind, we set out to explore experience-driven structural changes (remodeling) of neuronal tissue over a timescale of hours rather than days or weeks. We discovered that 2 hr of training on a spatial navigation task results in MRI changes indicative of structural plasticity in specific brain regions.

MRI provides a handful of qualitative and quantitative structural measures. The most commonly used is $T_1$-weighted imaging, which provides the best contrast for studies of gross anatomy (macrostructure). Recent studies have addressed the relationship between tissue microstructure and cognitive performance using diffusion MRI (Klingberg et al., 2000; Moseley et al.,

2002; Sasson et al., 2010), a technique considered to be a microstructural probe. Diffusion tensor imaging (DTI), a framework of diffusion MRI, provides a multitude of quantitative indices that reflect the micron-scale density and organization of the tissue (Assaf and Pasternak, 2008; Basser, 1995). Indices derived from DTI include the mean diffusivity (MD) and fractional anisotropy (FA), which serve respectively as measures of tissue density and fiber organization/directionality (Pierpaoli and Basser, 1996).

In this study, we used DTI to detect structural changes in brain tissues of individuals after they had performed a spatial learning and memory task based on a computer car race game (Electronic Arts). A cohort of 46 volunteers was divided into a learning group and 2 control groups. The learning group (n = 17) repeated a single track 16 times, divided into 4 sessions of 4 trials each. Their objective was to learn the track and achieve better lap times. To enhance memorization, at the end of each session, subjects were given snapshots of locations in the track, which they had to arrange in the correct order. In addition they were asked to sketch an outline of the track at the end of each session. Subjects were engaged in the overall task for 90 min on average. Each subject underwent a DTI scan before and immediately after the task (i.e., the interval between the two scans was approximately 2 hr).

Because the task included procedural learning (control of the car) as well as spatial learning and memorization, a group of 15 subjects was used to control for this aspect. These subjects played the car game for the same duration as the learning group, but the track was different in each trial. Therefore, compared to the learning group, the memorization of a single track was limited, and spatial learning was apparently attenuated. The second control group (n = 14) did not perform any task, and waited between scans for the equivalent duration of the car racing tasks.

## RESULTS

### Short-Term Learning Structural Plasticity

All subjects in the learning group showed improvement in the task. Their lap times decreased significantly (Figure 1; decrease in normalized lap time [mean ± SEM] was 20% ± 0.4%, $p < 0.0001$; for absolute values see Figure S1A available online), and their arrangement of snapshots improved ($p < 0.0001$; Figure S1). The active control group showed no improvement in their normalized lap time (Figure 1). In order to compare between the two groups that played the computer game, we used the normalized average score in the first and last sessions as a measure of performance. Repeated-measures analysis of variance (ANOVA) was conducted for this measure, with learning versus active control group as a categorical factor. This analysis revealed a significant group by time interaction effect ($F(1,29) = 4.59$; $p < 0.05$) because the learning group showed improvement, whereas no changes were observed for the active control (Figure S1B).

In order to characterize the effect of the task in the learning group and compare it with the control groups, three statistical analysis procedures were performed.

(1) t test: A voxel-wise paired t test between the pre- and post-FA and MD maps of the learning group only was used to assess the regional brain changes that occurred in this group due to the task.

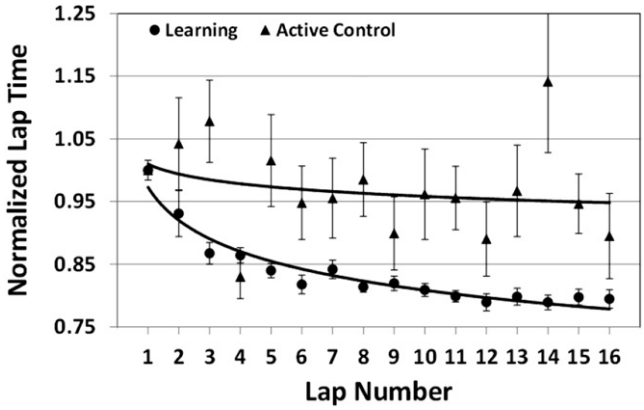


**Figure 1. Behavioral Data**

This figure shows the normalized lap time for each trial averaged for the learning and active control groups (mean ± SEM). In the learning group most of the improvement in performance is achieved within the first session. Smaller improvements are observed in the following sessions, despite the fact that those changes require a substantial increase in skill (control of the car and memorizing the track). In the active control group, no significant improvement in performance was observed.

See also Figure S1.

(2) Planned comparisons: Following previous experiments on long-term memory of spatial learning task in rats (Blumenfeld-Katzir et al., 2011; Lerch et al., 2011) and the behavioral data of the current study, we could assume two patterns of DTI changes. The first is that MD reduction or FA increase will occur in the learning group, and not in any of the controls; the second is that MD reduction or FA increase will occur mostly in the learning group, to a lesser extent in the active control, and none in the passive control (linear effect). To test these hypotheses, we employed learning versus control group ad hoc-planned comparisons with respect to scan time with weighted contrasts that check for the aforementioned hypotheses.

(3) ANOVA: a three (experimental groups) by two (scan time) mixed design ANOVA with repeated measures on the second factor was performed to search for a time by group interaction effect. We used this analysis to verify that our ad hoc comparison hypotheses indeed represent the tissue changes and that there are no other regional effects unrevealed.

Figure 2 shows the results of the aforementioned statistical analyses. In all statistical analyses we report only on regions where significant differences were obtained after correction for multiple comparisons (Supplemental Experimental Procedures); for purposes of illustration, however, we also show maps where differences were significant without such correction. The analyses indicated the following regional changes in the learning group: reduction in MD in the left hippocampus (Figures 2A and 2E) and the left and right parahippocampus (Figures 2F and 2J). These results were found also in another cohort of subjects that performed the same task (replica; Figure S2A). Similar analysis was conducted on FA maps (Table S1) in which effect (increase in FA) was found in the left parahippocampus,

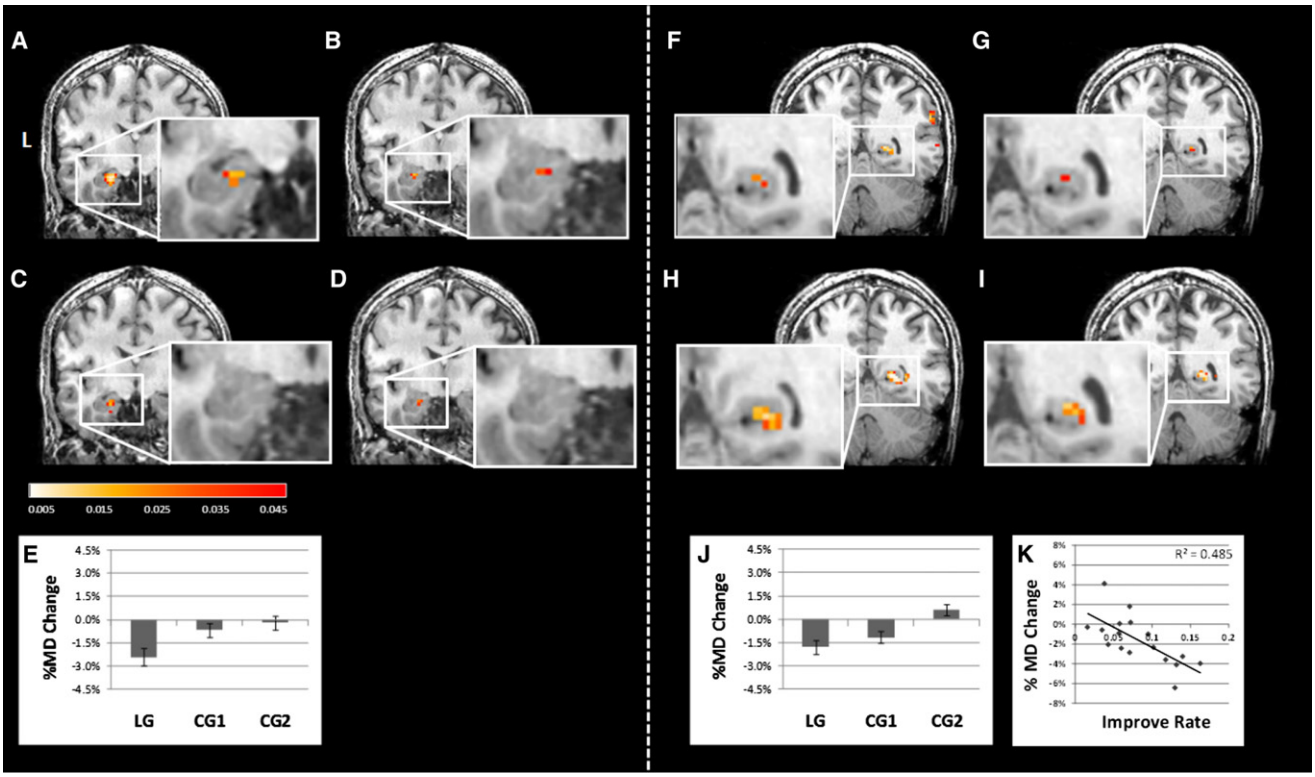


**Figure 2. Structural Remodeling of Brain Tissue, Measured by DTI as Changes in MD after 2 hr of Training on a Spatial Learning and Memory Task**

The following statistical analyses were employed: paired t tests between the MD maps before and after the task in the learning group (A and F); planned comparisons analysis of the learning versus control groups with respect to scan time with predicated effect in the learning group only (B and G); and linear effect between groups (C and H) as well as a group by time interaction following ANOVA (D and I). The effects were found in the left hippocampus (A–D) and right parahippocampus (F–I). The parametric maps in these images were generated at a significance level of $p < 0.005$ (uncorrected). The enlarged subset in those images indicates the significant voxels following correction for multiple comparisons ($p < 0.05$, corrected). In the enlarged subset the corrected p value color scale is between 0.005 and 0.05. L indicates the left side of the brain. (E) and (J) show the MD values in the clusters in the subset of (A) and (F) (mean ± SEM). (K) shows the correlation analysis between subjects' improvement rates (see Figure 1) and decrease in MD in the right parahippocampus (of the cluster in F).
See also Figure S2 and Table S1.

right supramarginal/angular cortex, right superior temporal gyrus, right amygdala, and left pulvinar. The planned comparison analysis (with the learning versus control group contrast) indicates that the learning group MD reduction is significantly different from the control groups in both the hippocampus and parahippocampus (Figures 2B and 2G).

Although the behavioral results indicate that the active control group did not significantly improve in its performance, task-related brain changes in this group may have occurred that are not reflected by our behavioral measures. The linear effect planned comparisons (Figures 2C and 2H) in which the control groups are differently weighted test this issue. Indeed, this analysis shows that the effect in the hippocampus and parahippocampus is slightly different. Although in the hippocampus both control groups do not show any effect, in the parahippocampus the active control group is different from the passive one. There, as can be also seen in Figure 2J, is a reduction in MD, although not as large as in the learning group.

We have also performed a group by time interaction (Figures 2D and 2I), which was found to be significant in the parahippocampus. It is noteworthy that other regional effects were not observed in this analysis, indicating that the planned comparisons contrasts adequately represent tissue changes in our study design.

In the regions found to change in the planned comparisons test, correlation analysis between the behavioral measures and the pre- and post-task differences in DTI measures was performed. This analysis revealed significant negative correlations between improvement rates in the car racing task and MD reduction in the left hippocampus ($r = 0.49$; $p < 0.05$) and right parahippocampus ($r = 0.70$; $p < 0.005$; Figure 2G). The improvement rate and starting performance (lap time in the first trial) were found to be highly correlated ($r = 0.84$; $p < 0.001$). Therefore, we performed partial correlation between MD reduction and improvement rate controlling for the starting performance. In this analysis the parahippocampus showed significant correlation ($r = 0.56$; $p < 0.05$).

## Verification of Observations

Further analysis excluded the possibility that our observations (Figure 2) were derived from artifact bias caused by image

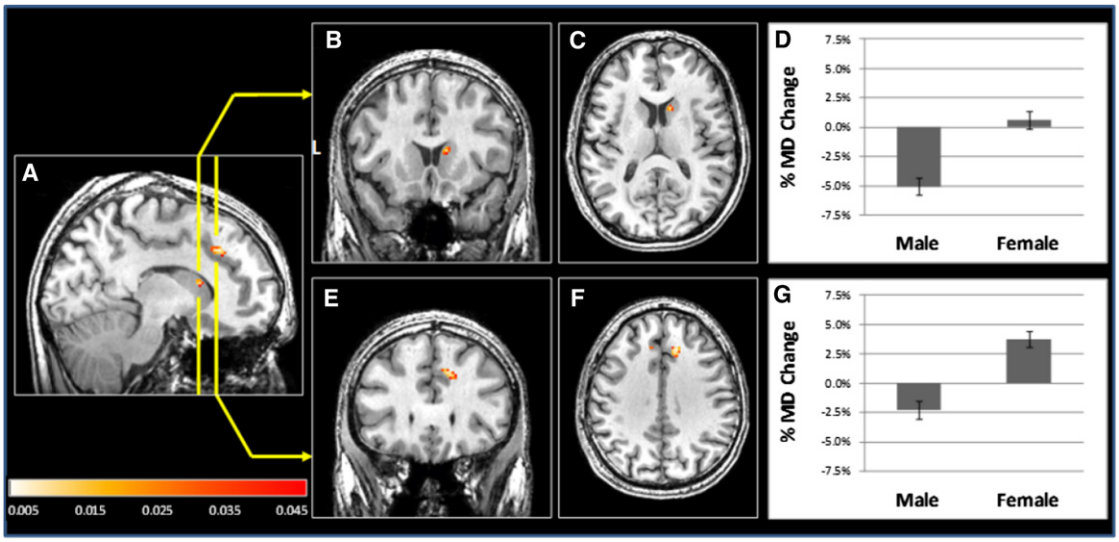


**Figure 3. Gender Effect**

DTI shows gender-related changes in the caudate head and the superior frontal gyrus after 2 hr of training on a spatial learning and memory task. (A–C) and (E–F) are parametric MD maps of the interaction effect of a 2 × 2 repeated-measures ANOVA, with gender and scan time as factors. The parametric maps are corrected for multiple comparisons ($p < 0.05$, corrected). (A) Sagittal slice depicting clusters in the right caudate and right superior frontal gyrus. These regions are also shown in (B) and (E) in coronal sections and in (C) and (F) in axial sections.(D) and (G) depict the interaction effect of the ANOVA between MD changes and gender (mean ± SEM). Note that in the caudate the interaction is due to an effect in males, whereas in the superior frontal gyrus, it is due to an effect in females. L indicates the left side of the brain.

preprocessing and the registration and normalization procedures (Supplemental Experimental Procedures; Figures S2B and S2C). This included overlaying our results on a single-subject FA map to verify that the effect does not include border regions between gray and white matter (Supplemental Experimental Procedures; Figure S2B). In addition, we verified the MD reduction in the hippocampus by region of interest analysis in the native space of each subject (Supplemental Experimental Procedures; Figure S2C).

To verify the statistical analysis (performed with parametric test), in addition to the paired t test, we performed the nonparametric Wilcoxon signed-rank test on the whole brain. This test is applicable if the distribution of the data is unknown, and is less sensitive to outliers than the paired t test. The same statistical threshold ($p < 0.05$, corrected) was used for both tests, and both yielded similar results, namely a decrease in MD and an increase in FA in the same regions (data not shown).

To verify that the diffusion changes do not originate from volumetric or residual blood flow/activity traces, we performed voxel-based comparison of T1 and T2* maps (Supplemental Experimental Procedures) that were measured on the replication group. Voxel-based morphometry (VBM) analysis of the $T_1$ scans before and after the task did not reveal any affected brain regions excluding the possibility that the DTI observations are due to gross anatomical changes in the tissue. Voxel-based analysis (VBA) of the T2* maps before and after the task did not reveal any significant changes excluding the possibility that the DTI observations are due to changes in tissue susceptibility that may be caused by traces of neuronal function or blood vessel volume.

## Gender Effect

The learning group was composed of young individuals of both genders. Behaviorally, no significant difference in improvement between the genders was obtained. However, it should not necessarily be inferred that the brain mechanisms that underlie the behavioral results were similar (Schweinsburg et al., 2005; Speck et al., 2000). Thus, to test for gender differences in regional structural plasticity, we performed a 2 × 2 repeated-measures ANOVA of gender by scan time. Parametric maps of the interaction between gender and scan time for the learning group reveal differences in regional changes between the two genders (Figure 3): the MD is decreased in the right caudate head in males but not in females (Figure 3D), and increased in the superior frontal gyrus in females but not in males (Figure 3G).

## Supporting Rat Experiment

Further investigation of the biological correlates of the DTI changes observed in humans necessitates an animal study with similar short-term memory protocol. Previous studies on rodents focused on long-term training (Blumenfeld-Katzir et al., 2011; Lerch et al., 2011). In order to provide supporting biological relevance to the current human study, we conducted a short-term water maze study on rats. A cohort of 24 rats underwent two MRI scans 1 day a part. Between the MRI scans a water maze task was performed including 12 trials performed within 2 hr. As in the human study, two control groups were also examined: a passive group that did not perform any task between the MRI scans, and a cued group that performed the water maze but with a visible platform (for more details see Supplemental Experimental Procedures; Figure S3). In the statistical analysis (same

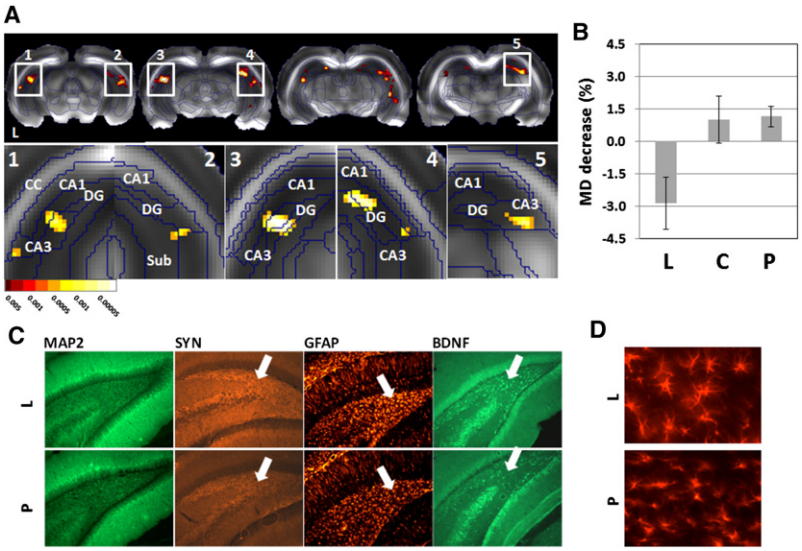


**Figure 4. Structural Remodeling of the Rat Hippocampus following 2 hr of Water Maze Task**

Planned comparisons analysis between the MD maps before and after the water maze task in learning group (L) versus the cued control (C) and the passive control (P) groups shows effects in both hippocampi, mostly in the posterior part (A).

(A) The parametric maps in the top row of (A) were generated at a significance level of $p < 0.005$ (uncorrected). The enlarged maps in the bottom row of (A) show the same analysis but with correction for multiple comparisons ($p < 0.05$, corrected).

(B) shows the results of planned comparison analysis for a representative cluster (A1) indicating MD decrease in L group with no apparent change in any of the control groups (mean ± SEM).

(C) shows immunohistochemical images (at magnification level ×10) for the following markers: MAP2, Synaptophysin (SYN), GFAP, and BDNF for one slice at the posterior hippocampus showing the hilus of the dentate gyrus for representative rats from the learning (L) and passive control (P) groups. Although no apparent difference in the immunoreactivity of MAP2 was found between the two groups, for the SYN, GFAP, and BDNF stainings, the immunoreactivity in the L group was much higher than in the P group. The most pronounced effect was observed in the GFAP staining (for numerical values, please refer to Figure S8). Arrows indicate areas with high immunoreactivity in the learning group compared with the control.

(D) shows the GFAP staining at magnification level ×40 depicting the significant morphometric change that the astrocyte underwent in the L group versus the P group.

See also Figure S3.

as in the human study), we found MD decrease in the posterior parts of the hippocampus (Figures 4A and 4B). Histological analysis of the brain following the second MRI scan revealed an increase in the immunoreactivity of the following markers in the learning group compared with the control group: synaptophysin, glial fibrillary acidic protein (GFAP), and brain-derived neurotrophic factor (BDNF) (Figures 4C and S3). No immune-reactivity differences were observed when staining for microtubule-associated protein 2 (MAP2), a marker of dendrites. This result indicates that within the regions of MD decrease, the following occurred: an increase in the number of synaptic vesicles, astrocyte activation (reflected also by increase in the number of astrocytic processes; Figure 4D), as well as increase in BDNF expression, which may be indicative of LTP.

## DISCUSSION

The results of this study indicated that short-term learning (2 hr) in humans leads to significant changes in diffusion MRI indices. This surprising observation was strengthened by a rigorous statistical analysis, was repeated in a replica of the study (Figure S2A), and was obtained in a supporting study in rats (Figures 4 and S3). It is reasonable to assume that this MRI observation reflects structural aspects of neuroplasticity. Because DTI can be considered to be a marker of tissue microstructure, structural remodeling of the tissue will lead to a change in its water-diffusion properties (Assaf and Pasternak, 2008; Barazany et al., 2009; Blumenfeld-Katzir et al., 2011; Scholz et al., 2009). These diffusion changes are likely to be manifested as an increase in tissue density (due to reshaping of neuronal or glial processes), or enhancement of tissue organization (strengthening of axonal or dendritic backbones and surrounding tissue; Assaf and Pasternak, 2008). Reports indicate a strong link between structural changes (neuronal or cellular or both) and diffusion indices. The best studied of these links is the reduction in MD after stroke (Assaf, 2008; Benveniste et al., 1992; Johansson, 2004; Le Bihan et al., 2001), attributed to swelling of cells in this pathological condition. Another indication is the transient MD reduction following neuronal depolarization (Darquié et al., 2001; Latour et al., 1994).

### Localization of Neuroplasticity

The localization of the structural changes that we traced in this study, though expected, nevertheless, has some surprising aspects. Animal and human studies in vivo, as well as histological, functional, and anatomic observations, point to a central role of the hippocampus in short-term memory processes (Bliss and Collingridge, 1993; Bruel-Jungerman et al., 2007a, 2007b). The main finding of our study is indeed in line with such knowledge of hippocampal function. In addition, structural changes are shown here, as expected, in other parts of the limbic system, namely the parahippocampus, amygdala, and other temporal regions (Table S1). The paired t test of the learning group only indicated some other regions that might be related to the task but have not been found in planned comparisons that included the control groups. These regions include some parietal and frontal regions and the insula. The exact meaning and relevance of these regions to spatial navigation need further studies, but the literature may suggest that those are not unrelated to the task as was used in the current study (Maguire et al., 1999). Additional noteworthy observation is that the left hippocampus appears to be more strongly affected than the right. However, a similar effect was also found in the right hippocampus when analyzed using region of interest approach (data not shown),

indicating that both hippocampi have a role in the task used in this study and that laterality is not significant.

Another interesting regional observation was the gender effect in the caudate head. The dopaminergic system, which is active in decision making and error prediction, functions differently in this region between genders (Becker, 1999). Although decision making was an aspect of the task in the present study, it was not quantified here. The decrease in MD seen in the caudate for males but not for females points to a gender-related difference in the effect of the behavioral task on this region. This finding raises the question of the gender effect on the memory mechanism and the role of hippocampal versus stimulus-response learning (Xu et al., 2009). However, the task used in this study could not dissociate the underlying different memory mechanisms. Although it is tempting to speculate on the cellular origins of this striking result, a more meaningful discussion would require a study designed to yield quantitative data on the behavioral aspects of the gender effect.

## Statistical Considerations

This study included a design of three experimental groups and two scan times. When hypothesizing the direction of effect, the most appropriate analysis of such design is ad hoc-planned comparisons (as shown in Figures 2B, 2C, 2G, and 2H). According to previous experiments (Blumenfeld-Katzir et al., 2011; Lerch et al., 2011) and the behavioral data, we examined two hypotheses using a learning versus control group contrast and a linear contrast (see Results and Experimental Procedures). To verify that no other effects that are not hypothesized in our planned comparisons test are present, an ANOVA test (Figures 2D and 2I) was also performed indicating that indeed our hypotheses are valid.

## Effect across Species

To strengthen our observations, we carried out a supporting experiment in rodents. Similar studies of that kind were performed before but focused on long-term memory (Blumenfeld-Katzir et al., 2011; Lerch et al., 2011). The molecular as well as the structural mechanisms of short- and long-term memory are different (Lamprecht and LeDoux, 2004). Therefore, the relevance of previous rodent studies to this human study is limited. Although significant structural plasticity is expected following long-term learning procedures, it should be demonstrated that even short-term training leads to significant structural effects that can account for the DTI changes reported here on humans. Thus, in order to provide appropriate rodent data to this human study, we conducted a short-term memory experiment on rats. As in the human study, we scanned three groups of rats twice using a DTI protocol. The first group (learning group) underwent a short spatial memory test (water maze task) of 2 hr; the second group (cued group) underwent a cued test in which the effects of memory were minimized, and the third group was not required to perform a task (passive group) (for a comprehensive description of the rat experiment, please refer to Supplemental Experimental Procedures, section 2 [Methods (rats experiment)]). A similar statistical analysis to the one employed in the human study disclosed a decrease in MD in both hippocampi of the learning group rats, with no effect in the other groups (Figure 4). This result is similar to the findings in humans (Figure 2), indicating that the phenomenon is observed across species.

## The Biological Correlates of DTI Observations

The relationship between structural remodeling and changes in MD or FA is complex and does not lend itself to intuitive explication. Some studies indicated that acute neuronal activity may lead to MD changes (Darquié et al., 2001); however, in this study we excluded the possibility that trace of neuronal activity at the time of the scan is the source for the observations. Understanding which cellular processes lead to a decrease in MD and which lead to an increase in FA is not feasible in a human study. At the most basic level, such changes in healthy tissues may be related, respectively, to an increase in tissue density and in fiber organization. The extracellular matrix could conceivably serve as a mediator between MD reduction and tissue remodeling. Previous studies have indeed indicated that changes in the extracellular matrix following structural tissue remodeling might be responsible for changes observed in the diffusion properties of the tissue (Benveniste et al., 1992; van der Toorn et al., 1996). Possible structural manifestations of these changes are synaptogenesis, changes in the morphometry of axons, dendrites, and glial processes, and alterations in cell body size and shape (Blumenfeld-Katzir et al., 2011; Lerch et al., 2011). Indeed, the histology performed in the supporting rat study as well as previous studies on long-term memory (Blumenfeld-Katzir et al., 2011; Lerch et al., 2011) revealed significant physiological and morphological effects induced by spatial learning procedures. Although the histology in the current study was performed 1 day following the task, increase in BDNF level (which may be indicative of LTP) as well as in the amount of synaptic vesicles (reflected by the immunoreactivity of synaptophysin) was observed. It is unlikely that DTI is sensitive to structural changes at the level of existing synapses (due to their small volumetric contribution). It is more likely that other cellular changes, which accompany the formation or reshaping of synapses, make more sizeable contributions to the observed changes. Indeed, the histological analysis revealed a robust change in the activation of astrocytes indicated by increased levels of GFAP immunoreactivity and remodeling of the glial processes (Figures 4C, 4D, and S3). This histological evidence might suggest tissue (cellular) swelling or changes in the ratio between intra/extracellular volumes following long episodes of neural activation (Le Bihan, 2007; Theodosis et al., 2008) that may be the base of MD reduction. More studies on the relation between cell swelling following neural activation and diffusion changes should explore this hypothesis.

Correlation analysis reveals that the magnitude of changes in the right parahippocampus is correlated with an improved rate of task performance, suggesting that individual microstructural changes (as measured by MRI) in this specific region are indicative of improvement in the task. This observation suggests that structural remodeling is strongly related to ability to improve in the task. It is not surprising, therefore, that longer periods of training lead to gross volumetric changes in the tissue both in humans (Draganski et al., 2004) and rodents (Lerch et al., 2011). However, volumetric changes were not found in the current short-term memory study.

Because DTI follow-up examinations point to microscopic rearrangement in the density and organization of cellular structures, DTI findings may be indicative of sites of induction of LTP (Matsuzaki et al., 2004; Muller et al., 2002). Such microscopic rearrangements, related to short-term plasticity, are known to occur following or concomitantly with LTP induction. In addition the rat study showed that within the regions of MD decrease, an increase in BDNF, a marker of LTP, was observed. Thus, we argue that DTI examinations before and after cognitive tasks can be used to indirectly localize microstructural changes that might be indicative of LTP. Combined electrophysiological and MRI studies are needed in order to back up this hypothesis.

## Conclusions

The microstructural correlates of diffusion imaging are not well understood, and further studies should be directed at elucidating the exact relationship between them. Nevertheless, given the ability to follow tissue plasticity with DTI, and particularly over such short timescales, it should be possible to gain new insights into the dynamics of structural brain plasticity. Although not proven, it is possible that the structural changes obtained by DTI are related to long-lasting electrophysiological effects such as LTP, and further study should be directed toward exploring this relationship. In summary, DTI may offer a novel measure of short-term brain plasticity, better characterization of the underlying tissue changes in the tissue, and as a consequence, new insights into the dynamics of learning and memory.

## EXPERIMENTAL PROCEDURES

All experiments in this study were approved by the relevant ethics committee for humans (institutional review board) or animals (institutional committee on animal care and use).

### Subjects

The study participants were 46 healthy adult volunteers (21 males and 25 females, all right-handed). The age range was 20–36 (mean, 26.7; SD 3.5). The subjects were divided into 3 experimental groups: learning group (n = 17, 8 males and 9 females, mean age 26.8); a nonspatial learning group (n = 15, 7 males and 8 females, mean age 26.9); and a passive control group (n = 14, 6 males and 8 females, mean age 26.1). The research protocol was approved by the Institutional Review Board of the Tel Aviv Sourasky Medical Center. All participants signed an informed consent form. None of the subjects had a history of neurological disease, psychological disorders, drug or alcohol abuse, or use of neuropsychiatric medication. All had intact vision.

### Learning Tasks

Tasks were based on a computer car racing game (Need for Speed; Electronic Arts). The learning group underwent a learning task consisting of 16 laps (trials) of the same car game track, divided into 4 sessions. Their objective was to learn the track and achieve better lap times. To enhance memorization, at the end of each session, subjects were given snapshots of locations in the track and were required to arrange them in the correct order. In addition they were asked to sketch an outline of the track at the end of each session.

The control groups were of two types. For the first control group, the task was similar to that of the learning group, except that the track was different in each trial. Therefore, memorization of a track was limited, and spatial learning was attenuated. The second control group did not perform any task but waited for the equivalent duration of the car racing tasks between the two MRI scans.

### Normalized Lap Time

Because the active control group played a different track in each trial (different lengths and scenery), evaluating their improvement during the task necessitates normalization. This normalization was crucial because the tracks were randomized between active control subjects. The normalization procedure, performed for each subject, included normalizing the lap time to the track length and dividing by the performance in the first trial. The same procedure was applied to the learning group.

### MRI Acquisition

MRI was performed at the Tel Aviv Sourasky Medical Center with a 3T (GE, Milwaukee, WI, USA) MRI system. All subjects underwent two series of scans approximately 2 hr apart. Between the two sessions a task was administered to the learning group and the first control group; the second control group did not perform any task. The MRI protocol of the first series of scans included conventional anatomy sequences, and DTI was acquired with an eight-channel head coil. In the second series only DTI scans were administered.

### Conventional Anatomic Sequences

$T_1$-weighted images were acquired with a 3D spoiled gradient-recalled echo (SPGR) sequence with the following parameters: up to 155 axial slices (whole-brain coverage), TR/TE = 9/3 ms, resolution 1 × 1 × 1 $mm^3$, scan time 4 min. In addition to the $T_1$ scan, $T_2$-weighted images (TR/TE = 6,500/85) and FLAIR images (TR/TE/TI = 9,000/140/2,100) were acquired. The entire anatomical data set was used for radiological screening.

### DTI Protocol

Double-refocused, spin-echo diffusion-weighted, echo-planar imaging sequences were performed with up to 70 axial slices (to cover the whole brain), and resolution of 2.1 × 2.1 × 2.1 $mm^3$ was reconstructed to 1.58 × 1.58 × 2.1 $mm^3$ (field of view was 202 $mm^2$, and acquisition matrix dimension was 96 × 96 reconstructed to 128 × 128). Diffusion parameters were $\Delta/\delta$ = 33/26 ms; b value of 1,000 s/$mm^2$ was acquired with 19 gradient directions, and an additional image was obtained with no diffusion weighting ($b_0$ image). The double-refocused sequence was used in order to minimize eddy currents and susceptibility artifacts. The DTI scan was repeated three times to increase signal-to-noise ratio. For details on the DTI analysis routine, please refer to section 1.2 (Image analysis) of the Supplemental Experimental Procedures.

### Statistical Analysis

VBA is a whole-brain technique that allows regionally specific differences in quantitative MRI indices (such as FA or MD) to be computed on a voxel-by-voxel basis. The statistical VBA design included three groups (learning and controls) and two scan times (with repeated measures on the second factor). On this design we applied the following procedures.

(1) A paired t test on the learning group only (comparing the pre and post-learning scans).
(2) Planned comparisons to examine the following hypotheses: A contrast of learning versus control effect hypothesizing that microstructural changes occurred in the learning group, and not in any of the control groups. In this test the contrast weights on the experimental groups were 2 −1 −1 and 1 −1 on the scan time factor. A linear effect contrast between the experimental groups (learning > active control > passive control) with respect to scan time. In this test the contrast weights on the experimental groups were 1 0 −1 and 1 −1 on the scan time factor.
(3) An ANOVA group by time interaction.

To avoid partial volume bias in the statistical analysis, we applied a non-cerebrospinal fluid (CSF) mask. Segmentation was performed (using the standard routine in SPM software) on each subject's normalized MD images. Voxels with a probability of 0.2 of containing CSF in any of the subjects were excluded from the non-CSF mask, which was applied to the statistical maps as an explicit mask. In that way, areas of partial volumes, such as those surrounding the ventricles and the borders around the cortex, were masked out.

The sequential Hochberg correction (Hochberg, 1988) was used to correct for multiple comparisons. This procedure uses a step-up ranking of the

p values and then corrects for the p value threshold by dividing it by the rank of the comparison. A voxel was considered significant only if it exceeded the corrected statistical threshold ($p < 0.05$). The statistical parametric maps are superimposed on a template T1 image, providing an anatomical informative reference.

In addition, for the learning group, we performed a mixed-design ANOVA of 2 × 2 (gender × scan time) with repeated measures on the second factor. This design allowed us, by observing the interaction effect, to identify voxels that were changed differently over time for the males and females in the learning group.

## SUPPLEMENTAL INFORMATION

Supplemental Information includes three figures, one table, and Supplemental Experimental Procedures and can be found with this article online at doi:10.1016/j.neuron.2012.01.025.

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