White Matter Integrity of Specific Dentato-Thalamo-Cortical Pathways is Associated with Learning Gains in Precise Movement Timing

Robert Schulz, Maximilian J. Wessel, Máximo Zimerman, Jan E. Timmermann, Christian Gerloff and Friedhelm C. Hummel

Brain Imaging and Neurostimulation (BINS) Laboratory, Department of Neurology, University Medical Center Hamburg-Eppendorf, 20246 Hamburg, Germany

Address correspondence to Dr Friedhelm C. Hummel, Department of Neurology, University Medical Center Hamburg-Eppendorf, Martinistr. 52, 20246 Hamburg, Germany. Email: f.hummel@uke.de

The dentato-thalamo-cortical tract (DTCT) connects the lateral cerebellum with contralateral motor and nonmotor areas, such as the primary motor cortex (M1), the ventral premotor cortex (PMv), and the dorsolateral prefrontal cortex (DLPFC). As the acquisition of precisely timed finger movements requires the interplay between these brain regions, the structural integrity of the underlying connections might explain variance in behavior. Diffusion tensor imaging was used to 1) reconstruct the DTCT connecting the dentate nucleus with M1, PMv, and DLPFC and 2) examine to which extent their microstructural integrity (tract-related fractional anisotropy) relates to learning gains in a motor-sequence learning paradigm consisting of a synchronization and continuation part. Continuous DTCT were reconstructed from the dentate nucleus to all cortical target areas. We found that the microstructural integrity of the DTCT connecting the left dentate nucleus with the right DLPFC was associated with better early consolidation in rhythm continuation ($R = -0.69$, $P = 0.02$). The present data further advances the knowledge about a right-hemispheric timing network in the human brain with the DLPFC as an important node contributing to learning gains in precise movement timing.

Keywords: cerebello-thalamo-cortical tract, diffusion, FA, sequence, timing

Introduction

Perceptual and productive timing processes require the interplay between the cerebellum, cortical and subcortical brain regions. Neuroimaging (Penhune et al. 1998; Ivy et al. 2002; Lewis and Miall 2003; Wiener et al. 2010), lesion (Ivy et al. 1988; Gooch et al. 2010), and electrophysiological studies (Del Olmo et al. 2007) have confirmed the particular role of the lateral cerebellum in fast motor-timing down to the subsecond range.

The dentato-thalamo-cortical tract (DTCT) connects the lateral cerebellum with contralateral motor areas, such as the primary motor cortex (M1) and ventral premotor cortex (PMv) as well as nonmotor areas such as the dorsolateral prefrontal cortex (DLPFC) (Middleton and Strick 1994, 2001; Hoover and Strick 1999; Dum and Strick 2003). These brain regions are not only involved in mere performance of timing (Halsband et al. 1993; Rao et al. 1997; Onoe et al. 2001; Schubotz and von Cramon 2001; Constantinidis et al. 2002; Lewis and Miall 2003, 2006a, 2006b; Smith et al. 2003; Verstynen et al. 2006; Chen et al. 2009; Wiener et al. 2010), but are also important for the acquisition of novel motor skills (Doyon et al. 1996, 2003; Hikosaka et al. 2002; Floyer-Lea and Matthews 2005; Lehéricy et al. 2005; Steele and Penhune 2010).

Learning in the temporal domain, that is the acquisition of precisely timed finger movements with variable time intervals and one constant effector, has only been scarcely addressed. Imaging data have suggested the involvement of the lateral cerebellum (Ramnani and Passingham 2001; Schubotz and von Cramon 2001; Sakai et al. 2002; Penhune and Doyon 2005) in the early phase of learning (Penhune and Doyon 2002, 2005) and overnight consolidation (Lewis et al. 2011). Delayed recall of novel timing skills has involved M1 and premotor cortices as well as parietal cortical areas (Penhune and Doyon 2002). The DLPFC has been found to be relevant when the cognitive demand was increased (Sakai et al. 2002). Numerous studies have associated the DLPFC with planning and spatial working memory processes (Smith and Jonides 1999; Wager and Smith 2003). However, particularly, the right DLPFC has been increasingly considered an important node within a right-hemispheric timing network, not only for the integration of time and working memory (Onoe et al. 2001; Constantinidis et al. 2002; Lewis and Miall 2003; Smith et al. 2003; Wiener et al. 2010), but also for primary time estimation (Lewis and Miall 2006a, 2006b) in cognitive timing processes among others for coordinated movements.

The structural integrity of the underlying network pathways is an important basis for cerebellar–cortical information throughput and might explain at least some of the behavioral variance between healthy participants during motor skill acquisition: Preferred learning in a visuomotor grip force task has been related to the microstructure of precentral and cerebellar white matter (WM) adjacent to the dentate nuclei (Tomassini et al. 2011). The level of synchronization after days of rhythmic finger tapping training has recently been associated with the microstructural integrity of WM underlying the sensorimotor cortices (Steele et al. 2012). Similarly, the integrity of prefrontal–subcortical connections has been found to predict success in implicit sequence learning (Bennett et al. 2011). However, in regard of the acquisition of precisely timed finger movements and the integrity of the different DTCT pathways, this specific structure–function relationship has still not been addressed in detail. Moreover, in regard of cerebellar–cortical connections in the human brain, previous structural imaging studies have focused on either single segments of the tracts (Habas and Cabanis 2007; Yamada et al. 2010; Kwon et al. 2011) or M1 (Yamada et al. 2010) as the only cortical target area specified. To our knowledge, the entire extent of the DTCT has not been assessed in the human brain by structural imaging.

In the present study, diffusion tensor imaging (DTI) was used to 1) reconstruct the whole extent of 3 DTCT connecting the dentate nucleus with M1, PMv, and DLPFC, 2) to quantify the microstructural integrity of these fiber tracts, and 3) to examine how this measure relates to learning gains in precise movement timing.
Materials and Methods

Participants
Twenty right-handed young healthy volunteers (mean age 25 ± 0.6 years, range 20–32 years, 8 males) were recruited for this study. Participants were naive to the experimental purpose, and none of them were professional piano players or trained as typists. None of the participants were taking any CNS-active medication. The study design was approved by the local Ethics Committee of the University of Hamburg. All participants gave their written informed consent according to the ethical declaration of Helsinki.

Learning Task and Behavioral Data Analysis
As a temporal sequence task, we used an established motor synchronization–continuation paradigm adapted from Lewis et al. (2011), which the participants had to perform with the index finger of their dominant hand. Training consisted of 7 blocks (lasting 140 s each) separated by short breaks. Each block was divided in 2 parts; synchronization and continuation (see Supplementary Fig. 1 for visualization). In the synchronization part, the participants had to tap their index finger on a standard computer mouse in line with auditory cues (300 ms each). Two different interstimulus interval sequences have been adopted from a previous study (sequence A was 640-160-560-960-320-400-240-720 ms and B 320-1040-800-160-480-560 ms, Lewis et al. 2011), which were introduced in a pseudorandomized allocation for baseline block or training blocks between participants. In each block, the synchronization part consisted of 8 subsequent trials of the training sequence. In the following continuation part, the participants had to reproduce the preceding temporal sequence without any cues 4 times. After familiarization and 1 baseline block, participants performed 7 blocks of training (T1–T7, 20-min duration). Skill consolidation was assessed in 2 follow-up blocks, F1 90 min (early consolidation) and F2 24 h (delayed consolidation) after the last training block. The stimulus presentation and the recording of the behavioral tapping data were performed with a standard personal computer using Presentation Software (Neurobehavioral Systems, Inc., USA) and analyzed offline. For the behavioral analysis, we calculated the tapping errors. In synchronization, this was the absolute (abs) time interval where the acoustic cue and the key press did not overlap (tapping error [ms] = abs(cueON – keyON) + abs(cueOFF – keyOFF)). For continuation, the played tapping interval (interkeypress interval, IKI) was aligned with the referring interstimulus interval (ISI) to calculate the absolute difference (tapping error [ms] = abs(ISI – IKI), see Supplementary Fig. 1) (Steele and Penhune 2010). Tapping errors were averaged for each block and participant. For further correlative analyses, the relative change in both synchronization and continuation tapping error over time served as behavioral outcomes, which are T1/ T7 for training, F1/F7 for early consolidation and F2/F7 for delayed consolidation. Notably, small numbers indicate reduction in tapping error, hence improvement over time.

Brain Imaging
A 3T Siemens Skrya MRI scanner (Erlangen, Germany) was used to acquire diffusion-weighted images. Axial slices (75; 2-mm3 isotropic, acquisition matrix of 189 × 120 × 80; FOV 256 mm) were also collected along 64 noncollinear directions. The complete dataset consisted of 2 × 64 b1500 images, 2 b0 images were also collected, one at the beginning and the other after the first 64 images. T1-weighted images (1-mm3 isotropic, acquisition matrix of 208 × 256, FOV 256 mm) were also collected.

The image analysis was conducted using the FSL 4.1.9 software package (http://www.fmrib.ox.ac.uk/fsl). After correction for eddy currents, head motion and brain extraction, local diffusion directions were estimated for each voxel using Markov Chain Monte Carlo sampling (Behrens et al. 2007). Fractional anisotropy (FA) maps were calculated using tensor estimation models at each voxel. These individual FA maps were then registered nonlinearly to the Montreal Neurological Institute (MNI) standard space using the available FA template. This transformation was also applied to the anatomical image after its nonlinear co-registration to the individual FA map (in diffusion space). Proper registration and normalization have been checked in every participant by visual inspection before further statistical analysis.

Using probabilistic tractography, we aimed to reconstruct dentato- thalamic-cortical trajectories between the dentate nucleus and contralateral cortical target areas M1, PMv, and DLPFC. The seed mask for the dentate nucleus was manually drawn behind the floor of the fourth ventricle on axial slices in MNI standard space according to published topographical data (Dimitrova et al. 2002, 2006; Diedrichsen et al. 2011), approximately covering a cuboid extending from x = ±90 to x = ±23, from y = ±46 to y = ±64 and from z = ±28 to z = ±42 bilaterally. To allow for increased cortical connectivity variability, the following methodology was used to define individual cortical target regions (see Supplementary Fig. 2 for illustration). First, brain segmentation into WM and gray matter (GM) with subsequent cortical anatomical parcellation was conducted in an automated fashion using raw T1 structural images and the Freesurfer image analysis suite (Fischl et al. 2002). Surface-based cortical GM masks were then transferred to the FSL MNI 1-mm T1 standard space and used to calculate individual masks related to the boundary between WM and GM covering the extent of M1, PMv, and DLPFC. Their extents were determined based on anatomical and connectivity-based suggestiveness (Geyer et al. 1996; Tomassini et al. 2007) as well as borders suggested by functional data (Rushworth and Owen 1998; Mayka et al. 2006) and probabilistic cytoarchitectonic maps (Eickhoff et al. 2005). For DLPFC, we decided to limit its extent to Brodmann area 46 that is approximated as the middle third of medial frontal gyrus with the adjacent rostral portion of the inferior frontal gyrus and the middle frontal sulcus (Rushworth and Owen 1998). Cortical masks were multiplied with binarized, normalized FA maps (thresholded and binarized at FA values > 0.15) to provide target voxels with a reasonable connection to WM trajectories. This value was also consistently found to prevent streamlines crossing corticospinal fluid. Second, in order to bias the fiber reconstruction to M1 towards the hand representation, functional imaging data were included. The 500 nearest voxels within the M1 GM/WM boundary mask adjacent to published peak coordinates (xyz in MNI: ±37, −25, 62 Talarach-MNI converted, Mayka et al. 2006) were selected using an in-house Matlab script (The Math Works, Natick, MA, USA). Recently, this approach has been introduced for the reconstruction of the corticospinal tract (Schulz et al. 2012). For tractography, a thalamic mask (calculated in each individual participant using Freesurfer image analysis suite) and an interhemispheric exclusion mask in the midsagittal plane were used to guide the tract reconstruction. Each individual output connectivity distribution was constrained by 0.1% of the overall successful streamlines (25 000 sent from each voxel in the seed mask) and binarized. Thresholding was applied to calculate the group average for each tract: Only those voxels were kept as part of the common trajectories that were found in at least 65% of the participants as previously applied (Schulz et al. 2012). Tract-related mean FA was calculated based on these tract group averages and correlated with the training gains. Notably, the parts of the tracts located within thalamic GM was not included to calculate mean FA values. In order to confirm a plausible spatial distribution of structure–function relationship along the tract of interest, binarized common trajectories were filled with individual FA values and fed into voxel-wise spatial statistics.

Statistics
Training gains in both synchronization and continuation were evaluated using repeated-measures (RM) ANOVA (Greenhouse–Geisser corrected, within factors BLOCKS T1–T7, F1, F2) with subsequent individual paired Student’s t-tests (T1 vs. T7, F1 vs. T7, and F2 vs. F1). RM-ANOVA (Greenhouse–Geisser corrected, within factors TARGET and HEMISPHERE) with post hoc paired Student’s t-tests were used to compare tract-related FA values. Partial correlation analyses (corrected for age) were conducted between tract-related mean FA and relative improvement (ratios T1/T7, F1/T7, and F2/F1). Statistical significance was assumed at P < 0.05, fully corrected for multiple comparisons via false-discovery rate (FDR) via the Benjamini–Hochberg procedure (Benjamini and Hochberg 1995) as implemented in a freely available Matlab script under the BSD License (http://www.mathworks.com/matlabcentral/fileexchange/27418-benjamini-hochberg-yekutieli-procedure-for-controlling-false-discovery-rate), valid for independent or positively dependent tests.
Results

Probabilistic Tractography of Dentato-Thalamo-Cortical Connections

Probable trajectories connecting dentate nucleus and contralateral target areas M1, PMv, and DLPFC were successfully reconstructed in all 20 participants. Across the group, there was good spatial reproducibility of all 6 trajectories connecting the cerebellar seed mask with the cortical target masks as indicated by the weighted trajectory map (Fig. 1). In agreement with previous reports, the fiber bundles ascended into the midbrain through the ipsilateral superior cerebellar peduncle (SCP) and crossed obliquely and dorsocaudally the mesencephalic tegmentum ($z = -17$ in MNI). Passing around the red nucleus, trajectories ascended and entered the thalamus. Fibers of the DTCT targeting M1 were located most posteriorly in the thalamus and within the adjacent internal capsule. Trajectories to PMv and DLPFC were located more anteriorly. All fibers then entered the corona radiata. Fibers to PMv converged laterally into the anterior part of the precentral gyrus. Fibers to M1 could be followed continuously to the hand knob area in a posterior position. The trajectories to DLPFC continued their course in an anterior direction (Fig. 1). In order to evaluate the quality of the tract reconstruction in more detail, the course of the DTCT targeting M1 was exemplarily assessed at the bicommissural level and above as recently suggested (Kwon et al. 2011). Notably, the ventrolateral thalamic location of the tract was in good agreement with the work of Kwon et al. (Supplementary Fig. 3) and compatible with the course of the DTCT in animals (Holsapple et al. 1991; Hoover and Strick 1999; for a review see Middleton and Strick 2000). Tract-related microstructural integrity did not show any differences between homolog tracts of both hemispheres (for tract-related FA values see Supplementary Table 1).

Behavioral Results

We found a significant improvement in both synchronization ($F_{3.4,65.0} = 68.9, P < 0.001$) and continuation ($F_{3.6,68.3} = 8.5, P < 0.001$) over time. Subsequent testing of the relative temporal components of learning gains revealed a significant reduction in tapping error during training ($T_7/T_1$) for both synchronization and continuation ($P < 0.05$), delayed consolidation of motor skills for synchronization ($F_3/F_1$), and early improvement in tapping error for continuation ($F_7/T_1$, Fig. 2). These 4 temporal components of improvement served as the behavioral measures for the correlative analyses.

Tract-Related WM Integrity and Behavioral Improvement

Correlation analysis revealed a significant association between early consolidation in continuation ($F_7/T_1$) and WM integrity of the DTCT connecting the left dentate nucleus and the right DLPFC ($R = -0.69, P = 0.02$, Fig. 3A, Table 1). Neither gains in active training ($T_7/T_1$) in continuation nor the temporal components of interest in synchronization revealed significant associations with WM integrity of any of the tracts investigated ($P > 0.08$, Table 1). Notably, there was no correlation between tract-related FA and mere motor performance ($T_7, P > 0.14$).

To confirm this tract-related structure–function relationship in a voxel-wise fashion, we aimed to test whether the significant association between tract-related mean FA and early consolidation was paralleled by significant correlations between local FA values along the course of the entire tract connecting the left dentate nucleus and the right DLPFC. Indeed, we found a widespread distribution of clusters with a positive correlation between local FA and behavior throughout the whole tract suggesting that the correlation found for tract-related mean FA was not driven by one single local brain region (such as SCP) which would hinder inference of tract specificity (Fig. 3B).

Discussion

Using DTI, we reconstructed continuous DTCT pathways connecting the dentate nucleus of the lateral cerebellum with the contralateral primary motor cortex (M1), ventral premotor cortex (PMv), and the DLPFC. Previous animal tracing data (Middleton and Strick 1994, 2001; Hoover and Strick 1999; Dum and Strick 2003) have suggested the existence of such connections. To the best of our knowledge, this is the first time to show noninvasive reconstructions of the entire extent of each individual tract from the dentate nucleus to the neocortical targets in the human brain. Previous DTI studies have focused on either single segments of the tracts (Habas and Cabanis 2007; Yamada et al. 2010; Kwon et al. 2011) or M1 (Yamada et al. 2010) as the only cortical target area specified. After successful reconstruction of the DTCT, the aim of the present study was to examine the extent to which tract-related microstructural integrity would relate to learning gains in precise movement timing.

The structural integrity of the network pathways is an important basis for interregional interactions in the human brain. DTI allows not only the noninvasive reconstruction of probable fiber pathways, but also allows the estimation of the microstructural integrity of the underlying WM via FA reflecting its biophysical properties such as the axonal density, diameter, myelination, and orientation coherence of WM bundles (Beaulieu 2002). Precentral and cerebellar FA adjacent to the dentate nuclei has been recently associated with training gains in a visuomotor grip force task in young healthy participants (Tomassini et al. 2011). Two studies have investigated this structure–function relationship in sequence learning: Focusing on the effector domain, it has been shown that tract-related FA of connections between DLPFC and caudate nucleus as well as hippocampus was well correlated with gains in an alternating serial reaction time task (Bennett et al. 2011). Focusing on the time domain (applying a one-finger tapping timing sequence), the level of synchronization reached after days of rhythmic finger tapping training has been correlated with FA of long association fibers such as the superior longitudinal fascicle underlying the sensorimotor cortices as M1 and S1 (Steele et al. 2012).

With the present data evidence for tract-related structure–function relationships between multiple DTCT and training gains in skill acquisition of precisely timed motor sequences is provided. Applying an established finger tapping timing task
with a synchronization and continuation part (Lewis et al. 2011), we found that the microstructural integrity of the DTCT connecting left dentate nucleus and right DLPFC was associated with early consolidation in rhythm continuation.

In agreement with the present results, neuroimaging data (Penhune et al. 1998; Ivry et al. 2002; Lewis and Miall 2003; Wiener et al. 2010), lesion studies (Ivry et al. 1988; Gooch et al. 2010), and electrophysiological experiments (Del Olmo et al. 2007) have documented the role of the lateral cerebellum in perceptual and productive fast timing processes. Moreover, the lateral cerebellum and the dentate nucleus are also critically important for sequence learning in the spatial domain, meaning sequences with variable effectors, and constant time intervals (Doyon et al. 1996, 2003; Hikosaka et al. 2002; Floyer-Lea and Matthews 2005; Lehéricy et al. 2005). Increases in activations in the lateral cerebellum have also been reported for sequential learning in the time domain with variable timing intervals and one constant effector (Ramnani and Passingham 2001; Schubotz and von Cramon 2001; Sakai et al. 2002; Penhune and Doyon 2005; Steele and Penhune 2010), with a particular contribution in the early phase of learning (Penhune and Doyon 2002, 2005; Steele and Penhune 2010) and also overnight consolidation (Lewis et al. 2011). It has been shown that the decrease of right cerebellar activity over time correlated positively with improvement in synchronization (Penhune and Doyon 2005; Steele and Penhune 2010). A simultaneous increase in activity in M1 has suggested a relevant role of the functional connectivity between cerebellum and M1.

Figure 1. "Probabilistic tractography of dentate-thalamo-cortical connections." Population trajectory probability maps (A) illustrate the group average of the cerebellar–cortical connections of interest between the dentate nucleus and contralateral primary motor cortex (M1), dorsolateral prefrontal cortex (DLPFC), and ventral premotor cortex (PMv). Color bars indicate the number of participants in which the relevant voxel was found as part of the individual tract (range 6–20, red–yellow from right dentate nucleus, blue–light blue from left dentate nucleus). For visualization purpose, images were thresholded to show only voxels common to at least 6 of 20 participants. Axial slices (superimposed on MNI T1 template) are presented in radiological convention (R-L). Thresholded binarized tracts used for individual tract-related FA calculation are presented in (B).
imaging has revealed that the right DLPFC was specifi-
cally involved in timed motor-sequence learning and 
functional in time control of neuronal activities in 
time (Penhune and Doyon 2005; Steele and Penhune 2010).

The present data showed a predominant association 
with the DTCT targeting the right DLPFC and gains in 
early consolidation in rhythm continuation. How can this 
finding be interpreted? There is converging evidence for 
the existence of a right-hemispheric motor-timing network in 
the human brain, not only involved in mere performance of 
perceptual and productive timing processes, but also in the 
acquisition of temporal motor skills. The ventral portion of the left 
dentate nucleus connects to the contralateral right DLPFC 
Electrophysiological (Constantinidis et al. 2002) and functional 
imagining data in animals (Onoe et al. 2001) as well as functional 
(Jueptner et al. 1997; Rao et al. 2001; Lewis and Miall 2003; 
Smith et al. 2003; Wiener et al. 2010), structural (Ullén et al. 
2008), inactivation (Koch et al. 2003, 2009; Jones et al. 2004), 
and lesion data (Harrington et al. 1998; Mangels et al. 1998; 
Koch et al. 2002; Gooch et al. 2010) in humans have continu-
ously enhanced the understanding of the right DLPFC not only 
as an integral in more cognitive timing and working memory 
processes, but also as a site for primary time estimation (Lewis 
and Miall 2003). For skill acquisition of precisely timed motor sequences, functional 
imaging has revealed that the right DLPFC was specifically 
involved when the cognitive demand was increased, for instance, by combining effector-dependent and time-dependent 
learning components. The authors have concluded that the 
DLPFC would act as an integrator between effector and tem-
poral information of a motor sequence with implementation of

Figure 2. “Learning gains in synchronization and continuation.” Tapping error [ms] 
plotted against the 7 training blocks T1–T7, follow-up 1 [F1, 90-min post-T7] and 
follow-up 2 [F2, 24-h post-T7] for the synchronization and continuation part. RM-ANOVA 
revealed a significant training effect over time (P < 0.001). Significant temporal 
components for training (ratio T7/T1), early (ratio F1/T7), and late consolidation (ratio 
F2/T7) are indicated by asterisks (P < 0.05, FDR-corrected).

Table 1

| Tract-related mean FA (θ) of the tract connecting the left dentate nucleus and right 
DLPFC was found significantly associated with early skill consolidation (F1/T7) in 
the continuation part (P < 0.05, FDR corrected for 24 correlations). Voxel-wise spatial 
statistics (θ) confirmed a widespread distribution of clusters within the tract of interest 
associated with learning gains (r-values significant at P < 0.01). Notably, strongest 
associations were located in ipsilateral superior cerebellar peduncle and frontal WM. 
Spatial statistics were conducted within common tract of interest (see Fig. 1B).

| Table 1 Tract-related white matter integrity and learning gains in synchronization and continuation |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Dentate to       | Right DLPFC     | Left DLPFC      | Right DLPFC     | Left DLPFC      |
| Synch            |                 |                 |                 |                 |
|                  | M1              | PMv             | DLPFC           | M1              | PMv             | DLPFC           |
| T7/T1            | P 0.38          | 0.37            | 0.32            | 0.34            | 0.39            | 0.27            |
| F1/T7            | P 0.23          | 0.23            | 0.28            | 0.28            | 0.23            | 0.35            |
| Cont             | P 0.16          | 0.30            | 0.19            | 0.11            | 0.24            | 0.18            |
|                  | P 0.52          | 0.39            | 0.51            | 0.66            | 0.41            | 0.52            |

Exploratory multiple correlations between tract-related FA of dentato-thalamo-cortical tracts of 
interest and learning gains. First 2 lines indicates target area of tract from contralateral dentate 
nucleus. M1, primary motor cortex; PMv, ventral premotor cortex; DLPFC, dorsolateral prefrontal 
cortex; Synch, synchronization; Cont, continuation. T7/T1, active training; F1/T7, early consolidation 
90 min post. F2/T7, delayed consolidation 24 h post. Partial correlation coefficient R is given. 
*Significant correlation at P < 0.05 (fully FDR-corrected for 24 comparisons).
an action-oriented representation (Sakai et al. 2002). The present results support this concept and add structural data which point toward a critical role of a distinct cerebellar–prefrontal right-hemispheric circuit for temporal sequence learning in humans.

Why did the analyses reveal this DLPFC-related DTCT involvement only for the continuation part, but not for the synchronization? There has been converging evidence that the right DLPFC is particularly involved in timing tasks with suprasecond intervals when the cognitive load is increased (Lewis and Millar 2006b; Wiener et al. 2010). However, lesion data have also indicated that cognitive timing processing does not necessarily mean suprasecond timing intervals (Nichelli et al. 1995). While subsecond intervals were applied in the present study, participants had to play the sequence from memory in the continuation part suggesting a significant working memory load. This might contribute to the association found for WM integrity of the DTCT targeting the right DLPFC in the continuation part, but not in the synchronization part. Notably, the involvement of the DTCT targeting the right but not the left DLPFC also seems to argue for a specific role of the right DLPFC and, on the other hand, against a rather unspecific working memory effect of both frontal brain areas. However, by contrasting temporal sequence learning tasks (including a relevant cognitive load) with working memory tasks, future structural and functional imaging studies are needed to investigate this issue in more details.

The dorsal dentate nucleus densely projects to the contralateral primary (M1) and ventral premotor cortex (PMv) (Hoover and Strick 1999; Dum and Strick 2003). Both M1 (Penhune and Doyon 2005; Verstynen et al. 2006; Steele and Penhune 2010) and PMv are involved in a broad range of fast perceptual (Schubotz and von Cramon 2001; Chen et al. 2008; Coull et al. 2008; O’Reilly et al. 2008) and productive timing processes (Halsband et al. 1993; Rao et al. 1997; Thaut 2003; Schubotz 2007; Chen et al. 2009). The present data have not revealed any significant correlations between the microstructure of the DTCT to M1 or to PMv and training gains, neither in synchronization nor in continuation. On the one hand, functional imaging has shown M1 activation in early learning and its association with later consolidation only in accuracy, but not synchronization in timed motor-sequence learning (Penhune and Doyon 2005; Steele and Penhune 2010). In this regard, the present data might argue for a task-specific role of M1 and its underlying tracts connecting M1 with the cerebellum in temporal motor-sequence learning. On the other hand, studies of sequential motor learning both in the effector (Karni et al. 1995) and time domain (Penhune and Doyon 2002) have reported particular activation in M1 and premotor cortices in delayed recall only: increases in M1 activation have been found in a motor-timing experiment in delayed recall after 4 weeks following 5 days of training (Penhune and Doyon 2002). As the present training paradigm has covered an active training period of only 20 min duration, an early consolidation after 90 min and delayed consolidation after 24 h, it is likely that it has not covered the extended period of learning needed to uncover M1 and premotor cortex involvement. Future studies with prolonged periods of training and follow-up are needed to assess this in detail.

There are some limitations of the present study. Due to limited spatial resolution in the diffusion-weighted images, the trajectories show considerable spatial overlap at smaller MNI z-values, which might have biased tract specificity in mean FA values and correlations. However, the association between the mean FA of the DTCT connecting the left dentate nucleus and the right DLPFC is not only driven by local FA in a single segment or overlap region arguing for a reasonable contribution of the entire extent of the tract. Future studies with increased image resolution are needed to address this issue. Furthermore, DTI-based probabilistic tractography does not distinguish between ascending and descending fibers forming the tracts of the sensorimotor systems. Moreover, common trajectories used for further analysis might also contain trajectories starting and ending along the course of the tracts of interest, such as corticorubral, dentate-thalamic, and other thalamo-cortical fibers, which might have affected their topology and mean FA values.

Taken together, the present results add to the insight and understanding of the acquisition of precise movement timing in young adults by providing structural connectivity data of the underlying brain networks. Thereby, the involvement of a right-hemispheric cerebellar–cortical circuit does not seem to be random: indeed, brain imaging in dystonia mutation carriers has recently shown a significant relationship between left cerebellar outflow pathway connectivity and task-related brain activation in right prefrontal brain areas in motor-sequence learning (Carbon et al. 2011). Hence, the combination of behavior, functional, and structural imaging in a multimodal framework might open new ways to study the neurobiology of timing, both in healthy participants, aging processes, and neurological diseases.

Supplementary Material
Supplementary material can be found at: http://www.cercor.oxfordjournals.org/.

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Notes
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References


