Neuron
Article

Both the Hippocampus and Striatum Are Involved in Consolidation of Motor Sequence Memory

Geneviève Albouy,[1,2,3,4] Virginie Sterpenich,[1] Evelyne Balteau,[1] Gilles Vandewalle,[1] Martin Desseilles,[1] Thanh Dang-Vu,[1] Annabelle Darsaud,[1] Perrine Ruby,[3,4] Pierre-Hervé Luppi,[2,4] Christian Degueldre,[1] Philippe Peigneux,[1,5] André Luxen,[1] and Pierre Maquet[1,*]

[1]Cyclotron Research Centre, University of Liège, B-4000 Liège, Belgium
[2]Centre National de la Recherche Scientifique, Unité Mixte de Recherche 5167, Institut Fédératif des Neurosciences de Lyon, F-69372 Lyon, France
[3]Institut National de la Santé et de la Recherche Médicale, Unité 821, Institut Fédératif des Neurosciences de Lyon, F-65500 Lyon, France
[4]University of Lyon, F-69622, Lyon, France
[5]Present address: Neuropsychology and Functional Neuroimaging Research Unit, University of Brussels, B-1050 Bruxelles, Belgium.
*Correspondence: pmaquet@ulg.ac.be
DOI 10.1016/j.neuron.2008.02.008

SUMMARY

Functional magnetic resonance imaging (fMRI) was used to investigate the cerebral correlates of motor sequence memory consolidation. Participants were scanned while training on an implicit oculomotor sequence learning task and during a single testing session taking place 30 min, 5 hr, or 24 hr later. During training, responses observed in hippocampus and striatum were linearly related to the gain in performance observed overnight, but not over the day. Responses in both structures were significantly larger at 24 hr than at 30 min or 5 hr. Additionally, the competitive interaction observed between these structures during training became cooperative overnight. These results stress the importance of both hippocampus and striatum in procedural memory consolidation. Responses in these areas during training seem to condition the overnight memory processing that is associated with a change in their functional interactions. These results show that both structures interact during motor sequence consolidation to optimize subsequent behavior.

INTRODUCTION

Motor sequences constitute an integral part of a number of everyday life activities such as writing, typing, speaking, knitting, or playing a musical instrument. Motor skills are usually learned through repeated practice. As a rule, performance considerably improves during initial training, whereas subsequent practice sessions are associated with slow and progressive improvements in performance (Karni et al., 1995). Importantly, sequential skills improve between practice sessions, suggesting that motor memory undergoes a process of consolidation that evolves offline, in the absence of any additional practice. Consolidation of motor sequence memory depends on various experimental factors such as posttraining interval (Hauptmann and Karni, 2002; Walker et al., 2003), sleep (Fischer et al., 2002; Korman et al., 2003; Maquet et al., 2000; Peigneux et al., 2003; Walker et al., 2002), circadian rhythms (Cajochen et al., 2004), and subject's awareness of the sequential material (Robertson et al., 2004).

The neural correlates of the early, fast learning phase have been extensively characterized and involve the cerebellum, the basal ganglia, the supplementary motor area, and motor and premotor cortices (Doyon et al., 2003). Recently, it was established that the hippocampus is also implicated in motor sequence learning, due to its ability to associate temporally discontiguous but structured information (Schendan et al., 2003). Although the hippocampus is classically associated with explicit learning in the amnesia literature, hippocampal responses were recorded during sequence learning irrespective of whether the sequential knowledge was implicitly or explicitly acquired (Schendan et al., 2003).

In contrast, the neural correlates of motor sequence memory consolidation have not yet been comprehensively characterized. Various changes in responses were reported in distributed cortical areas, in the cerebellum, and in the basal ganglia, 12 to 72 hr after a single training on a motor sequence learning task (Walker et al., 2005), after sleep or sleep deprivation (Fischer et al., 2005; Maquet et al., 2003). An influential model of motor sequence memory consolidation presently posits that long-lasting retention of motor sequential skills relies upon striato-cortical rather than cerebello-cortical networks (Doyon et al., 2003). In addition, the recruitment of the hippocampus during motor sequence learning led to the hypothesis that this structure might also participate in motor sequence memory processing (Doyon and Benali, 2005). Although the hippocampus plays a key role in consolidation of declarative memories (Alvarez and Squire, 1994; Frankland and Bontempi, 2005; Nadel and Moscovitch, 1997), its participation in consolidation of motor sequence learning has received little experimental support so far. Nevertheless,

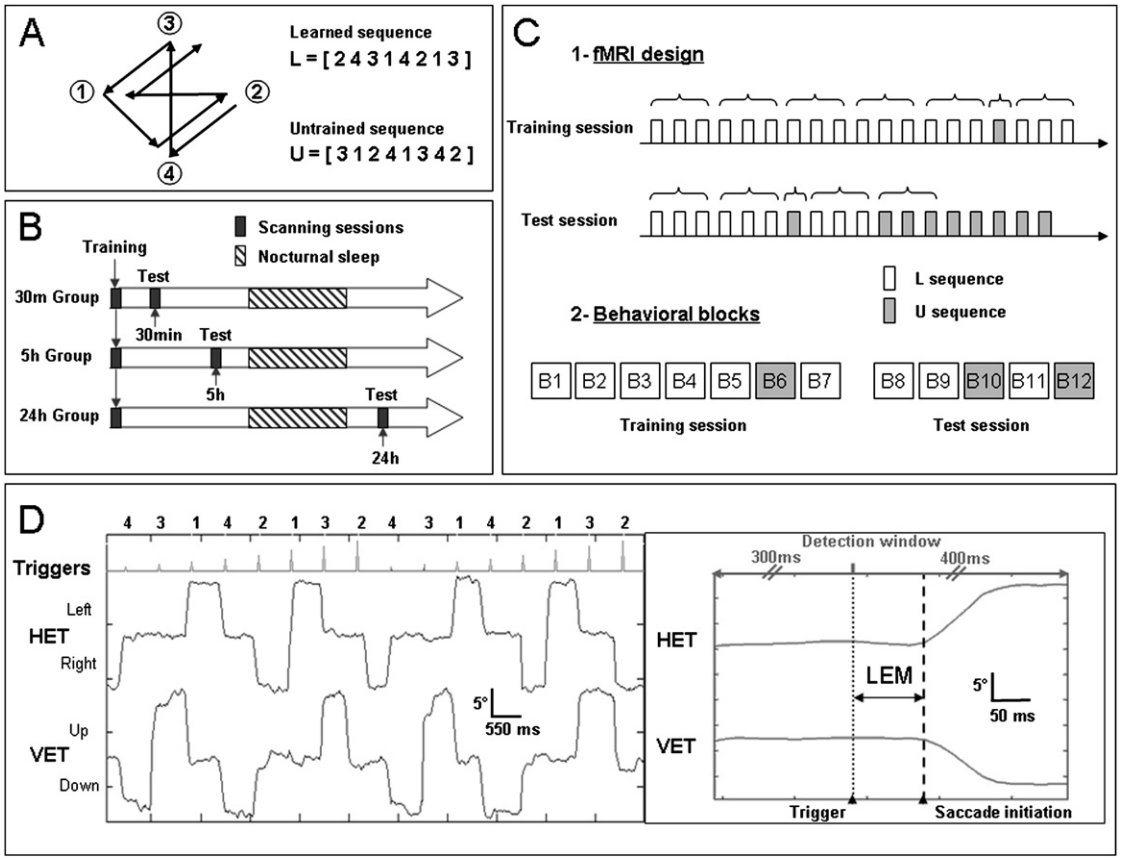

Figure 1. Experimental Protocol

(A) Trajectory followed by the dot between the possible locations in the SORT task. Arrows and numbers depict the trajectory of the learned sequence (L). In order to allow a direct measure of sequence learning, another sequence (untrained sequence, U) was presented.

(B) Experimental groups. All subjects were trained to the SORT in the scanner. They were tested afterwards in the scanner, according to the group they belong to (30 min, 5 h, or 24 h including sleep), after the training has ended.

(C) Experimental design. (1) fMRI design: training and test sessions consisted of 19 and 18 blocks, respectively, with each block consisting of five sequences. The untrained sequence was proposed once during training and nine times during testing. (2) Behavioral blocks: the mean LEMs were computed over three consecutive blocks. The untrained sequence was proposed to the participants on behavioral blocks 6, 10, and 12.

(D) Saccade detection. (Left) Raw Eye Tracking (ET) horizontal (HET, first row) and vertical (VET, second row) recordings during practice of the SORT. The triggers indicate dot movements and positions (1, 2, 3, or 4) described in Figure 1A. (Right) Automatic detection of LEMs computed the delay between the dot movement (vertical dotted line: trigger) and the first saccade in the right direction (vertical dashed line: saccade initiation) in a 700 ms detection window.

recent behavioral data suggest that consolidation of motor sequence memory depends on sleep when sequence learning requires contextual associations, a process assumed to rely on hippocampal formation (Spencer et al., 2006).

The aim of the present study was to characterize, at the macroscopic systems level, the cerebral correlates of implicit oculomotor sequence learning, and to understand the latter's subsequent offline processing. Offline memory processing was indirectly revealed by a change in the neural representation of motor memories, during repeated practice of the learned task at a later date. Using functional magnetic resonance imaging (fMRI), we recorded regional cerebral activity during practice of the serial oculomotor reaction time (SORT) task (Albouy et al., 2006), both during initial training and at specific posttraining delays (after 30 min, 5 hr, or 24 hr), with the longest delay including a period of nocturnal sleep (Figure 1B). In the SORT task, participants have to visually track a dot which, at any point in time, is displayed at one of four possible positions (Figure 1A), and the color of which can briefly change. Participants are explicitly instructed to detect the changes in dot color (Supplemental Information I). However, unbeknownst to them, the succession of dot positions follows an eight-element sequence (learned sequence, or L) which is repeated during several practice blocks. A different but structurally equivalent sequence (untrained sequence, or U) is presented once at the end of training and during testing to allow a direct measure of sequence learning (Figure 1C). During both training and testing, performance was measured by the latencies of eye movements (LEMs), defined as the time interval between the change in dot position and the first saccade initiated in the direction of the target (Figure 1D). LEMs reflect the development of an implicit sequential knowledge, as they become progressively shorter with repetition of the learned sequence, and slow down when the untrained sequence is presented (Albouy et al., 2006).

Our results show that the hippocampus and the ventral striatum are not only recruited during training in the implicit motor sequence learning task, but also seem to condition subsequent overnight gain in performance. Importantly, the competitive interaction observed between hippocampus and striatum during training turns to cooperation overnight when the memory trace is deemed consolidated.

RESULTS

Population

Based on our previous behavioral study, we expected that some subjects would learn the task only to a limited extent (Albouy et al., 2006). The proportion of these subjects would possibly be increased given that the task had to be adapted to the fMRI design. Consequently, a prospective survey of behavioral data was conducted during the study. This was pursued until a sufficient number of subjects who showed an undisputable improvement during training were available in each delay group. To set an objective threshold to identify such participants, we examined the distribution of LEMs over all participants and across the entire training. It turned out to be bimodal with a median at 100 ms (see Figure S1A, available online). We defined participants who presented mean LEMs shorter than 100 ms on the last block of training (B5) as fast learners, and the others (LEMs > 100 ms) as slow learners (Figure S1B). In the following sections, we will consider two *categories* [fast (F) versus slow (S) learners] and three test *intervals* [(30 min, 5 h, and 24 h)]. Subjects were thus distributed in six *groups* (30 mS, 30 mF, 5 hS, 5 hF, 24 hS, and 24 hF).

Some subjects were discarded from the analyses because of either technical failures or the emergence of an explicit sequential knowledge (Supplemental Information III and V). Eventually, 14 subjects were included for analysis in the 30 mF group (8 females), and 4 subjects were included in the 30 mS group (2 females). Twelve subjects were included for analysis in the 5 hF group (seven females) and fifteen subjects were included in the 5 hS group (four females). Thirteen subjects were considered for analysis in the 24 hF group (seven females) and twelve were considered in the 24 hS group (six females).

Results reported in the main text focus on the three groups of fast learners (30 mF, 5 hF, and 24 hF) because their performance during training followed a learning curve similar to the one observed in our previous study and their motor sequential skill improved overnight, as also reported in our previous study (Albouy et al., 2006). In contrast, no evidence for an overnight gain in performance was observed in slow learners, suggesting that their poor initial performance did not allow any efficient overnight consolidation process. For the sake of completeness, behavioral and brain imaging results on slow learners (30 mS, 5 hS, and 24 hS) and the comparison between the two categories of learners are available in Supplemental Information VII and VIII.

Behavior

Initial Training

An analysis of variance (ANOVA) was conducted on LEMs, with delay (30 mF, 5 hF, or 24 hF) and repetition (five blocks of learned sequence, B1 to B5, see Figure 1C) as factors. The LEMs became significantly shorter with practice in all groups [F(4,144) = 125.68, p < 0.0001]. The improvement did not differ between the three groups [F(8,144) = 1.07, p = 0.38]. At the end of training, a novel but structurally equivalent sequence was presented (on block B6, see Figure 1C). An ANOVA computed on LEMs tested the effect of sequence type ($L_{Training}$ versus $U_{Training}$, i.e., B5 versus B6) and delay (30 mF, 5 hF, or 24 hF). LEMs were shorter for the learned than for the untrained sequence [F(1,36) = 403.01, p < 0.0001]. This difference in LEMs, reflecting the acquisition of the learned sequence, did not differ between groups [F(2,36) = 0.39, p = 0.67].

An ANOVA conducted on LEMs of blocks (B5 versus B7) by delay (30 mF, 5 hF, or 24 hF) tested the saturation effect on the last two training blocks of learned sequence. The LEMs did not decrease between the two blocks [F(1,36) = 0.06, p = 0.7]. This saturation effect did not differ between groups [F(2,36) = 0.5, p = 0.6].

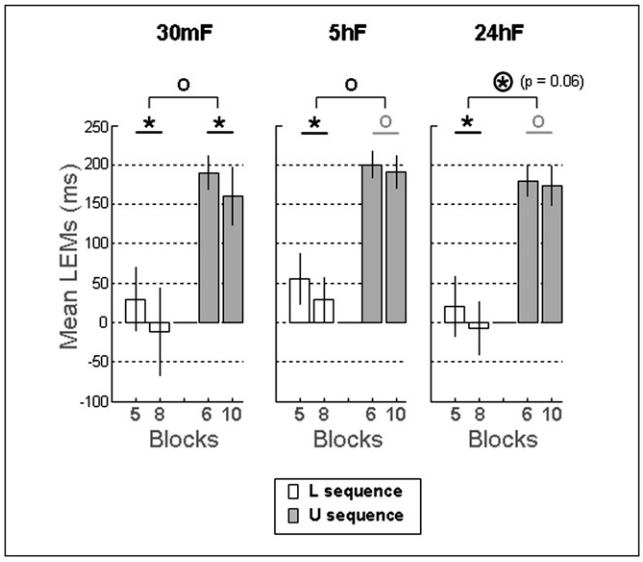

Figure 2. Behavioral Results

Changes in LEMs (ms) between training and test sessions (left- and right-hand bar of each pair, respectively), for the learned (white bars; B5 and B8) and the untrained (gray bars; B6 and B10) sequences, for the three groups of fast learners. Bars represent standard error of the mean (SEM).

Posttraining Changes in Performance

An ANOVA was computed on mean LEMs for blocks B5, B6, B8, and B10 (Figure 1C) using sequence type (L versus U) and session (Training versus Test) as within-subjects factors, and delay (30 mF, 5 hF, or 24 hF) as a between-subjects factor (Figure 2 and Table 1). The LEMs significantly differed between sequences [F(1,36) = 508.07, p < 0.0001] and sessions [F(1,36) = 43.10, p < 0.0001]. The differences in LEMs between the

Table 1. Average (SD) Mean of LEMs (ms) for Blocks B5, B6, B8, and B10

	B5	B6	B8	B10
Group 30 mF	29 (45)	189 (52)	−12 (69)	160 (51)
Group 5 hF	55 (42)	200 (25)	29 (36)	191 (32)
Group 24 hF	19 (46)	178 (26)	−7 (44)	173 (32)

Negative values denote anticipation (see text).

learned and the untrained sequences were larger during testing than training [(B5 versus B6) by (B8 versus B10)], F(1,36) = 4.92, p = 0.03), and the delayed performance gain differed between groups [F(2,36) = 3.30, p = 0.04]. In subjects tested 24 hr after training (24 hF Group; n = 13), LEMs significantly decreased for the learned sequence (B5 versus B8, planned comparisons, p < 0.001), but not for the untrained sequence (B6 versus B10, planned comparisons, p = 0.45), although the session by sequence interaction fell short of significance [F(1,12) = 4.18, p = 0.06]. These results suggest that the delayed gain observed at 24 hr posttraining tended to be specific to the learned sequence. In contrast, the performance gain between training and testing did not differ between the learned and untrained sequences when subjects were tested 30 min after the end of training [30 mF Group; n = 14; F(1,13) = 0.58, p = 0.45]. Planned comparisons indicated that 30 min after training, LEMs similarly decreased from training to test session for both the learned sequence (p = 0.001) and the untrained sequence (p = 0.02). Five hours after training, there was no evidence for a selective improvement for the learned sequence [5 hF Group; n = 12; F(1,11) = 2.11, p = 0.17], although oculomotor performance significantly improved on the learned (p = 0.026), but not on the untrained (p = 0.12), sequence.

Finally, and for the sake of completeness, behavioral results on slow learners are available in Supplemental Information VII. Behavioral results on color detection scores are reported in Supplemental Information VI.

Basic Properties of Recorded Eye Movements

The angular velocity of eye movements is ruled by a stable relationship between the saccade duration and its angular amplitude (Leigh and Zee, 1999). An ANOVA was computed on saccadic duration for blocks B5, B6, B8, and B10 using sequence type (L versus U) and session (Training versus Test) as within-subjects factors, and interval (30 mF, 5 hF, or 24 hF) as a between-subjects factor. Saccadic duration did not decrease between sessions [F(1,36) = 0.45, p = 0.50], but tended to decrease between sequences [F(1,36) = 3.61, p = 0.06]. These effects did not differ between groups [session by delay interaction, F(2,36) = 0.40, p = 0.66; sequence by delay interaction, F(2,36) = 1.71, p = 0.19]. The sequence by session and sequence by session by delay interactions were not significant [F(1,36) = 2.62, p = 0.11; F(2,36) = 0.71, p = 0.49, respectively].

These results suggest that the changes in performance reported in the present study mainly pertain to the progressive and implicit acquisition of sequential knowledge, as reflected by LEMs, rather than to a mere speeding of oculomotor responses.

Brain Imaging Data

Main Effect of Learning during Training

To characterize the cerebral correlates of sequence learning, the cerebral responses to the learned and untrained sequences were compared ($L_{Training}$ versus $U_{Training}$). For fast learners, this analysis revealed significantly larger responses for the learned than for the untrained sequence in both caudate nuclei and the left anterior and posterior hippocampus (Figure 3A, Table 2-1). No significant responses were observed in slow learners (Supplemental Information VIII, Table S1-1, available online).

In addition, we assessed whether the learning effect during training would predict the gain in performance of the learned sequence between training and testing sessions. Learning-related responses were linearly related to the delayed gain in performance in bilateral posterior hippocampus, right anterior hippocampus, bilateral ventral putamen, and the brainstem (Table 2-2, Figure 3B), but only for fast learners tested at 24 hr posttraining and not for the other groups (Table 2-2). The regressions based on the hippocampal and putaminal responses were also significantly better for the 24 hF group than for the two other groups of fast learners (Table 2-2). Supplemental analyses did not show any learning-related responses correlated with the subsequent gain in performance in the hippocampus in slow learners (Table S1-1). Furthermore, the regression observed with the posterior hippocampus responses was also significantly better for the fast learners of the 24 hr group than for the three groups of slow learners (Supplemental Information VIII, Table S1-1).

Modulation of Cerebral Activity by Performance during Training

During the training session, the main effect of performance on cerebral activity was estimated by identifying brain areas in which BOLD responses were modulated by LEMs. These results are summarized in Table 2-3. First, we looked for areas in fast learners where responses increased as performance improved over the training session. Such a response pattern was observed bilaterally in the putamen on both its ventral and dorsal portions. Conversely, responses in the left cerebellum decreased as performance improved over the training session.

In addition, responses in left posterior hippocampus (−38 −34 −2 mm, Z = 3.62, p_{svc} = 0.009) and cerebellum progressively decreased throughout training in fast learners, whereas these responses increased in slow learners (Supplemental Information VIII, Table S1-2, Figure S2). Furthermore, in fast learners, functional connectivity analysis showed a negative connectivity between the left posterior hippocampus described above and the left ventral putamen such that the functional connectivity decreases as performance improves (Table 2-4, Figure 3C).

Main Effect of Learning during Testing

For the sake of completeness, we reported the main learning effect of the test session (L_{Test} versus U_{Test}) and the learning by session interaction ([L versus U] by [Test versus Training]) for each of the six groups (fast and slow learners), in Tables S2 and S3 and Figure S3 for fast learners.

Here, we focus on interaction effects showing larger responses for a given delay with respect to another in the learned sequence as compared with the untrained sequence during testing (learning × delay interactions, Table 3-1). For fast learners, differences in responses for the learned sequence as compared with the untrained sequence were essentially observed 24 hr after training as compared with other delays, and were mainly located in the striatum and the hippocampus (Figure 4, Table 3-1). Indeed, learning-related responses were larger in bilateral ventral putamen, right dorsal putamen, left (anterior and posterior) hippocampus, the anterior cingulate cortex, the bilateral thalamus, and the left cerebellar hemisphere 24 hr after training, as compared with 30 min after training. Likewise, learning-related responses were larger in bilateral dorsal putamen, ventral

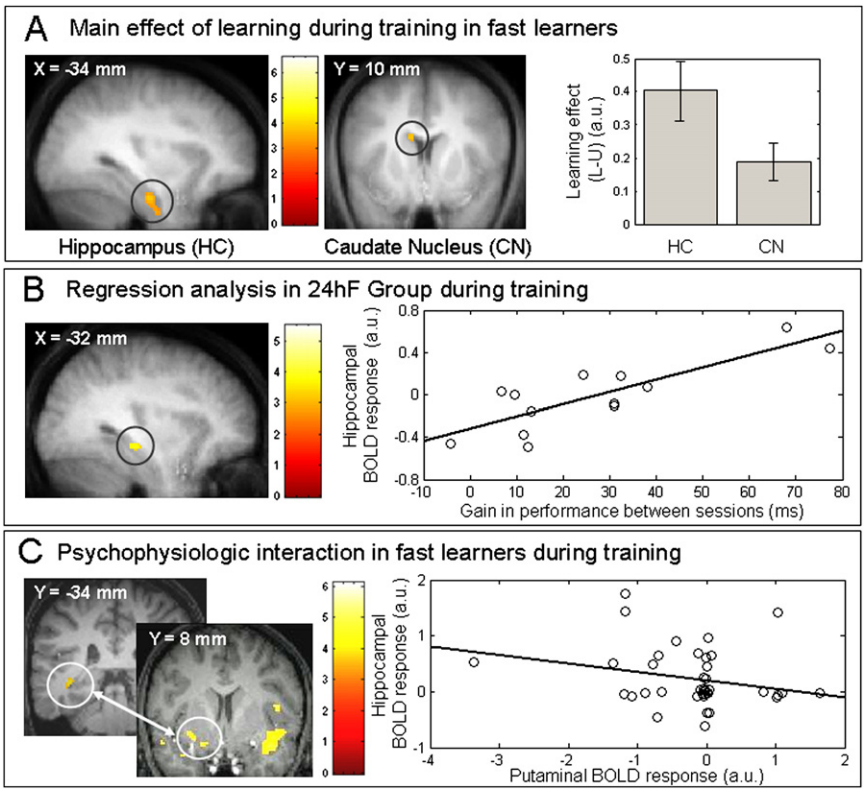

Figure 3. Functional Imaging Results for the Training Session in Fast Learners

Functional data are shown over the mean structural image of all subjects (A and B) or over the structural MR image of one representative subject (C) normalized to the same stereotactic space ($p < 0.001$, uncorrected).

(A) Main effect of learning during training in fast learners ($L_{Training} - U_{Training}$). (Left) The main effect of learning was observed in the hippocampus and the caudate nucleus during training. (Right) Differential ($L_{Training} - U_{Training}$) mean parameter estimates. a.u., arbitrary unit. Bars represent SEM.

(B) Regression analysis between cerebral activity during training and overnight gain in performance in fast learners. (Left) Learning-related responses in the left posterior hippocampus during training are correlated with the subsequent overnight gain in performance on the learned sequence for fast learners tested at 24 hr only ([$L_{Training} - U_{Training}$] regressed against overnight gain in performance). (Right) Regression plot of the BOLD learning-related responses in the left posterior hippocampus during training against the overnight gain in performance on the learned sequence. Each data point represents a single subject of the 24 hF Group.

(C) Psychophysiological interaction in fast learners during training. (Left) The left posterior hippocampus and the left ventral putamen are connected in proportion with LEMs during the training session. (Right) Plot of the performance-related signal changes from the left posterior hippocampus against the left ventral putamen that exhibited significant negative correlation with the hippocampus in functional connectivity analysis. Each data point represents a single subject in the fast learners' category.

putamen, and the left thalamus 24 hr after training than 5 hr after training. At shorter delays, learning-related responses in the cerebellum, the right thalamus, and the anterior cingulate cortex were larger 5 hr after training relative to 30 min after training.

The interaction effects showing larger responses for a given delay with respect to another, in the learned sequence as compared with the untrained sequence, and during testing as compared with training (learning × delay × session interactions), gave similar results (Supplemental Information IX and Table S5).

The comparison between categories (Supplemental Information VIII, Table S4, and Figure S4) revealed that 24 hr after training, fast learners recruit the hippocampus to accurately perform the oculomotor sequential memory task, as compared with slow learners, in whom only the cerebellum was preferentially recruited.

Finally, during testing in fast learners at 24 hr posttraining, the linear relationship between responses in the putamen and the left posterior hippocampus was found to be significantly tighter for the learned sequence than for the untrained one (Table 3-2). This regression was also significantly larger 24 hr than 30 min after training. There was no evidence that this pattern of functional connectivity was present during training or in the sooner testing sessions (30 min and 5 hr, Table 3-2). Responses in the putamen were more tightly related to those of the dentate nucleus of the cerebellum, 24 hr posttraining as compared with 5 hr posttraining.

DISCUSSION

In this study, we aimed at characterizing the neural correlates of implicit oculomotor sequence learning both during training and during testing at three time delays during the first 24 posttraining hours. The changes in responses from training to testing, taking place without any further practice, were regarded as an indication of the offline motor memory processing occurring during the delay period. We focused on results observed in fast learners because their motor skill performance improved overnight, indicating a successful memory consolidation. Our results confirm and further highlight the involvement of the striatum and the hippocampus during initial training on a motor sequence learning task. More importantly, they show that the early recruitment of these structures predicts the overnight improvement in performance. Finally, the competitive interaction observed between hippocampus and striatum during training turns to cooperation overnight when the memory trace is deemed consolidated.

Slow Learners

In contrast to fast learners, slow learners did not show any behavioral improvement overnight, although they did so over the day, suggesting an unsuccessful overnight processing of motor sequence memory. Accordingly, brain responses in slow learners were conspicuously different from those recorded in

Table 2. Functional Results for the Main Effect of Learning during Training in Fast Learners

Area	x mm	y mm	z mm	Z	p_{svc}
1. Main learning effect ($L_{Training} - U_{Training}$)					
Left anterior hippocampus	−16	−14	−28	3.80	0.005
Left posterior hippocampus	−42	−34	−12	3.57	0.011
Left caudate nucleus	−12	10	22	3.19	0.032
Right caudate nucleus	18	22	12	3.38	0.019
2. Regression analysis with the subsequent gain in performance (B5 – B8)					
30 mF					
(No significant responses)	–	–	–	–	–
5 hF					
(No significant responses)	–	–	–	–	–
24 hF					
Left posterior hippocampus	−32	−28	−10	3.54	0.011
Right posterior hippocampus	26	−34	−6	3.99	0.003
Right anterior hippocampus	30	−10	−20	3.13	0.036
Left ventral putamen	−20	6	−26	3.16	0.033
Right ventral putamen	26	4	−24	3.53	0.012
Pons	−4	−16	−28	4.66	0.049*
24 hF – 30 mF					
Left posterior hippocampus	−30	−24	−10	3.52	0.012
Right posterior hippocampus	26	−34	−6	4.03	0.002
Left ventral putamen	−30	−14	−10	3.23	0.028
Right ventral putamen	28	−14	−8	3.48	0.014
24 hF – 5 hF					
Right posterior hippocampus	40	−26	−20	3.70	0.015
	26	−34	−4	3.16	0.033
Right anterior hippocampus	30	−10	−20	3.56	0.011
Right ventral putamen	26	6	−20	3.42	0.034
	28	−16	−4	3.19	0.031
3. Modulation by the performance effect					
Regions wherein responses increase with performance improvement					
Right occipital gyrus	8	−90	8	5.02	0.013*
Left ventral putamen	−14	10	−10	4.99	0.014*
Right ventral putamen	20	12	−10	4.73	0.043*
Left dorsal putamen	−28	−6	10	3.73	0.012
Right dorsal putamen	26	6	4	3.61	0.010
Right cerebellar hemisphere	16	−48	−20	3.49	0.015
Regions wherein responses decrease with performance improvement					
Left cerebellar hemisphere	−4	−64	−44	3.17	0.036
4. Psychophysiological interaction on the left posterior hippocampus					
Right insular gyrus	42	−4	−6	4.97	0.015*
Left ventral putamen	−28	8	−14	3.72	0.015
	−18	8	−22	3.65	0.022
Right cerebellar hemisphere	16	−54	−26	4.03	0.003

Brain activations significant after correction over the entire volume (*) or over a small volume of interest (svc) are reported here.

fast learners in that they significantly and differentially recruited the hippocampus during training and during subsequent testing 24 hr later. These findings have two important implications. First, they show that a visuomotor skill can be learned in more than one way and can be related to recruitment of different neural systems, resulting in different forms of knowledge representation. These results echo the variability in approaches in higher-level cognitive learning tasks that was reported in both normal volunteers (Foerde et al., 2006; Poldrack et al., 2001) and neurological patients (Shohamy et al., 2007). Second, the difference between slow and fast learners in terms of behavior and brain responses speaks for the importance of initial memory formation for subsequent consolidation process. Early studies had already identified differences in learning rates between slow- and fast-learning rats, which also differ in their posttraining sleep and subsequent memory consolidation (Ambrosini et al., 1992; Leconte et al., 1973).

Comparison with Previous Behavioral Results

The behavioral results in fast learners largely confirmed our previous behavioral study (Albouy et al., 2006). A significant gain in oculomotor performance was observed at 30 min on both the learned and untrained sequences. In addition, performance improved 24 hr after training. In the present study, this gain in performance tended to be specific to the learned sequence, although the sequence by session interaction fell short of significance in contrast to our previous behavioral study. The main difference between the present results and our previous study is observed 5 hr after training. In the present study, performance improved on the learned sequence at that time, whereas we had not observed any gain in performance in our previous study. These differences are probably explained by the adaptation of the SORT task to the fMRI design, which necessitated shorter practice blocks and the absence of behavioral feedback. The changes might have promoted implicit learning, known to be associated with gains in performance over the day (Press et al., 2005).

Hippocampal Recruitment during Training

During training, sequence learning was associated with a significant hippocampal response in fast learners. The hippocampus was recruited early on during training, but its contribution decreased over time. It appears unlikely that the hippocampal recruitment during practice of the SORT task is due to the emergence of an explicit knowledge of the sequence. Indeed, explicit awareness of the sequence was tested at the end of the experiment on the basis of a generation task, and subjects showing any evidence for an explicit knowledge of the sequence were discarded from the analyses. In humans, the hippocampal formation is not classically considered mandatory for procedural motor learning because amnesic patients with damage to this area are thought to be able to acquire and retain motor skills (Gabrieli et al., 1993; Reber and Squire, 1994). However, further studies indicated that amnesic patients with hippocampal damage are impaired in some aspects of implicit learning (Chun and Phelps, 1999; Yang et al., 2003), and in particular, learning higher-order associations included in second-order conditional sequences (Curran, 1997), such as the one used in the present study. In addition, functional neuroimaging studies in healthy volunteers have revealed significant hippocampal responses during implicit learning (Degonda et al., 2005; Henke et al., 2003). The hippocampus would participate in the formation of

Table 3. Functional Results for the Main Effect of Learning during Testing in Fast Learners

Area	x mm	y mm	z mm	Z	p_{svc}
1. Main learning by group effects ([L_{Test} − U_{Test}] × delay)					
30 mF – 5 hF					
(No significant responses)	–	–	–	–	–
30 mF – 24 hF					
(No significant responses)	–	–	–	–	–
5 hF – 30 mF					
Left anterior cingulate cortex	−4	6	40	3.47	0.015
Right thalamus	24	−18	2	3.24	0.029
Left cerebellar hemisphere	−22	−38	−36	3.11	0.037
5 hF – 24 hF					
(No significant responses)	–	–	–	–	–
24 hF – 30 mF					
Left ventral putamen	−24	8	−18	3.25	0.028
Right ventral putamen	22	8	−16	3.67	0.008
Right dorsal putamen	30	0	12	3.39	0.019
Left posterior hippocampus	−22	−34	0	3.14	0.037
Left anterior hippocampus	−26	−16	−12	3.73	0.007
	−26	−28	−22	3.25	0.028
	−30	−28	−20	3.19	0.033
Left anterior cingulate cortex	−2	6	42	3.85	0.005
Left thalamus	−20	−12	18	3.48	0.015
Right thalamus	22	−22	6	3.52	0.025
Left cerebellar hemisphere	−4	−60	−16	3.51	0.014
24 hF – 5 hF					
Left dorsal putamen	−22	6	6	3.97	0.003
Right dorsal putamen	32	−2	10	3.58	0.001
	26	−8	10	3.19	0.033
Left ventral putamen	−22	−6	−2	3.27	0.026
Right ventral putamen	22	4	−14	3.34	0.022
Left thalamus	−20	−12	22	3.55	0.012
2. Psychophysiological interaction with the right dorsal putamen					
30 mF					
(No significant responses)	–	–	–	–	–
5 hF					
Left cerebellar hemisphere	−6	−74	−22	4.25	0.001
Right cerebellar hemisphere	14	−70	−28	3.26	0.027
Left middle frontal gyrus	−28	22	30	3.28	0.026
24 hF					
Left posterior hippocampus	−36	−26	−12	3.61	0.015
Left dentate nucleus	−12	−62	−34	3.21	0.031
24 hF – 30 mF					
Left posterior hippocampus	−34	−26	−12	3.55	0.012
24 hF – 5 hF					
Left dentate nucleus	−14	−60	−34	3.35	0.021

Brain activations significant after correction over a small volume of interest (svc) are reported here.

higher-order temporal associations required in sequence learning (Fletcher et al., 2005; Kumaran and Maguire, 2006; Schendan et al., 2003), irrespective of whether the sequence is implicitly or explicitly learned (Schendan et al., 2003).

Hippocampal and Striatal Recruitment during Training Forecast Successful Motor Memory Consolidation

An important finding of the present study is that the learning-related hippocampal recruitment during training in fast learners predicts the overnight behavioral improvement observed 24 hr later, suggesting that early hippocampal responses induced during training influence subsequent offline memory processing. One possibility would be that the recruitment of the hippocampus triggers an offline memory processing that goes on for hours and eventually leads the following day to increased learning-related responses in the same area, and to a specific gain in performance for the learned sequence. This hypothesis is not supported by our data because there is no evidence for a persisting hippocampal learning-related response over the day. Although a learning-related response is observed in the hippocampus at 30 min posttraining in fast learners (Table S2), the hippocampus does no longer differentially respond to trained and untrained sequences 5 hr after training. Moreover, learning-related activity in the hippocampus during training did not relate to changes in performance observed over the day, i.e., after 30 min or 5 hr in either fast or slow learners. These results suggest that the hippocampal involvement during training is specifically related to late, overnight memory processing. The early hippocampal response might act as a tag for the neuronal populations that would participate in offline memory processing at a later date. Such a hippocampal tag might trigger memory processing during wakefulness at delays longer than 5 hr (Press et al., 2005; Robertson et al., 2004). This explanation cannot be ruled out by our design, which does not specifically address this issue, but the hypothesis that hippocampal neuronal ensembles, tagged during training, participate in memory processing during sleep is consistent both with a possible synaptic downscaling during sleep (Tononi and Cirelli, 2006) and with evidence of experience-dependent replay of neuronal activity during posttraining sleep in rodents (Ji and Wilson, 2007) and humans (Maquet et al., 2000; Peigneux et al., 2004; Rasch et al., 2007). Our hypothesis is also consistent with recent behavioral data obtained in humans using a manual serial reaction time task, showing that implicit sequence learning is improved after a night of sleep only when learning benefits from the formation of contextual, hippocampus-dependent associations (Spencer et al., 2006).

Our hypothesis is challenged by a report on three amnesic patients with lesions of the mesio-temporal structures whose performance did improve overnight after training to mirror tracing (Gabrieli et al., 1993). It was not specified if the overnight gain in performance was similar in amnesic patients and normal controls, and these results need to be confirmed by prospective studies on larger patient populations. However, these preliminary neuropsychological observations suggest that the hippocampal tag might not hold for motor learning at large but only for sequence learning. In addition, amnesic patients might still rely on the more conventional cortico-striatal system to achieve delayed gains in performance.

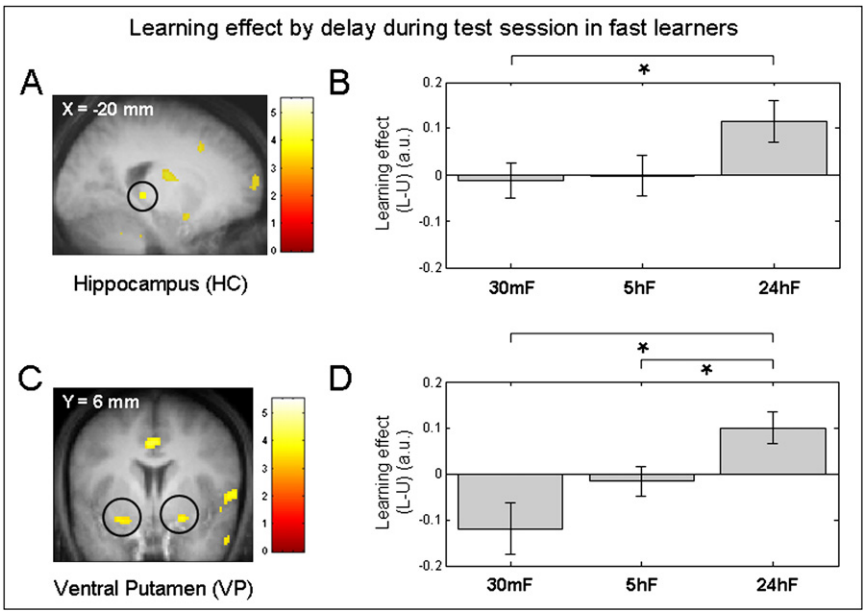

Figure 4. Functional Results of the Main Learning Effect by Delay Interaction for the Test Session in Fast Learners ([L_{Test} − U_{Test}] × Delay)

Functional data are shown over the mean structural image of all subjects ($p < 0.001$, uncorrected).

(A) Responses in left posterior hippocampus.

(B) Differential (L_{Test} − U_{Test}) mean parameter estimates indicated that the responses in the left posterior hippocampus are significantly larger at 24 hr as compared with 30 min. a.u., arbitrary unit. Bars represent SEM.

(C) Responses in bilateral ventral putamen.

(D) Differential (L_{Test} − U_{Test}) mean parameter estimates indicated that the responses in the left ventral putamen are significantly larger at 24 hr as compared with 30 min and 5 hr. a.u., arbitrary unit. Bars represent SEM.

Indeed, oculomotor sequence learning also involved striatal structures. A shift in activation within the striatum was observed during the course of sequence learning in fast learners. The involvement of the striatum in motor sequence learning has been extensively reported with manual tasks (Doyon and Benali, 2005; Doyon et al., 2003; Peigneux et al., 2000). Learning-related responses in fast learners were detected in the caudate nucleus during training. As a rule, the caudate nucleus and rostro-dorsal striatal areas are activated early on during learning of new motor sequences (Lehericy et al., 2005; Toni et al., 1998). Furthermore, as training progresses and LEMs decrease, responses increase mainly in the ventral putamen, as reported for manual motor sequence learning (Doyon et al., 1996, 2003), although sometimes only after extended practice (Lehericy et al., 2005). In addition, a linear relationship was observed between the learning-related responses in the ventral striatum and the overnight gain in performance in fast learners, but not with improvements achieved over the day (30 min and 5 hr delays). As for the hippocampus, our data are not consistent with the persistence of continuous striatal activity during the first 24 posttraining hours. Learning-related activity is still observed in fast learners in the ventral striatum after a delay of 30 min, but not after 5 hr. This time course suggests again the importance of offline processes taking place after delays longer than 5 hr, possibly during sleep. Consistent with the latter hypothesis, functional connectivity of the striatum has been shown to be modified during REM sleep following motor sequence learning (Peigneux et al., 2003).

Hippocampal and Striatal Recruitment 24 hr Posttraining

Twenty-four hours after training, responses in fast learners to the learned sequence again involve the hippocampal formation and the striatum. The joint activation of hippocampal and striatal structures seems to characterize consolidated motor sequence memory because it is associated with an improvement in performance that tends to be specific to the learned sequence.

The involvement of hippocampus during retrieval indicates that the hippocampus not only participates in initial motor sequence learning (Schendan et al., 2003), but also in motor skill consolidation. We cannot rule out the possibility that hippocampal activity at 24 hr was related to some level of recognition of the repeated sequence, although the results of the generation tasks speak for a limited explicit sequential knowledge in our subjects. The recruitment of the putamen generalizes to oculomotor learning: previous reports on manual sequence learning have suggested that caudo-ventral putamen contributes to the long-term storage of motor sequences (Doyon and Benali, 2005; Doyon et al., 2002; Lehericy et al., 2005).

The present results are barely comparable with the few neuroimaging studies that have investigated the processing of oculomotor sequence learning. They focused on explicit sequence learning and emphasized the recruitment of frontal and parietal areas during both sequence learning (Kawashima et al., 1998) and the execution of newly learned sequences (Grosbras et al., 2001; Petit et al., 1996). In contrast, to the best of our knowledge, the neural correlates of memory consolidation in human oculomotor sequence learning have not yet been investigated.

Evidence from both animal and human studies suggest that memory systems interact during learning (Poldrack and Packard, 2003; Poldrack and Rodriguez, 2004). In normal subjects, probabilistic classification learning is accompanied by a decreased response in the medial temporal lobe structures, contrasting with an activation of basal ganglia (Poldrack et al., 1999, 2001; Seger and Cincotta, 2005). Our results confirm the antagonistic activity between mesio-temporal structures and the basal ganglia during learning in fast learners. As for the classification task (Poldrack et al., 1999, 2001), the negative interaction between the hippocampus and the putamen was related to a progressive decrease in hippocampal responses during the course of training, contrasting with the monotonic increase in putaminal activity. This competitive interaction does not seem to persist significantly over the day, but changes overnight.

Indeed, in fast learners, the interaction between the hippocampus and the putamen becomes cooperative 24 hr after training, when the gain in performance tends to be specific to the learned sequence. These results speak for the conversion, after time (and possibly sleep), of the competing interaction into cooperative interaction between two brain areas crucially involved in sequential memory. This cooperative interaction might support the gain in performance specific to the learned sequence, although we are not in a position to causally relate the change in functional connectivity to changes in performance.

This finding suggests that offline memory processing taking place at delays longer than 5 hr, and possibly during sleep, can change functional interactions between the hippocampus and the striatum. Such cooperation would foster both the detection of sequential associations by the hippocampus (Eichenbaum, 2004) and the sequential motor prediction in the basal ganglia (Seger, 2006), thereby improving sequential performance.

Again, we stress that the involvement of hippocampus and striatum might be of specific importance for oculomotor sequence learning rather than for motor learning at large. Although this should be formally established by future research, we do not expect that similar hippocampo-striatal interactions would necessarily apply to visuomotor adaptation tasks, let alone to elementary basic motor behaviors (Muellbacher et al., 2002). On the other hand, it is equally important to note that striatum and hippocampus also interact during higher-level cognitive learning (Nagy et al., 2007; Shohamy et al., 2007). The recruitment of both structures and their interactions might be required for optimizing motor and nonmotor learning and behavior.

To conclude, our results provide evidence for the importance of the hippocampus and ventral striatum in motor sequence learning. Not only do they respond to implicit motor sequence learning during training, but their early recruitment can predict subsequent sequence-specific overnight gain in performance. In addition, offline memory processes taking place overnight are associated with the emergence of a cooperative interaction between the hippocampus and the striatum, two structures that interacted competitively during training. Future research is needed to confirm the generality of these findings and specify the role of sleep in these memory processes.

EXPERIMENTAL PROCEDURES

Ninety young (range: 19–28 years), right-handed (Oldfield, 1971), healthy volunteers were recruited by advertisement. They had no history of medical, neurological, or psychiatric disease. None of them were on medication. The quality of their sleep was normal as assessed by the Pittsburgh Sleep Quality Index questionnaire (Buysse et al., 1989) (Supplemental Information IV). They all gave their written informed consent to take part in the study, which was approved by the Ethics Committee of the Faculty of Medicine of the University of Liège.

Task and General Experimental Design

Subjects were scanned during two separate sessions, referred to as the Training and Test sessions, while they performed the SORT task (Albouy et al., 2006) coded using Cogent2000 (http://www.vislab.ucl.ac.uk/Cogent/) and implemented in MATLAB (Mathworks Inc., Sherbom, MA). In this task, adapted from the serial reaction time (SRT) task described by Nissen and Bullemer (1987), a yellow dot (0.38°) is constantly displayed at one of four possible positions (1–4, visual angle 16° × 18°, see Figure 1A). The dot stays at any given position for a constant duration of 550 ms, then abruptly disappears and instantaneously reappears in another position. Unbeknownst to the subjects, the trajectory of the dot, i.e., the sequence of its positions, follows a second-order eight-element sequence, 2 4 3 1 4 2 1 3 (learned sequence) or 3 1 2 4 1 3 4 2 (untrained sequence). The two sequences are identical in terms of dot locations and transition frequency, but differ by the subsequences of three elements they contain.

To ensure that the subjects fixated the dot at all time, they were engaged in a color detection task. The only instructions to the subjects concerned the detection of a change in the color of the dot. There was a 20% chance at each position that the color of the dot would turn to an isoluminant orange color for 34 ms. Subjects were instructed to press a key on a keyboard when they detected the orange dot (Supplemental Information I). Key presses were recorded with an ~3 ms precision. Ocular movements were recorded online using an eye-tracking (ET) system (ASL, Model 504; Bedford, MA), at a sampling rate of 60 Hz. Dot moves were precisely marked on the ET recording by a trigger transmitted by the Cogent script (Figure 1D). The most efficient strategy to detect the color change was to keep one's gaze on the moving dot at all times. However, no mention was made in the instructions to the subjects concerning any reaction time or sequence of dot positions (Supplemental Information I).

The task was run in successive 22 s blocks separated by 15 s rest periods, during which the yellow dot remained at the center of the screen but could also transiently turn to orange. During the training session, subjects performed 19 consecutive blocks. During 18 of them, a given sequence, referred to as the learned sequence, was repeated five times. During the 16th block, the other sequence, referred to as the untrained sequence, was repeated five times (Figure 1C-1). Despite the low sample of the untrained sequence imposed by the constraints of the task, the overall design was still differentially sensitive to the brain responses evoked by the learned and untrained sequences (Supplemental Information X and Table S6). The test session took place after a variable delay, depending on the experimental group. Three delays were considered: 30 waking minutes (n = 25), 5 waking hours (n = 36), or 24 hours including a period of nocturnal sleep (n = 29, Figure 1B, see details in Supplemental Information II). The test session consisted of 18 further blocks, with 9 blocks each for the learned and untrained sequences. In each block, a given sequence was repeated five times. To match the analysis of a previous behavioral study (Albouy et al., 2006) where blocks included 15 sequence repetitions, the mean LEM was computed over three consecutive blocks (curly brackets in Figure 1C-1; Training, learned sequence: B1–B5 and B7; untrained sequence: B6; Test, learned sequence: B8, B9, and B11; untrained sequence: B10 and B12, Figure 1C-2). During test session, the fixed succession of the blocks of learned and untrained sequences was designed to minimize any proactive interference of the untrained sequence on the learned one. It also maximized the sensitivity of the experiment, ensuring that the fundamental frequency of any given trial type was in the useful frequency range to maintain a reasonable signal to noise ratio in fMRI data. At the end of the test session, subjects were debriefed using a standardized procedure to assess their level of explicit knowledge of the sequence gained during SORT practice (Supplemental Information V).

Behavioral Data Analysis

LEMs were automatically computed from ET recordings as the delay between the onset of a dot movement and the first eye movement in the correct direction, as described in (Albouy et al., 2006) (Figure 1D). As learning progressed, subjects were more likely to (implicitly) anticipate the next dot position and perform their saccades before the dot moved. In such a case, a negative LEM was computed. A repeated-measure ANOVA on mean LEMs per block with block repetition [B1 to B5, learned sequence] as a within-subjects factor and test delay [30 min, 5 h, and 24 h] as a between-subjects factor assessed the practice-related changes in LEMs during the training session. Another ANOVA using changes in LEMs between the trained (B5) and the untrained (B6) sequences as within-subjects factors and test delay [30 m, 5 h, and 24 h] as between-subjects factors was done to test the acquisition of sequence knowledge. We additionally checked for any difference in performance between B5 and B7 (trained sequences). Another ANOVA explored the between-session changes in performance on blocks B5, B6, B8, and B10, using sequence

[Learned versus Untrained] and session [Training versus Test] as within-subjects factors, and test delay [30 min, 5 h, and 24 h] as between-subjects factors. The difference in LEMs between blocks B5 and B8 reflected the gain in performance on the learned sequence between sessions. B5 was preferred to B7 for statistical analysis to avoid any possible interference effect due to the untrained sequence. B7 was presented in order to end the training with a learned sequence, avoiding any possible interference effect during the retention delay. The between-session change in performance related to the untrained sequence was assessed by the difference in LEMs between blocks B6 and B10. These two gains, as well as their differences, i.e., the sequence by session interaction, were subsequently used to explore the specific sequence knowledge acquisition. Similar analyses were performed on data of slow learners, and supplemental ANOVAs with a category factor were done to compare the effects between categories (Supplemental Information VII). Identical statistical analyses were conducted on saccadic duration in order to check that the changes in performance (LEMs) pertain to the progressive and implicit acquisition of sequential knowledge, rather than to the speeding of motor responses. The same statistical analyses were conducted in color detection scores (Supplemental Information VI).

fMRI Data Acquisition and Analysis

Functional MRI series were acquired using a head-only 3T scanner (Siemens, *Allegra*, Erlangen, Germany). Multislice T2*-weighted fMRI images were obtained with a gradient echo-planar sequence using axial slice orientation (TR = 2130 ms, TE = 40 ms, FA = 90°, 32 transverse slices, 3 mm slice thickness, 30% interslice gap, FoV = 220 × 220 mm^2, matrix size = 64 × 64 × 32, voxel size = 3.4 × 3.4 × 3.0 mm^3). Training sessions consisted of 350 scans, and test sessions, of 320 scans. A structural T1-weigthed 3D MP-RAGE sequence (TR = 1960 ms, TE = 4.43 ms, TI = 1100 ms, FA = 8°, 176 slices, FoV = 230 × 173 mm^2, matrix size = 256 × 192 × 176, voxel size = 0.9 × 0.9 × 0.9 mm^3) was also acquired in all subjects. Head movements were minimized using a vacuum cushion.

The three initial scans were discarded to allow for magnetic saturation effects. Functional volumes were preprocessed and analyzed using SPM2 (http://www.fil.ion.ucl.ac.uk/spm/software/spm2/; Wellcome Department of Imaging Neuroscience, London, UK). Preprocessing included the realignment of functional time series, the coregistration of functional and anatomical data, a spatial normalization to an EPI template conforming to the Montreal Neurological Institute space, and a spatial smoothing (Gaussian kernel, 8 mm full-width at half-maximum [FWHM]).

The analysis of fMRI data, based on a mixed effects model, was conducted in two serial steps, accounting for fixed and random effects. For each subject, changes in brain regional responses were estimated by a general linear model including the following factors: responses to the learned and untrained sequences and their modulation by LEMs, and motor responses to the changes in dot color. These regressors consisted of box cars (or step functions, for motor responses) convolved with the canonical hemodynamic response function. Movement parameters derived from realignment of the functional volumes were also included as covariates of no interest. High-pass filtering was implemented in the matrix design using a cutoff period of 128 s to remove slow drifts from the time series. Serial correlations in the fMRI signal were estimated using an autoregressive (order 1) plus white noise model and a restricted maximum likelihood (ReML) algorithm.

For the training session, linear contrasts tested the main learning effect ($L_{Training} - U_{Training}$). Linear contrasts tested the main effect of practice of the learned sequence modulated by LEMs ($\pm L_{TrainingLEM}$). These contrasts looked for regional responses that vary in proportion to LEMs of each block. They identified regions where responses decrease (or increase) as oculomotor performance improves during training. Another contrast (reported in Supplemental Information X and Table S6) tested the effect of the untrained sequence as compared with the learned one ($U_{Training} - L_{Training}$). For the test session, a linear contrast tested the main learning effect ($L_{Test} - U_{Test}$). Finally, a linear contrast tested the main learning effect by session [($L_{Test} - U_{Test}$) − ($L_{Training} - U_{Training}$)], i.e., [(L − U) × (Test − Training)]. These linear contrasts generated statistical parametric maps [SPM(T)]. These images were then further spatially smoothed (Gaussian kernel 6 mm FWHM) and entered in a second-level analysis, corresponding to a random-effects model, accounting for intersubject variance. In this second-level analysis, the participants were split into two categories, slow or fast learners, based on their performance during training. The results of fast learners are reported in the main text, whereas the results of slow learners and comparison between categories are reported in Supplemental Information VIII.

For the training session, one-sample t tests were separately run on the data of each category. A first analysis characterized the learning effect, a second one characterized the performance effect, and a third one consisted of an ANOVA (Supplemental Information VIII) wherein the six groups were separately specified (three intervals, two categories). Inclusive masks based on the effect of interest in the category of interest were applied for each contrast to isolate the effects within each category. A correction for nonsphericity was applied on the data to account for possibly unequal variance between groups. T contrasts characterized the learning by category interaction [($L_{Training} - U_{Training}$) × category] and the performance by category interaction [($\pm L_{TrainingLEM}$) × category]. Furthermore, to assess the relationship between brain activity during training and the subsequent gain in performance on the learned sequence at a later date, we regressed the individual within-subject contrasts images (learning effect) against the gain in performance on the learned sequence (B5–B8), separately for each group. A final ANOVA compared this regression between the three groups of fast learners (main text) and between the two categories (Supplemental Information VIII). Finally, psychophysiological interaction (PPI) analyses were computed to test the functional connectivity of the left posterior hippocampus with the rest of the brain during training. A new linear model was generated at the individual level, using three regressors. One regressor represented the practice of the learned sequence modulated by the performance. The second regressor was the activity in the reference area. The third regressor represented the interaction of interest between the first (psychological) and the second (physiological) regressors. To build this regressor, the underlying neuronal activity was first estimated by a parametric empirical Bayes formulation, combined with the psychological factor and subsequently convolved with the hemodynamic response function (Gitelman et al., 2003). The design matrix also included movement parameters. A significant PPI indicated a change in the regression coefficients between any reported brain area and the reference region, related to performance changes during training. The voxels identified in this analysis show a pattern of activity correlated with posterior hippocampus activity. The strength of this correlation is modulated by performance. Next, individual summary statistic images obtained at the first-level (fixed effects) analysis were spatially smoothed (6 mm FWHM Gaussian kernel) and entered in a second-level (random-effects) analysis using one-sample t tests. Inferences were conducted as for the main effect analysis.

For the test sessions, one-sample t tests characterizing the main learning effect were performed separately for each group (Table S2). An ANOVA compared the main learning effect between groups of fast learners (main text) and between categories (Supplemental Information VIII). T contrasts characterized the main learning effect by group of fast learners [($L_{Test} - U_{Test}$) × group] (main text) and the main learning effect by group by category [($L_{Test} - U_{Test}$) × group × category] (Supplemental Information VIII). Inclusive masks were also applied in order to isolate effect within each group. As above, a correction of nonsphericity was applied.

One-sample t tests comparing the main learning effect between sessions were performed separately for each group (Table S3). This analysis characterized the differential brain responses between the learned and the untrained sequences during testing as compared with training. A final ANOVA compared the main learning effect by session between groups of fast learners. T contrasts characterized the main learning effect by session by groups of fast learners [(L − U) × (Test − Training) × group] (Supplemental Information IX and Table S5). As above, a correction of nonsphericity was applied.

Finally, PPI analyses were computed to test the functional connectivity of the right dorsal putamen with the rest of the brain during different test sessions. The same method described above was used, but the first regressor represented practice of the learned sequence as compared with practice of the untrained sequence.

The resulting set of voxel values for each contrast constituted a map of the t statistic [SPM(T)], thresholded at $p < 0.001$ (uncorrected for multiple comparisons). Statistical inferences were performed at a threshold of $p < 0.05$ after

correction for multiple comparisons over either the entire brain volume or over small spherical volumes (10 mm radius), located in structures of interest reported in the literature (regions of interest in Supplemental Information XI).

SUPPLEMENTAL DATA

The Supplemental Data for this article can be found online at http://www.neuron.org/cgi/content/full/58/2/261/DC1/.

ACKNOWLEDGMENTS

This study was supported by the Belgian Fonds National de la Recherche Scientifique (F.N.R.S.), the Fondation Médicale Reine Elisabeth, the Research Fund of ULg, and PAI/IAP Interuniversity Pole of Attraction. G.A. is supported by a Ph.D. grant from the French "Ministère de la Recherche," and V.S., E.B., G.V., M.D., T.D.V., A.D., and P.M. are supported by F.N.R.S. P.R. was supported by a "Marie Curie Individual fellowship of the European Community." P.P. is now at the Université Libre de Bruxelles (ULB), Belgium.

Received: June 13, 2007
Revised: October 30, 2007
Accepted: February 4, 2008
Published: April 23, 2008

REFERENCES

Albouy, G., Ruby, P., Phillips, C., Luxen, A., Peigneux, P., and Maquet, P. (2006). Implicit oculomotor sequence learning in humans: Time course of offline processing. Brain Res. *1090*, 163–171.

Alvarez, P., and Squire, L.R. (1994). Memory consolidation and the medial temporal lobe: a simple network model. Proc. Natl. Acad. Sci. USA *91*, 7041–7045.

Ambrosini, M.V., Langella, M., Gironi Carnevale, U.A., and Giuditta, A. (1992). The sequential hypothesis of sleep function. III. The structure of postacquisition sleep in learning and nonlearning rats. Physiol. Behav. *51*, 217–226.

Buysse, D.J., Reynolds, C.F., 3rd, Monk, T.H., Berman, S.R., and Kupfer, D.J. (1989). The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res. *28*, 193–213.

Cajochen, C., Knoblauch, V., Wirz-Justice, A., Krauchi, K., Graw, P., and Wallach, D. (2004). Circadian modulation of sequence learning under high and low sleep pressure conditions. Behav. Brain Res. *151*, 167–176.

Chun, M.M., and Phelps, E.A. (1999). Memory deficits for implicit contextual information in amnesic subjects with hippocampal damage. Nat. Neurosci. *2*, 844–847.

Curran, T. (1997). Higher-order associative learning in amnesia: Evidence from the serial reaction time task. J. Cogn. Neurosci. *9*, 522–533.

Degonda, N., Mondadori, C.R., Bosshardt, S., Schmidt, C.F., Boesiger, P., Nitsch, R.M., Hock, C., and Henke, K. (2005). Implicit associative learning engages the hippocampus and interacts with explicit associative learning. Neuron *46*, 505–520.

Doyon, J., and Benali, H. (2005). Reorganization and plasticity in the adult brain during learning of motor skills. Curr. Opin. Neurobiol. *15*, 161–167.

Doyon, J., Owen, A.M., Petrides, M., Sziklas, V., and Evans, A.C. (1996). Functional anatomy of visuomotor skill learning in human subjects examined with positron emission tomography. Eur. J. Neurosci. *8*, 637–648.

Doyon, J., Song, A.W., Karni, A., Lalonde, F., Adams, M.M., and Ungerleider, L.G. (2002). Experience-dependent changes in cerebellar contributions to motor sequence learning. Proc. Natl. Acad. Sci. USA *99*, 1017–1022.

Doyon, J., Penhune, V., and Ungerleider, L.G. (2003). Distinct contribution of the cortico-striatal and cortico-cerebellar systems to motor skill learning. Neuropsychologia *41*, 252–262.

Eichenbaum, H. (2004). Hippocampus: cognitive processes and neural representations that underlie declarative memory. Neuron *44*, 109–120.

Fischer, S., Hallschmid, M., Elsner, A.L., and Born, J. (2002). Sleep forms memory for finger skills. Proc. Natl. Acad. Sci. USA *99*, 11987–11991.

Fischer, S., Nitschke, M.F., Melchert, U.H., Erdmann, C., and Born, J. (2005). Motor memory consolidation in sleep shapes more effective neuronal representations. J. Neurosci. *25*, 11248–11255.

Fletcher, P.C., Zafiris, O., Frith, C.D., Honey, R.A., Corlett, P.R., Zilles, K., and Fink, G.R. (2005). On the benefits of not trying: brain activity and connectivity reflecting the interactions of explicit and implicit sequence learning. Cereb. Cortex *15*, 1002–1015.

Foerde, K., Knowlton, B.J., and Poldrack, R.A. (2006). Modulation of competing memory systems by distraction. Proc. Natl. Acad. Sci. USA *103*, 11778–11783.

Frankland, P.W., and Bontempi, B. (2005). The organization of recent and remote memories. Nat. Rev. Neurosci. *6*, 119–130.

Gabrieli, J.D., Corkin, S., Mickel, S.F., and Growdon, J.H. (1993). Intact acquisition and long-term retention of mirror-tracing skill in Alzheimer's disease and in global amnesia. Behav. Neurosci. *107*, 899–910.

Gitelman, D.R., Penny, W.D., Ashburner, J., and Friston, K.J. (2003). Modeling regional and psychophysiologic interactions in fMRI: the importance of hemodynamic deconvolution. Neuroimage *19*, 200–207.

Grosbras, M.H., Leonards, U., Lobel, E., Poline, J.B., LeBihan, D., and Berthoz, A. (2001). Human cortical networks for new and familiar sequences of saccades. Cereb. Cortex *11*, 936–945.

Hauptmann, B., and Karni, A. (2002). From primed to learn: the saturation of repetition priming and the induction of long-term memory. Brain Res. Cogn. Brain Res. *13*, 313–322.

Henke, K., Treyer, V., Nagy, E.T., Kneifel, S., Dursteler, M., Nitsch, R.M., and Buck, A. (2003). Active hippocampus during nonconscious memories. Conscious. Cogn. *12*, 31–48.

Ji, D., and Wilson, M.A. (2007). Coordinated memory replay in the visual cortex and hippocampus during sleep. Nat. Neurosci. *10*, 100–107.

Karni, A., Meyer, G., Jezzard, P., Adams, M.M., Turner, R., and Ungerleider, L.G. (1995). Functional MRI evidence for adult motor cortex plasticity during motor skill learning. Nature *377*, 155–158.

Kawashima, R., Tanji, J., Okada, K., Sugiura, M., Sato, K., Kinomura, S., Inoue, K., Ogawa, A., and Fukuda, H. (1998). Oculomotor sequence learning: a positron emission tomography study. Exp. Brain Res. *122*, 1–8.

Korman, M., Raz, N., Flash, T., and Karni, A. (2003). Multiple shifts in the representation of a motor sequence during the acquisition of skilled performance. Proc. Natl. Acad. Sci. USA *100*, 12492–12497.

Kumaran, D., and Maguire, E.A. (2006). The dynamics of hippocampal activation during encoding of overlapping sequences. Neuron *49*, 617–629.

Leconte, P., Hennevin, E., and Bloch, V. (1973). Analysis of the effects of learning and the level of acquisition on consecutive paradoxical sleep. Brain Res. *49*, 367–379.

Lehericy, S., Benali, H., Van de Moortele, P.F., Pelegrini-Issac, M., Waechter, T., Ugurbil, K., and Doyon, J. (2005). Distinct basal ganglia territories are engaged in early and advanced motor sequence learning. Proc. Natl. Acad. Sci. USA *102*, 12566–12571.

Leigh, R.J., and Zee, D.S. (1999). The Neurology of Eye Movements (New York: Oxford University Press).

Maquet, P., Laureys, S., Peigneux, P., Fuchs, S., Petiau, C., Phillips, C., Aerts, J., Del Fiore, G., Degueldre, C., Meulemans, T., et al. (2000). Experience-dependent changes in cerebral activation during human REM sleep. Nat. Neurosci. *3*, 831–836.

Maquet, P., Schwartz, S., Passingham, R., and Frith, C. (2003). Sleep-related consolidation of a visuomotor skill: brain mechanisms as assessed by functional magnetic resonance imaging. J. Neurosci. *23*, 1432–1440.

Muellbacher, W., Ziemann, U., Wissel, J., Dang, N., Kofler, M., Facchini, S., Boroojerdi, B., Poewe, W., and Hallett, M. (2002). Early consolidation in human primary motor cortex. Nature *415*, 640–644.

Nadel, L., and Moscovitch, M. (1997). Memory consolidation, retrograde amnesia and the hippocampal complex. Curr. Opin. Neurobiol. *7*, 217–227.

Nagy, H., Keri, S., Myers, C.E., Benedek, G., Shohamy, D., and Gluck, M.A. (2007). Cognitive sequence learning in Parkinson's disease and amnestic mild cognitive impairment: Dissociation between sequential and non-sequential learning of associations. Neuropsychologia *45*, 1386–1392.

Nissen, M.J., and Bullemer, P. (1987). Attentional requirements of learning: evidence from performance measures. Cognit. Psychol. *19*, 1–32.

Oldfield, R.C. (1971). The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia *9*, 97–113.

Peigneux, P., Maquet, P., Meulemans, T., Destrebecqz, A., Laureys, S., Degueldre, C., Delfiore, G., Aerts, J., Luxen, A., Franck, G., et al. (2000). Striatum forever, despite sequence learning variability: a random effect analysis of PET data. Hum. Brain Mapp. *10*, 179–194.

Peigneux, P., Laureys, S., Fuchs, S., Destrebecqz, A., Collette, F., Delbeuck, X., Phillips, C., Aerts, J., Del Fiore, G., Degueldre, C., et al. (2003). Learned material content and acquisition level modulate cerebral reactivation during posttraining rapid-eye-movements sleep. Neuroimage *20*, 125–134.

Peigneux, P., Laureys, S., Fuchs, S., Collette, F., Perrin, F., Reggers, J., Phillips, C., Degueldre, C., Del Fiore, G., Aerts, J., et al. (2004). Are spatial memories strengthened in the human hippocampus during slow wave sleep? Neuron *44*, 535–545.

Petit, L., Orssaud, C., Tzourio, N., Crivello, F., Berthoz, A., and Mazoyer, B. (1996). Functional anatomy of a prelearned sequence of horizontal saccades in humans. J. Neurosci. *16*, 3714–3726.

Poldrack, R.A., and Packard, M.G. (2003). Competition among multiple memory systems: converging evidence from animal and human brain studies. Neuropsychologia *41*, 245–251.

Poldrack, R.A., and Rodriguez, P. (2004). How do memory systems interact? Evidence from human classification learning. Neurobiol. Learn. Mem. *82*, 324–332.

Poldrack, R.A., Prabhakaran, V., Seger, C.A., and Gabrieli, J.D. (1999). Striatal activation during acquisition of a cognitive skill. Neuropsychology *13*, 564–574.

Poldrack, R.A., Clark, J., Pare-Blagoev, E.J., Shohamy, D., Creso Moyano, J., Myers, C., and Gluck, M.A. (2001). Interactive memory systems in the human brain. Nature *414*, 546–550.

Press, D.Z., Casement, M.D., Pascual-Leone, A., and Robertson, E.M. (2005). The time course of off-line motor sequence learning. Brain Res. Cogn. Brain Res. *21*, 375–378.

Rasch, B., Buchel, C., Gais, S., and Born, J. (2007). Odor cues during slow-wave sleep prompt declarative memory consolidation. Science *315*, 1426–1429.

Reber, P.J., and Squire, L.R. (1994). Parallel brain systems for learning with and without awareness. Learn. Mem. *1*, 217–229.

Robertson, E.M., Pascual-Leone, A., and Press, D.Z. (2004). Awareness modifies the skill-learning benefits of sleep. Curr. Biol. *14*, 208–212.

Schendan, H.E., Searl, M.M., Melrose, R.J., and Stern, C.E. (2003). An FMRI study of the role of the medial temporal lobe in implicit and explicit sequence learning. Neuron *37*, 1013–1025.

Seger, C.A. (2006). The basal ganglia in human learning. Neuroscientist *12*, 285–290.

Seger, C.A., and Cincotta, C.M. (2005). Dynamics of frontal, striatal, and hippocampal systems during rule learning. Cereb. Cortex *16*, 1546–1555.

Shohamy, D., Myers, C.E., Kalanithi, J., and Gluck, M.A. (2007). Basal ganglia and dopamine contributions to probabilistic category learning. Neurosci. Biobehav. Rev. *32*, 219–236.

Spencer, R.M., Sunm, M., and Ivry, R.B. (2006). Sleep-dependent consolidation of contextual learning. Curr. Biol. *16*, 1001–1005.

Toni, I., Krams, M., Turner, R., and Passingham, R.E. (1998). The time course of changes during motor sequence learning: a whole-brain fMRI study. Neuroimage *8*, 50–61.

Tononi, G., and Cirelli, C. (2006). Sleep function and synaptic homeostasis. Sleep Med. Rev. *10*, 49–62.

Walker, M.P., Brakefield, T., Morgan, A., Hobson, J.A., and Stickgold, R. (2002). Practice with sleep makes perfect: sleep-dependent motor skill learning. Neuron *35*, 205–211.

Walker, M.P., Brakefield, T., Seidman, J., Morgan, A., Hobson, J.A., and Stickgold, R. (2003). Sleep and the time course of motor skill learning. Learn. Mem. *10*, 275–284.

Walker, M.P., Stickgold, R., Alsop, D., Gaab, N., and Schlaug, G. (2005). Sleep-dependent motor memory plasticity in the human brain. Neuroscience *133*, 911–917.

Yang, J., Weng, X., Guan, L., Kuang, P., Zhang, M., Sun, W., Yu, S., and Patterson, K. (2003). Involvement of the medial temporal lobe in priming for new associations. Neuropsychologia *41*, 818–829.