

An fMRI Study of the Role of the Medial Temporal Lobe in Implicit and Explicit Sequence Learning

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Summary

fMRI was used to investigate the neural substrates supporting implicit and explicit sequence learning, focusing especially upon the role of the medial temporal lobe. Participants performed a serial reaction time task (SRTT). For implicit learning, they were naive about a repeating pattern, whereas for explicit learning, participants memorized another repeating sequence. fMRI analyses comparing repeating versus random sequence blocks demonstrated activation of frontal, parietal, cingulate, and striatal regions implicated in previous SRTT studies. Importantly, medio-temporal lobe regions were active in both explicit and implicit SRTT learning. Moreover, the results provide evidence of a role for the hippocampus and related cortices in the formation of higher order associations under both implicit and explicit learning conditions, regardless of conscious awareness of sequence knowledge.

Introduction

Sequence learning is used for behaviors like typing, musical performance, and route navigation. Researchers have described the acquisition of perceptuomotor sequencing skills using either motor control (e.g., Hazeltine et al., 1997) or learning and memory frameworks (e.g., Reber and Squire, 1998). Both explanations agree that distinct brain processes support explicit learning, which occurs with awareness, and implicit learning, which occurs without awareness. However, the two accounts diverge over which brain systems are important. Most critically, only memory frameworks posit a role for the mediotemporal lobe (MTL) in sequence learning.

For explicit sequence learning, both motor control and memory accounts implicate the dorsolateral prefrontal cortex (DLPFC). This region, by motor accounts, supports conscious executive motor control to select goals or to select and maintain a spatial sequence in working memory (Willingham, 1998; Grafton et al., 1995; Hazeltine et al., 1997) or, by memory accounts, supports the manipulation and monitoring functions of working memory (Smith and Jonides, 1999). Memory models, however, also posit that the role of the DLPFC is limited

to sequences with attributes that do not exceed the storage capabilities of working memory (e.g., shorter, brief interstimulus delay, few intervening stimuli). For longer-term, explicit episodic retrieval of longer sequences, memory accounts implicate the MTL (Squire and Zola-Morgan, 1991). For implicit sequence learning, both frameworks implicate the striatum. While motor models also include the supplementary motor area (SMA), parietal lobe, and cerebellum (Willingham, 1998; Middleton and Strick, 2000), some memory accounts implicate MTL structures in certain types of implicit learning (Curran, 1997; Cohen and Eichenbaum, 1993).

We used fMRI to investigate the role of the human MTL in implicit and explicit sequence learning. fMRI data were acquired while subjects performed a serial reaction time task (SRTT; Figure 1A), developed originally by Nissen and Bullemer (1987). In the SRTT, learning results in faster response times (RTs) for repeated than for random sequences of cued locations.

For implicit SRTT learning, convergent evidence implicates subcortical and cortical components of frontostriatal pathways. Patients with striatal dysfunction are impaired on implicit SRTTs (Knopman and Nissen, 1991; Vakil et al., 2000; Jackson et al., 1995; Doyon et al., 1997, 1998). Neuroimaging studies using an implicit SRTT with healthy adults have shown activation in the caudate, putamen (Rauch et al., 1995, 1997a; Hazeltine et al., 1997; Grafton et al., 1995; Willingham et al., 2002; Peigneux et al., 2000) and ventral striatum (Berns et al., 1997; Doyon et al., 1996). The caudate has been proposed to be important for stimulus-response association (Poldrack et al., 2001) and cognitive abilities, such as working memory (Owen et al., 1998). Activation has also been found in cortical components of frontostriatal circuits, including the DLPFC, parietal lobe, premotor cortex, anterior cingulate, and SMA (Rauch et al., 1995, 1997b; Berns et al., 1997; Grafton et al., 1995, 1998; Hazeltine et al., 1997; Willingham et al., 2002; Peigneux et al., 2000).

For explicit SRTT learning, while striatal activation has rarely been noted, neuroimaging studies consistently find activation in cortical components of frontostriatal circuits, including the DLPFC, ventrolateral prefrontal, premotor, anterior cingulate, and dorsal and inferior parietal cortices (Hazeltine et al., 1997; Jenkins et al., 1994; Grafton et al., 1995; Rauch et al., 1995; Willingham et al., 2002). Motor accounts posit the DLPFC controls strategic processes throughout the explicit SRTT, and it can be recruited during an implicit SRTT, if participants become aware of a sequence (Willingham, 1998). This idea resembles an explicit-implicit variety of memory account but with the DLPFC, not MTL, being necessary for conscious sequence acquisition.

Memory, but not motor, accounts consider the MTL system to be necessary for learning sequences, especially those beyond the capacity of DLPFC processes of working memory. Each account, however, posits a somewhat different role for MTL. For explicit learning, explicit-implicit (or declarative-nondeclarative) memory accounts state that the MTL is necessary for long-term

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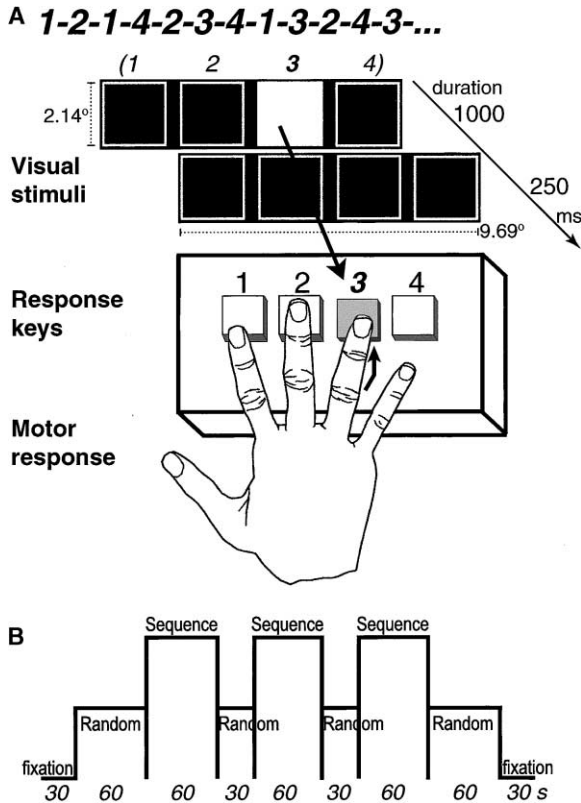


Figure 1. SRTT and fMRI Activation Paradigm

(A) In the SRTT, a filled white square at one of four locations cued a key press at the designated location (note, numerals were not shown). Responses were made as fast as possible using a dominant hand. At all times, a horizontal array of four white outline squares appeared on a black background (55.25 cm eye-to-screen). The implicit sequence (from Curran, 1997) is shown (top); for explicit learning, it was 2-3-2-4-1-3-1-4-3-4-2-1 (from Reber and Squire, 1998).

(B) On some SRTT blocks, one Sequence of locations repeated; on others, new sequences of Random locations were shown.

memory for facts and events that are consciously accessible (Schacter, 1997; Squire, 1992). The MTL is thought to support explicit memory by temporarily binding together distributed neocortical processing areas that jointly comprise a holistic representation of a remembered episode. In some versions, the MTL is necessary for conscious awareness of stimulus and task relationships, and this awareness is required for learning (Clark and Squire, 1998). By explicit-implicit accounts, MTL activity is a function of the degree to which participants become consciously aware of a sequence during learning.

In contrast, a relational memory account states that the MTL is critically involved in associative processes that bind multiple aspects of stimulus events into memory (Cohen and Eichenbaum, 1993; Wallenstein et al., 1998). Relational memory accounts thus expect the MTL to be involved in sequence learning whenever complex stimulus-stimulus associations are encoded, regardless of whether learning is explicit or implicit. Explicit-implicit and relational memory accounts thus diverge principally over MTL involvement in implicit learning.

While most prior work has not implicated the MTL in

implicit learning (Corkin, 1968; Knowlton et al., 1992, 1994), recent findings suggest the MTL is necessary for implicit learning of complex multi-event contingencies (Curran, 1997; Chun and Phelps, 1999; Clark and Squire, 1998; Poldrack et al., 2001; Rose et al., 2002). For the specific case of motor sequence learning, the MTL has not generally been implicated, even when learning becomes explicit. Only one prior neuroimaging study of the SRTT with healthy adults reported MTL activity, which decreased as both implicit and explicit learning progressed across blocks of a short repeating sequence (Grafton et al., 1995); note, sequence-specific learning could not be assessed in that study but can in ours by comparing repeated and random sequences.

In studies of patients with amnesia, implicit but not explicit SRTTs have been used. Nonetheless, amnesics are presumed to be impaired on explicit SRTT learning, as they perform poorly on explicit tests of sequence knowledge following implicit learning tasks (Reber and Squire, 1994, 1998). For implicit SRTT learning, amnesics are not generally impaired (Nissen and Bullemer, 1987; Nissen et al., 1987, 1989; Reber and Squire, 1994, 1998) but may show deficient learning with certain types of complex sequences. Curran (1997) compared *second-order conditional* (SOC) sequences that are equated for item and bi-item frequencies and the *first-order conditional* (FOC) sequences used in most SRTT studies, which are not so equated. A SOC sequence cannot be learned based on simple frequencies or contingencies between one location and a second, as the FOC variety can. Learning effects with SOC sequences thus reflect higher order associations between three or more successive locations. While amnesics and controls showed comparable mean RT learning effects in both FOC and SOC cases, finer grained RT analyses indicate amnesics can acquire the frequency or simple associative information of FOC sequences but are impaired at making the higher order associations between multiple locations required to learn the SOC variety. This finding motivated the use of SOC sequences and finer grained RT analyses in our study.

We examined MTL structures during implicit and explicit SRTT learning. While motor accounts predict no MTL involvement in either task, memory accounts predict MTL activity in explicit learning. Specifically, an explicit-implicit view predicts MTL activity throughout explicit SRTT learning, as participants are consciously acquiring a structured sequence at all times. It also predicts the MTL will not be involved in the implicit SRTT, except in individuals who become aware of the sequence. In this case, MTL activation should be greater later than earlier in implicit learning because of the emerging awareness. In contrast, a relational view predicts MTL activation in both explicit and implicit SRTTs, regardless of resultant conscious sequence awareness. It also predicts that MTL activity should decrease as the sequence is acquired because fewer or no new associations remain to be encoded later in learning. To test these predictions, we assessed the relationship between learning-related brain activity and conscious awareness of sequence knowledge by following both SRTTs with a comprehensive battery of explicit knowledge and sequence awareness tests. We also assessed

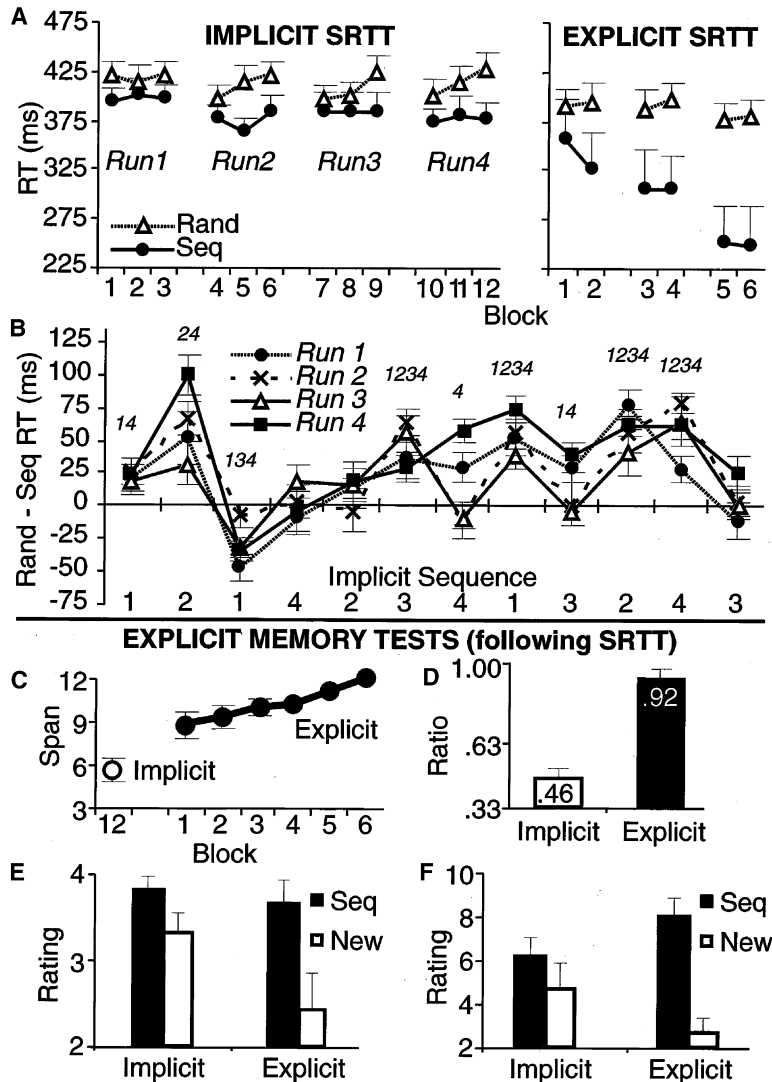


Figure 2. Performance on the SRTTs and Memory Tests of Conscious Sequence Knowledge

Bars show SE; Seq = Sequence; Rand = Random.

(A) RT learning effects occurred in implicit (left) and explicit (right) SRTTs.

(B) Higher order associative learning occurred in each run (labels at each Sequence position designate a reliable RT difference in that run 1-4; for all measures, $t > 2.9$; $p < 0.05$). A significant positive RT difference at one position (i.e., transition pair) demonstrates a higher order association among three consecutive locations, and at two or more consecutive positions demonstrates even higher order associations among four or more locations.

(C) Free generation was much better following each block of explicit learning compared with following all implicit blocks (Span = longest continuous section generated from the 12-location, repeating Sequence).

(D) Self-cued generation was twice as accurate following explicit compared with following implicit learning. This test yielded 96 key presses per subject that we coded as 94 consecutive response triplets. A ratio was calculated as $X/(X + I)$, referring to the number of triplets that were consistent (X) or inconsistent (I) with the Sequence.

(E) Triplet recognition ratings revealed participants reliably discriminated between New triplets and those from the Sequence ($F_{(1,9)} = 27$, $p < 0.001$); values on the vertical axis represent $(6 - \text{Rating})$.

(F) Full sequence recognition revealed that ratings for the actual Sequence and New SOC sequences were discriminated much better after explicit than implicit SRTTs.

higher order associations and changes in learning effects with increasing sequence experience.

Results

Performance

Repeated-measures ANOVAs and two-tailed t tests (Bonferroni α) assessed behavioral data of subjects included in parallel analyses of fMRI data; RT data of two subjects each were missing for explicit learning and implicit association analyses (due to technical problems).

Implicit and Explicit Learning

Median RTs (1250 ms cut-off) were faster in Sequence than Random blocks (Figure 2A) for both explicit, main condition (Sequence, Random) effect ($F[1,9] = 5.34$, $p < 0.05$) and implicit tasks ($F[1,14] = 42$, $p = 0.0001$; two short Random RT blocks analyzed as one "long" block). For implicit learning, RTs varied across blocks 1-3 ($F[2,28] = 4.95$, $p = 0.01$), block \times condition ($F[2,28] = 4.8$, $p = 0.02$), and run \times block \times condition ($F[6,84] = 2.49$, $p = 0.03$). For explicit learning, RTs varied across

run ($F[2,18] = 7.48$, $p = 0.004$) and run \times condition ($F[2,18] = 4.21$, $p = 0.032$).

Higher Order Association

Higher order association learning among three or more consecutive locations was assessed. As in Curran (1997), RTs to each pairwise transition of the implicit sequence were compared between Sequence and Random conditions. To do so, for all 12 pairs of location transitions, for each run, the median RT to the second location of each pair was determined, separately, for Sequence and for Random blocks (e.g., for transition pair 3-1, the RT to location 1 is taken if it is preceded by location 3). The median RT for each pairwise transition was then averaged across all runs. The main effect of condition (Sequence, Random) was reliable, $F(1,12) = 35$, and interacted with pairwise transition (1-12), $F(11,132) = 15$; the main transition effect was reliable, $F = 4.56$ (for all measures, $p < 0.0001$).

Critically, a difference score for each pairwise transition was calculated by subtracting the resultant mean RT for the Sequence condition from that for the Random condition, and planned contrasts (one-sample t tests)

defined pairs differing reliably from zero. Across all runs, we replicated the higher order associative learning curve obtained by Curran (1997): faster RTs in Sequence than Random conditions at transitions 4-1 ($t = 6.8$), 3-2 ($t = 6.34$), 2-4 ($t = 6.73$) (for all measures, $p < 0.0001$), 1-2 ($t = 5.16$), 2-3 ($t = 4.81$) (for all measures, $p < 0.001$), 3-1 ($t = 3.24$) (for all measures, $p < 0.005$), and 1-3 ($t = 2.75$, $p < 0.05$) and faster RTs to Random than Sequence conditions for 2-1 ($t = -7.46$, $p < 0.0001$). Analyses of runs 1-4, separately (Figure 2B), revealed higher order associative learning also in each run.

Explicit Memory Tests

To assess conscious knowledge of sequence structure, explicit memory tests followed learning. Overall, they revealed markedly greater sequence awareness following explicit compared with implicit SRTTs (implicit, I , $N_I = 15$; explicit, E , $n_E = 11$; implicit versus explicit, IE , $n_{IE} = 10$; data of one subject following the explicit SRTT were not collected).

Awareness. Explicit knowledge questions following only the implicit SRTT revealed little or no evidence that participants had become consciously aware of a repeating pattern. They reported that it was as likely that a sequence repeated ($M = 1.6$) as for locations to be random ($M = 1.7$, paired $t[14] = 0.34$, $p > 0.7$) or for task difficulty to vary ($M = 2$, $t = 1.23$, $p > 0.2$); $F(3,42) = 5.18$, $p < 0.005$. It was less likely, however, that some locations were more frequent ($M = 2.9$) than that a sequence was repeated ($t = 3.84$, $p < 0.005$), locations were random, or task difficulty varied.

Free Generation. Participants recalled a much longer correct sequence following explicit than implicit tasks (Figure 2C). Following implicit learning, mean generation span was slightly above chance (3.71); $t_{IE}(9) = 2.27$ ($M_{IE} = 5.6$), $t(14) = 2.42$ (for all measures, $p < 0.05$). For explicit learning, it reliably exceeded chance after every block (for all measures, $t > 4$, $p < 0.005$) and increased by Block (for all measures, $F[5,50] > 5$, $p < 0.001$).

Self-Cued Generation. Cued recall of the sequence was much better following explicit than implicit SRTTs (Figure 2D); $t_{IE}(9) = 5.69$, $p < 0.0005$. Participants generated consistent triplets above chance (0.3133) following explicit learning (one-sample $t_{IE}[9] = 14$; $t_E[10] = 12$; for all measures, $p < 0.0001$; $M_E = 0.89$) and possibly implicit learning ($t_{IE}[9] = 3.47$; $t_I[12] = 3.78$; for all measures, $p < 0.01$; $M_I = 0.45$; note, we used a conservative estimate of 0.31, but the chance ratio may be as high as 0.5; see Shanks and Johnstone, 1999).

Triplet Recognition. Sequence triplets were rated as more familiar than new ones (Figure 2E; $\alpha 0.025$) after implicit ($t_{IE}[9] = 3.13$, $p < 0.05$; $t[14] = 4.69$, $p < 0.0005$; $M_{Seq} = 2.2$; $M_{new} = 2.7$) and explicit tasks ($t_{IE}[9] = 3.5$, $p < 0.01$; $t_E[10] = 3.72$, $p < 0.005$; $M_{Seq} = 2.3$; $M_{new} = 3.8$). The third location was faster overall for old ($M_I = 467$ ms; $M_E = 521$ ms) than new ($M_I = 519$ ms; $M_E = 565$ ms) triplets (position \times repetition, $F[2,10] = 7.31$, $p < 0.05$; main effect of position, $F[1,5] = 19$, $p = 0.0004$), but this RT difference was marginal only after implicit learning ($t[5] = 2.45$, $p < 0.06$), suggesting motor fluency contamination of ratings following implicit SRTT but not after explicit SRTT. This was further supported by multiple regression analyses with mean triplet RTs and true old-new status ("repetition") as predictors; the correlation was reliable between RTs and Ratings for implicit

SRTT ($r = -0.58$, $z = -1.99$, $p < 0.05$, $n = 6$; note, for explicit SRTT, Repetition and Ratings $r = -0.57$, $z = -2.41$, $p < 0.05$, $n = 8$).

Full Recognition. Full-sequence recognition (Figure 2F) revealed the actual Sequence was reliably discriminated from new ones only after explicit learning (main effect of repetition $F_{IE}[4,36] = 11.7$, $F_E[4,40] = 9.52$, $p < 0.0001$; implicit $F_{IE} = 2.1$; $F_I[4,56] = 1.85$, $p > 0.1$); repetition \times task interaction ($F[4,36] = 3.49$, $p = 0.0165$), main effect of repetition ($F[4,36] = 8.51$, $p < 0.0001$), main effect of task ($F[1,9] = 4.57$, $p = 0.0613$).

fMRI

Using SPM99 software (Wellcome Dept. of Cognitive Neurology), preprocessing of fMRI BOLD data included motion correction, normalization to MNI305 stereotactic space (interpolating to 3 mm³ voxels; neurological convention), and spatial smoothing (8 mm³ gaussian kernel). Statistical analyses used the general linear model. High-pass filtering was applied, but global signal scaling was not used to avoid spurious deactivations. Design matrices were modeled using a boxcar function convolved with a canonical hemodynamic response function.

Learning-related activation was assessed in linear contrasts of Sequence relative to Random blocks for implicit and explicit runs, separately; note, this contrast serves to factor out skill effects that may be greater on the explicit SRTT (performed last), because baseline skill level is always equated between the Sequence and Random blocks. As we used SOC sequences, this contrast demonstrates sequence-specific activation. Contrast images for each subject were used in second-level analyses treating subjects as a random effect (one-sample t test for each implicit or explicit analysis; paired t test for contrasts between runs or tasks). Figure 3 shows the group averaged, statistical parametric maps (SPM) corrected ($p < 0.05$) across the whole brain for multiple voxel-wise comparisons using the false detection rate (FDR) procedure. To focus on regions of interest (ROIs), first, an automated algorithm extracted clusters of activation (5 mm radius, uncorrected $p_u < 0.05$) in the group averaged SPM of Sequence $>$ Random blocks for all implicit runs (implicit ROIs) or all explicit runs (explicit ROIs; excluding data of one subject who did not do the last explicit SRTT run), separately. Next, selection of ROI clusters in the MTL and striatum was based on mean anatomical T1-weighted images and human brain atlases (Duvernoy et al., 1999; Mai et al., 1998) and in the DLPFC was based on prior reports (Stern et al., 2000; Owen et al., 1998). Gaussian-field, small-volume correction (SVC) for multiple voxel-wise comparisons was applied in masks of all clusters occurring in each left and right MTL, striatal, or DLPFC ROI (i.e., multiple clusters were combined in each ROI to create each mask), with each SPM thresholded at $p_u < 0.05$ to exclude nonsignificant single voxels. Extent threshold was always 5 voxels. Next, we describe results that were reliable at $p \leq 0.05$ after SVC, which reports the lower of random field theory or Bonferroni family-wise corrections, and FDR p values.

Implicit Learning

All Runs. Learning-related activation was reliable in MTL, DLPFC, and striatal ROIs bilaterally (Table 1, Figure 4).

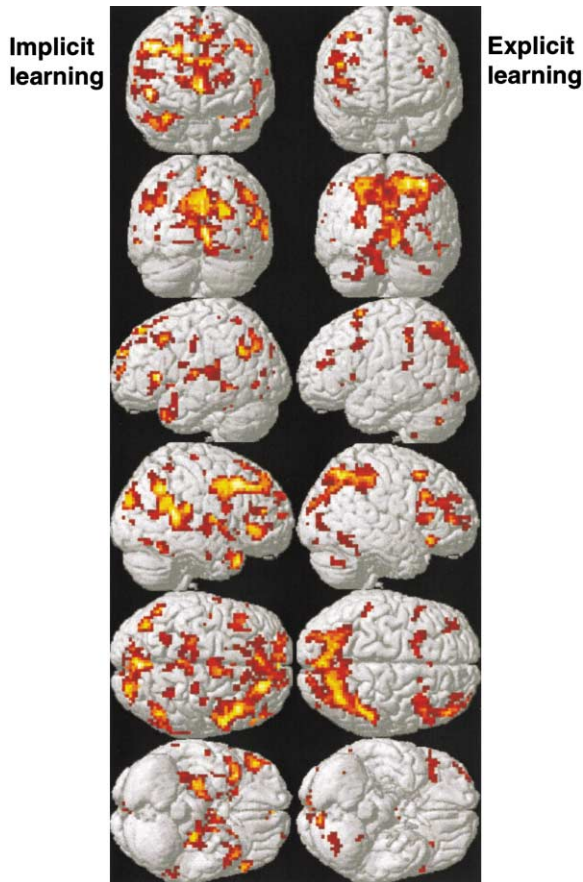


Figure 3. Learning-Related Activation Differed between Implicit and Explicit SRTTs

Activation shown for Sequence relative to Random conditions (FDR corrected $p < 0.05$) across all implicit runs (right) and all explicit (left) runs superimposed on rendered canonical brains.

Each Run. To compare earlier and later learning effects, contrasts of Sequence > Random blocks were analyzed separately for each run (Table 1, Figure 4). Activation of the MTL was reliable bilaterally in runs 1–3. In the striatum, while initially (run 1) effects were reliable in the putamen bilaterally and in the right caudate, later (runs 2 and 3) learning effects in both striatal structures were bilateral. The DLPFC showed reliable learning effects in only earlier runs 1 and 2. To further assess run 4, we applied the SVC to the nearest cluster centered at the coordinates of marginal 5 mm ROI clusters and found reliable activation in left hippocampus body, subiculum, and right amygdala (for all measures, $z[14] > 1.84$, $p < 0.05$).

Between Runs. SPMs were compared directly between runs 1 and 2 versus runs 3 and 4 and between run 1 and run 2, separately, versus run 4. Contrasts of Sequence > Random blocks showed that learning-related activation in left MTL, left putamen, and DLPFC was greater during earlier run 2 than later run 4 (Table 1). Caudate activation tended instead to be greater later in learning, as SVC on nearest clusters revealed that left caudate (head) activation was reliably greater in runs 3 and 4 than runs 1 and 2 ($[-12, 21, 3]$, $z = 3.39$, $p < 0.05$).

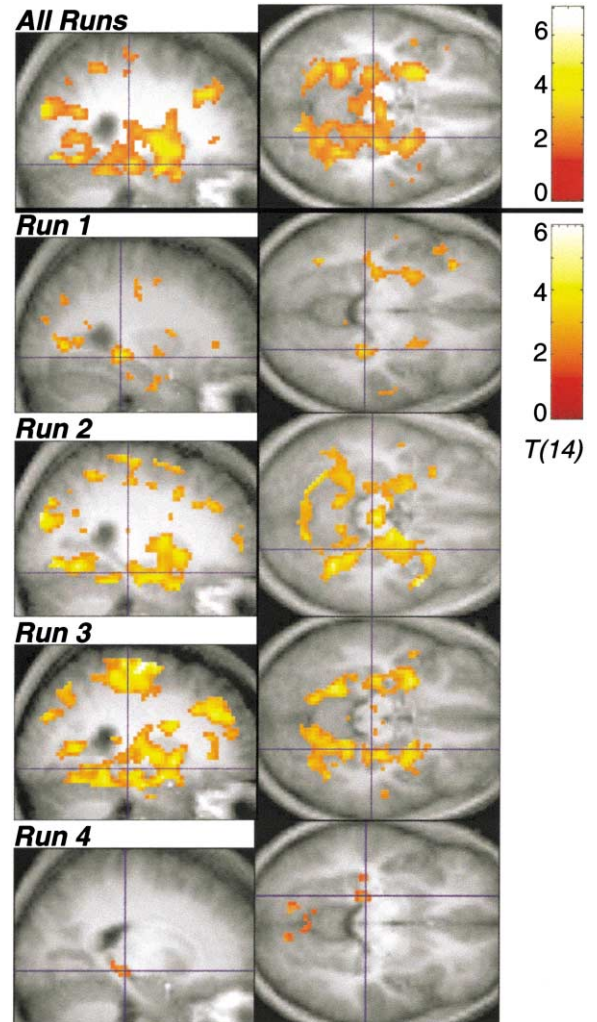


Figure 4. Implicit Learning-Related Activation in Mid-Anterior MTL and Putamen

fMRI results (Sequence > Random) superimposed on sagittal and axial slices of mean T1-weighted anatomical MRI. Activation is shown across all runs ($p_u < 0.0167$) and in each run ($p_u < 0.0167$), but is least in run 4 ($p_u < 0.05$).

Implicit Sequence blocks were also contrasted with each other between runs (as in Grafton et al., 1995). Sequence blocks showed greater activity in the last two than first two runs in the caudate bilaterally ($[-9, 3, 15]$, $[12, 6, 12]$; for all measures, $z > 2.81$, $p < 0.04$). By contrast, in left MTL and other striatal areas, sequence blocks in the first run showed more activity than in the last run (Table 1; SVC on nearest cluster centered in marginal ROI clusters).

Explicit Learning

All Runs. Learning-related activation was reliable in MTL, DLPFC, and caudate ROIs bilaterally (Table 2, Figure 5).

Each Run. To compare earlier and later learning effects, contrasts of Sequence > Random blocks were analyzed separately for each run; note, as respective Sequence and Random blocks were contrasted, these blocks were between scans for contrasts in so-called

Table 1. Implicit SRTT Learning: Z-Scores of Significantly Activated 5 mm Volume Clusters within the ROIs over All Runs, by Run, and between Runs

Region	x	y	z	Run					Sequence Only	
				All	1	2	3	2 > 4	1 > 4	
MTL										
Hh, A	L	-18	0	-21	3.69**	3.89**	3.40**	3.04**		
Hh, pHg		-33	-18	-27	3.11**	2.50*		3.58**	2.96*	
Hh		-30	-21	-9	3.30**	2.88**	3.15**	3.31**	2.73*	
Hh, pHg		-24	-27	-18	3.15**	3.45**	2.47*	2.44*		2.82*
A	R	21	6	-21	4.01**	2.56*	3.27*	3.64**		
Hh		30	-18	-15	3.15**	2.15*	2.53*	3.22*		
Hh, E		33	-18	-27	2.64**	2.24*	2.53*	2.95*		
Hh		27	-21	-18	3.31**	2.87*	2.76*	3.19*		
Hb		30	-24	-6	2.91**		2.12*	2.52*		
Hb, pHg, E		24	-33	-9	2.77**			2.60*		
Hb, pHg		30	-33	-9	2.65**	3.24*	2.08*			
Putamen	L	-27	6	-6	4.19***	2.67*	3.55**	3.30**	2.53*	2.71*
		-27	-9	9	3.39***	2.46*	2.97**	2.71*	2.67*	
	R	30	0	-3	4.02***	2.44*	2.85*	3.45**		
Caudate										
Tail, Body	L	-15	-6	18	3.40**		3.21*	3.47**		2.57*
Body		-12	9	12	3.39**		3.18*			
Body		-9	3	15	3.28**		2.56*	3.09*		
Head		-9	15	-3	2.28*		3.04*			
Body	R	12	6	12	3.89***		3.10**	3.18**		
Body		15	12	12	3.57***	3.61**		2.85**		
DLPFC	R	36	27	39	3.13**		2.95*		3.23**	
		45	33	36	2.80**	2.56*			3.12**	

Note: Sequence > Random contrast, except for Sequence Only column; MTL = medial temporal lobe; DLPFC = dorsolateral prefrontal cortex; Hh = hippocampus head (and subiculum); Hb = hippocampus body (and subiculum); pHg = parahippocampal gyrus; E = entorhinal cortex; A = amygdala; L = left hemisphere; R = right hemisphere. Corrected *p ≤ 0.05, **p < 0.01, ***p < 0.001.

“runs” 2 and 3 (also for RT data), but the within-scan contrast (Sequence 5-6 > Random 3-4) yielded results similar to “run” 3. Learning-related activation in the MTL occurred in run 1 reliably and run 2 marginally (Table 2, Figure 5); SVC to nearest clusters suggested reliable activation of right caudate head in run 1 (for all measures, $z > 2.32$, $p < 0.05$). The DLPFC showed activation in all runs but only on the right by run 3 (Table 2).

Between Runs. SPMs were compared directly between runs. Contrasts of Sequence > Random blocks suggested decreasing activity in MTL, striatum, and DLPFC as learning progressed. Run 1 showed marginally more activation in the MTL and caudate than in run 2 and more than in run 3 in these ROIs, as well as DLPFC; SVC of the nearest cluster revealed activation in run 1 was reliably (for all measures, $p < 0.05$) greater than in both later runs in the MTL (for all measures, $z > 2.66$) and left DLPFC (for all measures, $z > 1.9$). Run 2 showed marginally more activation than run 3 in the MTL and DLPFC; SVC of the nearest clusters revealed reliable (for all measures, $p < 0.05$) differences in left MTL ($z = 3.37$) and bilateral DLPFC (for all measures, $z > 2.4$).

Explicit Sequence blocks were also contrasted across runs. Table 2 shows that activation in Sequence blocks was greater during the first than the last two runs in the right MTL, bilateral DLPFC, and bilateral caudate.

Functional Connectivity

We predicted a negative correlation between MTL and caudate during implicit learning (Poldrack et al., 2001) and a positive correlation between MTL and DLPFC during explicit learning (Aggleton and Brown, 1999). fMRI signal change time courses were extracted from

each subject using masks of the respective implicit or explicit MTL clusters (coordinates in Tables 1 and 2; corrected for effects of interest; no temporal filter) and entered into a simple correlation for each subject. A SPM of voxels correlated with the MTL cluster for each subject was then entered into a one-sample t test (random effects, whole-brain family-wise $p < 0.05$). For implicit and explicit SRTTs, autocorrelations of each MTL cluster with itself were reliable, a homologous MTL region of the opposite hemisphere typically correlated positively, and, contrary to predictions, no areas negatively correlated with MTL activity.

Implicit Learning. Instead, MTL activity positively correlated most prominently with retrosplenial, cuneus, precuneus, and posterior cingulate cortex.

Explicit Learning. As expected, positively correlated regions included right DLPFC ROIs, but also lingual-fusiform, parieto-occipital, occipital, posterior cingulate, retrosplenial, intraparietal, postcentral, and superior temporal cortices, left insula and Sylvian fissure, medio-dorsal and anterior thalamic nuclei, and left cerebellum.

Earlier Implicit versus All Explicit Learning

We tested two hypotheses: learning-related activation in MTL and DLPFC is greater for explicit than implicit learning, whereas caudate activation is greater for implicit than explicit learning (Poldrack et al., 2001). SPMs of the Sequence > Random contrast were created for the first six implicit (runs 1 and 2) and all six explicit blocks (runs 1-4) to equate sequence exposure between tasks. SVC was applied in masks of implicit and explicit ROI clusters, separately.

Implicit > Explicit. Among implicit ROIs, no differ-

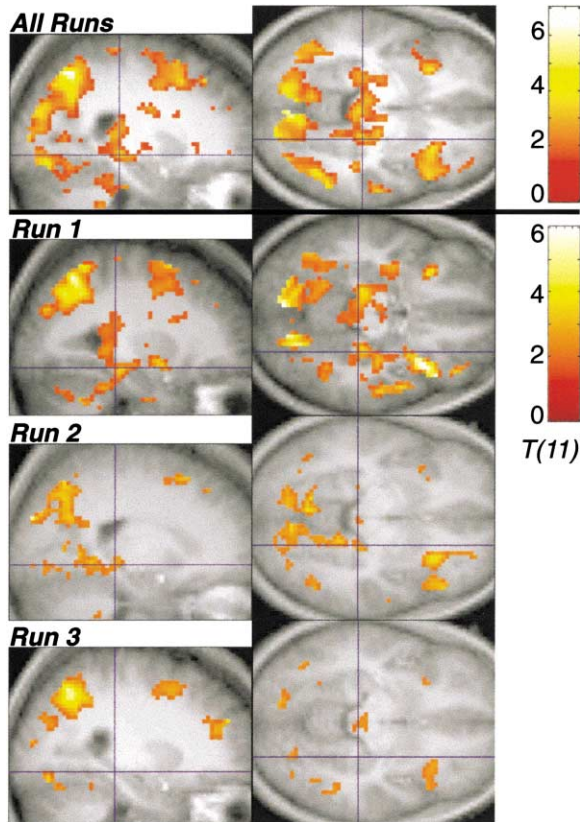


Figure 5. Explicit Learning-Related Activation in Mid-Posterior MTL and Caudate Tail

fMRI results (Sequence > Random; $p_u < 0.05$) superimposed on right sagittal and axial slices of mean T1-weighted anatomical MRI. Activation is shown across all runs and in each run.

ences were reliable; SVC of nearest clusters suggested learning-related activity was reliably greater for explicit than implicit learning at the left caudate head ($[-9, 21, -3]$, $z[10] = 2.85$, $p = 0.0262$) and an amygdala-ventral striatal area ($[-21, 3, -18]$, $[21, 9, -21]$; for all measures, $z \geq 2.1$, $p < 0.05$). To further assess this, learning-related activity was contrasted in all implicit versus all explicit runs, revealing reliable effects in a right amygdala-ventral striatal area ($z = 3.15$, $p = 0.0294$); SVC of nearest clusters suggested reliable left hemisphere effects in amygdala, hippocampus head, and putamen (for all measures, $z > 2.16$, $p < 0.05$). No explicit ROIs activated more for implicit than explicit learning.

Explicit > Implicit. Among explicit ROIs, learning-related activity was reliably greater during explicit than implicit learning in right DLPFC and right caudate (for all measures, $z \geq 2.06$, $p < 0.05$); SVC of the nearest clusters revealed marginally reliable differences (for all measures, $z \geq 2.05$) in left hippocampus body ($p = 0.0482$) and left caudate ($p = 0.0481$). No implicit ROIs activated more for explicit than implicit learning.

Later Implicit versus All Explicit Learning

To assess later implicit versus explicit learning, contrasts of Sequence > Random were averaged together for all six explicit blocks and the last six implicit blocks.

Implicit > Explicit. No differences were reliable in any ROIs.

Explicit > Implicit. In explicit ROIs, DLPFC was marginally more activated for explicit than later implicit learning; SVC of nearest clusters suggested reliable differences in bilateral DLPFC (for all measures, $z > 2.96$, $p < 0.04$). In implicit ROIs, right DLPFC was more activated for explicit than late implicit learning ($z = 2.91$, $p = 0.0211$).

Implicit SRTT: Sequence Awareness

Two sets of analyses assessed how sequence awareness related to MTL activity.

Unaware and Aware Groups. Implicit learning data

Table 2. Explicit SRTT Learning: Z Scores of Significantly Activated 5 mm Volume Clusters within the ROIs over all Runs, by Run, and between Runs

Region	x	y	z	Run				Sequence Only		
				All	1	2	3	1 > 2	1 > 3	
MTL										
Hb	L	-27	-30	-6	2.63*					
Hh, Hb	R	30	-24	-15	2.59*	2.84*			2.83*	3.01*
Hb, pHg		21	-27	-6	3.23*	2.10*			2.91*	2.39*
Ht		33	-39	0	2.64*	2.38*				
Caudate										
Tail	L	-21	-18	21	2.85*					
Body		-24	-27	27	2.37*					
Body		-21	6	24	2.54*				2.56*	
Tail	R	27	-33	6	3.04*					
Head		6	24	6	2.24*				2.56*	2.57*
DLPFC										
	L	-39	36	18	2.90**	3.27**			3.55**	3.67**
		-48	33	27	2.89**	3.15**	2.67*		2.39*	2.37*
	R	48	36	24	3.68**	2.89*	3.18*	2.96*	2.72*	2.61*
		33	48	30	3.29**		2.09*	2.84*		
		30	42	39			2.28*			
		39	33	39			2.56*		2.88*	3.24*

Note: Sequence > Random contrast, except for Sequence Only columns; MTL = medial temporal lobe; DLPFC = dorsolateral prefrontal cortex; Hh = hippocampus head (and subiculum); Hb = hippocampus body (and subiculum); Ht = hippocampus tail; pHg = parahippocampal gyrus; L = left hemisphere; R = right hemisphere. Corrected * $p \leq 0.05$, ** $p < 0.01$.

were analyzed separately for aware and unaware groups. Aware participants ($n = 7$) were defined as those who met the following criteria on at least two of the following three explicit tests: (1) free generation scores of 6 or more; (2) on cued generation, reliable or marginal ($p < 0.08$) difference from chance (0.3133) in t tests on the ratios for the 12 sets of triplets (e.g., 1-2-n, 1-3-n, 1-4-n, etc.) (Shanks and Johnstone, 1999); and (3) full recognition ratio was positive, and the actual Sequence was rated higher than all new sequences. For the unaware subjects ($n = 8$), six met no aware criteria, and two only narrowly met one criterion (one had a low full recognition ratio of 2.75; the other had a free generation score of 6).

Unaware subjects showed learning-related activation across all runs reliably in the right caudate and bilateral putamen (for all measures, $z > 2.55$, $p < 0.04$) and marginally in the MTL; SVC of the nearest cluster revealed reliable activation of right anterior MTL ($z = 2.87$, $p = 0.05$). In run 1, activation was reliable in right caudate ($z = 2.84$, $p = 0.0239$) and marginal in right putamen and MTL; SVC of nearest clusters revealed effects were reliable in MTL bilaterally (for all measures, $z > 2.06$, $p < 0.05$) and right putamen ($z = 1.81$, $p = 0.0499$). In run 2, activation was marginal in all ROIs (for all measures, $p < 0.19$). In run 3, activation was reliable bilaterally in MTL (for all measures, $z > 2.18$, $p < 0.03$) and putamen (for all measures, $z > 2.02$, $p < 0.05$) and marginal in bilateral caudate and right DLPFC (for all measures, $p < 0.15$). To increase power with this small subgroup, we examined runs 2 and 3 together, as these showed the most learning-related activation in full group analyses (Table 1); unaware participants showed reliable activation of all but one MTL cluster bilaterally (Figure 6; for all measures, $z > 2.37$, $p < 0.027$), bilateral putamen (Figure 6; for all measures, $z > 2.36$, $p < 0.01$), right caudate (for all measures, $z > 2.46$, $p < 0.03$), and right DLPFC (for all measures, $z > 3.32$, $p < 0.01$).

Aware participants showed reliable learning-related activation across all runs in bilateral MTL and putamen, and right caudate and DLPFC (for all measures, $z > 2.09$, $p < 0.04$). In run 1, activation was reliable in bilateral MTL and right caudate and DLPFC (for all measures, $z > 2.55$, $p < 0.05$). In run 2, MTL and striatal ROIs were marginal (for all measures, $p < 0.09$). In run 3, activation was reliable in left MTL ($z = 3.3$, $p = 0.0186$) and right caudate (for all measures, $z > 2.56$, $p < 0.023$). Examining runs 2 and 3 together, aware participants showed reliable bilateral activation of all but one MTL cluster (Figure 6; for all measures, $p < 0.04$), putamen (Figure 6; for all measures, $p < 0.005$), and caudate (for all measures, $z > 2.72$, $p < 0.02$).

Using random effects, two-sample t tests on Sequence > Random SPMS, direct contrasts of aware > unaware groups suggested no reliable differences across all runs or runs 2 and 3 together.

Regression. To assess further the relationship between learning-related activation and sequence awareness, a random effects, multiple linear regression analysis used SPMS of Sequence > Random across all implicit runs as a dependent variable and the five explicit memory test scores as predictors. Predictors were assessed using step-wise selection and the F_{ESS} statistic using the extra sum of squares principle that tests the

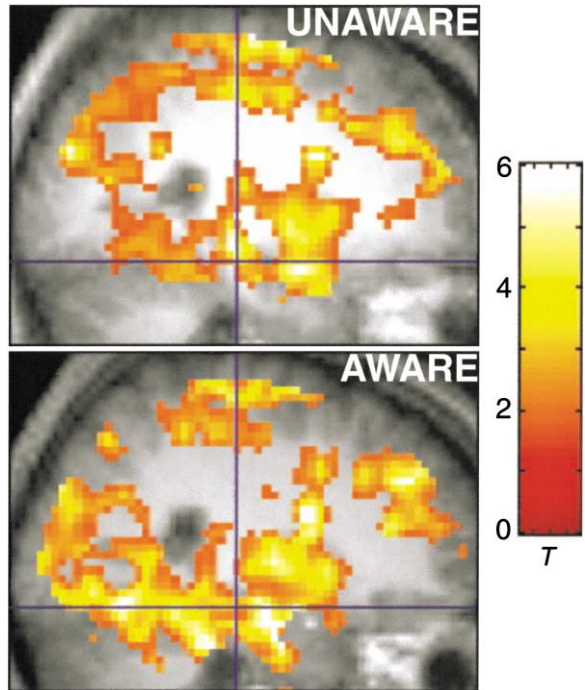


Figure 6. Implicit SRTT: Learning-Related MTL Activation and Sequence Awareness

fMRI results (Sequence > Random; $p_u < 0.05$) in runs 2 and 3 together show comparable MTL activation for unaware (upper) and aware (lower) groups superimposed on right sagittal slices of mean T1-weighted anatomical MRI.

omnibus hypothesis of no effects of interest. SVC ($\alpha 0.01$ with five predictors) was applied to left and right MTL ROIs. Results indicated that no predictor or set of predictors reached significance, even when analyses were done at the maximal location, SVC on nearest clusters was applied, or we used a summary statistic of all five scores.

Discussion

The present findings provide clear evidence that both implicit and explicit learning of higher order sequences involve the MTL structures implicated in memory functions, specifically, the hippocampus and adjacent subiculum and entorhinal and parahippocampal cortex. The MTL must thus be incorporated into a hybrid motor-memory theory of sequence learning. Prior studies report striatal activation on implicit but not explicit SRTTs (Rauch et al., 1995; Grafton et al., 1995) and DLPFC activation on explicit but typically not implicit SRTTs (Hazeltine et al., 1997). In contrast, we found learning-related activation in the striatum and DLPFC in both modes, albeit in somewhat distinct subregions for each mode, consistent with recent studies (Willingham et al., 2002).

Our findings demonstrate several features of MTL processing that should be incorporated into a hybrid motor-memory framework. First, the MTL is involved in higher order sequence learning, regardless of the implicit or explicit mode of knowledge acquisition. Second, while

implicit learning tends to recruit more anterior MTL regions (around hippocampus head and rostral body), explicit learning seems to involve more posterior regions (around hippocampus tail), and both engage the mid-MTL (around hippocampus body and caudal head). Connectivity results support functional segregation, as MTL activity correlates between hemispheres but not between anterior, mid, and posterior MTL regions. Third, MTL involvement is related to acquiring higher order associations among temporally distinct stimuli. Fourth, MTL processing has a weak or no relationship with subsequent ability to consciously access sequence knowledge. Finally, MTL activation is greater earlier than later in both implicit and explicit learning modes. These findings are consistent with animal and human research that implicates the hippocampus in the association of temporally discontinuous but structured information and events (Cohen and Eichenbaum, 1993; Stern et al., 2001; Rawlins, 1985; Fortin et al., 2002).

Conscious Sequence Knowledge

An explicit-implicit account posits that the primary role of the MTL is to form consciously accessible memories. The MTL should thus be recruited when learning is explicit but not when it is implicit. In addition, the MTL should be engaged more later in learning because conscious knowledge about the sequence improves in the explicit mode and becomes more likely to develop in the implicit mode as learning progresses. Consistent with this account, we found some evidence that mid-MTL is recruited slightly more during explicit than implicit learning, and, for some subjects, the implicit SRTT resulted in sequence awareness. Further, activity in the hippocampus tail seemed specific to our explicit SRTT, and posterior MTL has been implicated in explicit episodic encoding (Stern et al., 1996; Kirchoff et al., 2000; Wagner et al., 1998; Brewer et al., 1998).

The full pattern of our fMRI and performance findings, however, seems difficult to reconcile with an explicit-implicit account. Specifically, while this account predicts that the MTL should be recruited much more in an explicit than in an implicit mode, we found but a small focal effect in mid-MTL. It would also not predict the opposite pattern; yet, we found that activation in an anterior MTL area was greater during implicit than explicit learning. One concern may be that subjects develop awareness of the sequence over the course of implicit learning, resulting in MTL activity. However, our behavioral results suggest conscious sequence knowledge is minimal or absent following implicit learning and yet is completely accurate, or almost so, following explicit learning.

This account also predicts increasing MTL activity over successive runs, yet MTL activation in both SRTTs is greater earlier than later in learning. Indeed, MTL activation is high even in the first run of the implicit task, when all participants were the least likely to be aware of a repeating sequence, and, critically, even in an unaware subgroup of participants who demonstrate no consistent, conscious sequence knowledge.

An explicit-implicit view would predict higher MTL activation in aware than in unaware participants. To the contrary, we found that participants who were unaware

during implicit learning show significant MTL activation, which, moreover, is indistinguishable from that of aware participants. Also, implicit learning-related MTL activation does not significantly correlate with any of our tests of conscious sequence knowledge. Our results suggest that implicit learning-related MTL activity is thus unrelated to a greater conscious ability to remember the sequence.

In contrast, a relational memory account postulates that the binding of convergent inputs to the hippocampal system yields a unique associative representation of the relationship between perceptually salient features of a learning episode (Cohen and Eichenbaum, 1993). These representations can support, and are necessary for, flexible and explicit memory with conscious awareness. Because the binding process is thought to be automatic and obligatory, however, it can also be recruited during implicit learning, even without conscious awareness.

The relational view would predict our finding of greater MTL recruitment earlier than later in learning. More relational processing is required early on when more of the sequence structure needs to be detected and associated. Later, as a sequence is learned, fewer new relations remain. Critically, a main difference between prior SRTT studies that did not observe MTL activation and the present results (besides our focused MTL ROI analyses) has to do with whether data are collected and analyzed earlier or later in learning. We found MTL activation in an earlier acquisition phase of learning, whereas some previous reports examined subjects primarily after more sequence experience than in the present study and at a time when learning of the sequence may have been largely completed or the sequence was overlearned (Rauch et al., 1995; Willingham et al., 2002).

A relational account also predicts our finding of MTL involvement in both aware and unaware groups and in both implicit and explicit SRTTs, as relational processing is a task requirement in all cases. While this account does not specifically predict MTL differences between explicit and implicit modes, it can accommodate them based on differential relational processing, such as our observation of slightly more activity in anterior MTL areas in implicit than explicit learning. Because learning is slower in an implicit than in an explicit mode, more relations remain to be acquired after the same amount of sequence experience, and so the MTL may continue to be recruited for the implicit task.

Higher Order Associations

Overall, we conclude that the nature of the representation that is being acquired is the primary determinant of MTL involvement (Cohen and Eichenbaum, 1993; Wallenstein et al., 1998; Wood et al., 2000). Evidence that MTL amnesics are impaired at learning higher order, but not simple, associations (Curran, 1997) was obtained using a SRTT paradigm similar to ours in which repeating and random blocks of SOC sequences alternate. As in the amnesic study, learning effects in our study cannot be based on simple stimulus-stimulus associations because item and bi-item frequencies are equated between SOC sequences. Instead, they must be based on higher order associations among three or more consecutive locations. Our findings thus suggest that learning-

related MTL activation is related to the acquisition of higher order associations between successive elements of a sequence.

Moreover, results of our higher order association RT analyses demonstrate that these associations are acquired during implicit learning (Figure 2B). Critically, both higher order association learning and MTL activation occur during earlier implicit runs 1 and 2, when much of the structured pattern of locations remains to be discerned and associated. The RT results also demonstrate that triplets and quadruplets of sequential locations are acquired during runs 1–3 and are further associated into longer five- and eight-location sequences by run 4 (Figure 2B). Behavioral RT effects for a run (i.e., run 4) likely index underlying neural processes occurring prior to and/or within that run (i.e., run 3 or 4). Accordingly, learning-related MTL activation was strong throughout implicit runs 1–3 but less in run 4 (Figure 4), when most associations had already been acquired and so sequence acquisition was largely complete (see Curran, 1997).

Our explicit SRTT results also suggest that MTL activity is related to the acquisition of higher order associations. The free generation results indicate that most of the associations (i.e., 9 of 12 locations) are acquired in run 1 (Figure 2C). Similarly, learning-related MTL activation is strongest in the first run. Later on, only 1–3 locations remain to be associated, and the MTL activation is reduced. Note that MTL activation is strongest in run 1 of the explicit task but remains strong throughout runs 1–3 of the implicit SRTT (Figures 4 and 5). This task difference is consistent with the faster acquisition of higher order associative representations when the learning mode is primarily explicit (i.e., free generation of 9 of 12 locations in run 1) than when it is implicit (i.e., acquisition of higher order associations throughout runs 1–3). Finally, it is noteworthy that, in contrast to free generation performance, our finding of increasingly faster RTs to the explicit Sequence over successive runs (Figure 2A) is instead related to increasing right DLPFC activation across runs (Table 2), suggesting the RT improvement may reflect primarily the influence of DLPFC processes of goal selection or sequence monitoring in working memory, consistent with other research (Rauch et al., 1995; Grafton et al., 1995; Willingham et al., 2002).

As the mid-MTL region shows the greatest overlap between learning modes, we propose that it is the critical brain region for explicit and implicit learning of higher order associations (see also Small et al., 2001), regardless of awareness. The MTL may represent stimulus-stimulus associations early in learning that, once constructed, are coopted later on by the striatum, particularly the caudate, which associates them further with response-related representations (Poldrack et al., 2001; Peigneux et al., 2000). We found that caudate tends to be more active later in learning, as it is not recruited bilaterally until run 2, although putamen is recruited bilaterally from the outset. While most studies report right- (Rauch et al., 1995, 1997b, 1998; Doyon et al., 1996) or left-sided (Rauch et al., 1997a) striatal activations, ours are bilateral, perhaps due to higher sensitivity of fMRI at 3 T; this increased sensitivity might also contribute to the MTL activity in our study compared to other studies at 1.5 T (Rauch et al., 1997b, 1998) or with PET,

which requires intersubject averaging (Hazeltine et al., 1997; Grafton et al., 1995; Rauch et al., 1995, 1997a; Berns et al., 1997).

Temporal Sequence Learning

A SRTT entails both temporal and spatial processing. The role of the hippocampus may be to bind sequential events into a unique episodic experience, consistent with a relational account of memory. Convergent evidence from animal and human research indicates that the hippocampus is critical for processing temporally structured information and associating events separated in time and/or space (Stern et al., 2001; Wallenstein et al., 1998; Downes et al., 2002; Wallenstein and Hasselmo, 1997; Wise and Murray, 1999; Aggleton et al., 1986; Fortin et al., 2002; Wood et al., 2000; Agster et al., 2002).

The present and other brain imaging and neuropsychological studies of human memory also support an emphasis on relational processing of temporally discontinuous events. Amnesics can be impaired at estimating spatial and temporal intervals (Kesner and Hopkins, 2001). The hippocampus appears to be generally sensitive to temporal intervals, as it is involved with longer but not shorter delays (McGlinchey-Berroth et al., 1997; Elliott and Dolan, 1999), and electrical potentials in human MTL are modulated by temporal gaps in stimulus sequences (McCarthy et al., 1989).

Other evidence suggests that the hippocampus is necessary to bridge temporal gaps between stimuli not only within a trial but also over multiple trials, even when learning is implicit. Neuropsychological studies reveal that implicit context learning requires acquisition of an invariant complex spatial configuration over multiple trials, knowledge which is not consciously accessible, and this learning is impaired in MTL amnesia (Chun and Phelps, 1999). Also, amnesics with MTL lesions are impaired on multitrial, trace conditioning, a variant of classical conditioning where a temporal interval intervenes between CS and US, but not on delay conditioning with no CS-US time gap (McGlinchey-Berroth et al., 1997; Clark and Squire, 1998).

Brain imaging evidence, similarly, implicates the MTL, especially anterior regions, in the comparison, encoding, and maintenance of novel stimulus-stimulus covariations evolving over a substantial temporal interval or multiple intervening events (Stern et al., 2001). Anterior MTL is preferentially activated in (1) N-back working memory tasks with novel compared with familiar scenes, faces, and words (Stern et al., 2001; Ranganath and D'Esposito, 2001; Grunwald et al., 1998; Dolan and Fletcher, 1997), (2) temporally demanding associative learning tasks (Mitchell et al., 2000; Dolan and Fletcher, 1997; Sperling et al., 2001), and (3) multitrial learning tasks involving the acquisition of novel statistical regularities among familiar stimuli or responses, typically with feedback (Toni et al., 2001; Buchel et al., 1999; Rose et al., 2002; Goel and Dolan, 2000; Strange et al., 1999; Dolan and Fletcher, 1999; Poldrack et al., 2001). Consistent with the last, electrophysiological recordings from human hippocampus indicate it is sensitive to target probability (McCarthy et al., 1989; Halgren et al., 1998). Moreover, anterior hippocampus has been found

to be the most active early in learning when more stimulus contingencies remain to be detected and associated (Strange et al., 1999; Dolan and Fletcher, 1999; Poldrack et al., 2001). Notably, it is also recruited during encoding of auditory words for which salience develops over a temporal envelope (Saykin et al., 1999; Small et al., 2001).

In conclusion, the present findings are consistent with recent animal and human studies that have implicated the hippocampus and related structures in higher order associative learning, regardless of awareness. The results provide evidence that the early acquisition phase of temporal sequence learning, whether implicit or explicit, requires the hippocampus. Overall, we conclude that the associative and temporal structure of the representation that is being acquired is the primary determinant of hippocampal engagement.

Experimental Procedures

SRTT

Materials

Task. Figure 1A shows the SRTT, adapted from Rauch et al. (1997b). **Sequences.** Response cues were shown in SOC sequences which have 3 instances of each of the 4 spatial locations and 1 instance of each of the 12 possible transitions between locations (i.e., 1-2, 1-3, 1-4, 2-1, etc.). In the Sequence condition, one SOC sequence was shown repeatedly (see Figure 1A for sequences); in the Random condition, pseudorandomly generated (no immediate repetitions of locations; Emerson and Tobias, 1995) SOC sequences were shown once each.

Procedure

The implicit SRTT and respective explicit memory tests always preceded the explicit SRTT and respective explicit tests. Functional scans were acquired in four implicit and four explicit runs; each began and ended with fixation of a centered dot.

Implicit Runs. Before the first run, participants were instructed and practiced the SRTT for 12 trials of one random SOC sequence (note, they were not told about a repeating sequence). In all runs, Sequence and Random blocks alternated. Each Sequence block began at a unique random point of the sequence to further ensure learning was implicit, instead of using a dual (distractor) task (as in Grafton et al., 1995) which may confound results (Rah et al., 2000). The Sequence repeated four times in each of three blocks per run. Random blocks had two lengths: (1) two "short" blocks of two novel sequences separated the three Sequence blocks. (2) Two "long" blocks of four novel sequences, in run 1, both preceded all Sequence blocks; in runs 2 and 3 (Figure 1B), one preceded and the other followed; and, in run 4, both followed all Sequence blocks. Fixation rest (4 s) preceded some Random blocks.

Explicit Runs. Before the first run, participants were instructed to do a SRTT again, but this time to try to memorize another repeating sequence. They were taught that chunking helps, and their first exposure to the explicit sequence was in three four-location chunks just before the first Sequence block. In three runs, the Sequence repeated four times within each of two blocks (48 trials). Two Random blocks had four novel sequences (48 trials) each. A Free Generation test block (see below) assessed explicit sequence knowledge after each Sequence block. Of explicit scans, run 1 had two Random blocks followed by two Sequence and two Free Generation blocks alternating with each other, run 2 had two Sequence and two Free Generation blocks alternating, run 3 had two Sequence and two Free Generation blocks alternating followed by two Random blocks, and run 4 had two Random blocks. Immediately before each block type, instructions on the screen informed participants they would next experience Sequence, Free Generation, or Random blocks.

Explicit Memory Tests

Tests vary in sensitivity to conscious awareness and are described in the order of administration. No explicit test was given until after all four implicit runs were completed.

Awareness

Immediately following all implicit runs, participants pressed a key to respond "yes, probably, unlikely, or no" to four questions: "In the task you just performed, did you notice that: (1) the boxes lit up at random locations the entire time; (2) some boxes lit up more often than other boxes; (3) the task was easier at times and harder at other times; (4) there was a repeating pattern of locations some of the time?"

The next tests assessed explicit memory following both implicit and explicit tasks. For the next two, the four-square array was continuously shown.

Free Generation

Participants pressed response keys in the order of the repeating sequence until told to stop. This test also followed each explicit block.

Cued Generation

Each time participants pressed a key to generate the repeating sequence, the square at that location lit up until the next key press. They thus self-generated cues to predict the next location. Shanks and Johnstone (1999) devised this and the next test as more sensitive and objective explicit tests.

Triplet Recognition

Participants did the SRTT on three locations; half the triplets were in the repeating sequence, and half were new. After each triplet, they rated how similar it was to those in the actual Sequence on a scale from 1 (most) to 6 (least). Two 12-item SOC sequences were the source of new triplets, none of which overlapped with those in the respective implicit or explicit sequence.

Full Recognition

Participants did the SRTT with the entire implicit or explicit Sequence and four new 12-item SOC sequences. After each, subjects rated the 12-item SOC sequence on a scale from 0 (least) to 10 (most), if they had experienced it in the corresponding implicit or explicit SRTT (Reber and Squire, 1998).

MRI

High-resolution T1-weighted scans (MP-RAGE; 192×256) for anatomical localization and eight T2*-weighted functional BOLD scans were acquired at 3 T (Siemens Allegra). Using a gradient-echo, echo-planar pulse sequence, 21 AC-PC slices were acquired (5 mm thick; 1 mm skip; TR = 2 s, TE = 30 ms; flip angle = 90° , 64×64).

Participants

Participants were 17 neurologically normal people; data subsets were unusable due to scanner malfunction ($n = 3$), failure to obey instructions ($n = 1$), or time constraints ($n = 2$). Data were analyzed from 15 people for implicit SRTT ($M = 29$ years; 9 females); 12 people for explicit SRTT ($M = 27$ years; 8 females); and 11 people for implicit versus explicit contrasts ($M = 27$ years; 7 females).

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