

Implicit Learning–Explicit Knowing: A Role for Sleep in Memory System Interaction

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Abstract

■ There is evidence that sleep supports the enhancement of implicit as well as explicit memories (i.e., two memory systems that during learning normally appear to act together). Here, employing a serial reaction time task (SRTT) paradigm, we examined the question whether sleep can provide explicit knowledge on an implicitly acquired skill. At learning, young healthy subjects ($n = 20$) were first trained on the SRTT. Then, implicit knowledge was assessed on two test blocks, in which grammatically incorrect target positions were occasionally interspersed by the difference in reaction times between grammatically correct and incorrect target positions. To assess explicit sequence knowledge, thereafter subjects performed on a generation task in which they were explicitly instructed to predict the sequential target positions. In half the subjects, learning took place before a 9-hour retention interval filled with nocturnal sleep (sleep group), in the other half, the retention interval covered a 9-hour period of daytime wakefulness (wake group). At subsequent retesting, both testing on the generation task and the SRTT test blocks was repeated.

At learning before the retention interval, subjects displayed significant implicit sequence knowledge which was comparable for the sleep and wake groups. Moreover, both groups did not display any explicit sequence knowledge as indicated by a prediction performance not differing from chance on the generation task. However, at retesting, there was a distinct gain in explicit knowledge in the subjects who had slept in the retention interval, whereas generation task performance in the wake group remained at chance level. SRTT performance in the test blocks at retesting did not indicate any further gain in skill (i.e., unchanged reaction time differences between grammatically correct and incorrect target positions) independently of whether subjects had slept or remained awake after learning. Our results indicate a selective enhancement of explicit memory formation during sleep. Because before sleep subjects only had implicit knowledge on the sequence of target transitions, these data point to an interaction between implicit and explicit memory systems during sleep-dependent off-line learning. ■

INTRODUCTION

In humans learning can occur explicitly and implicitly. Explicit learning requires attention and relies on the conscious monitoring of the behavior to be acquired or retrieved, whereas implicit learning takes place without the need of any conscious capacities and without the subject being aware of having acquired or accessed any new information (Destrebecqz & Cleeremans, 2001; Willingham, 2001; Willingham & Goedert-Eschmann, 1999; Seger, 1994; Shanks & John, 1994; Squire, 1992; Reber, 1989). Although there is some evidence pointing to dissociable brain regions involved in implicit and explicit learning (Boyd & Winstein, 2001; Reber & Squire, 1998), these systems during learning normally appear to act in parallel (Willingham & Goedert-Eschmann, 1999).

The serial reaction time task (SRTT) has been widely used to assess mechanisms of implicit skill acquisition (Peigneux, Laureys, et al., 2003; Peigneux, Maquet, et al., 2000; Jimenez, Mendez, & Cleeremans, 1996; Cleeremans

& McClelland, 1991; Willingham, Nissen, & Bullemer, 1989; Nissen & Bullemer, 1987; Knopman & Nissen, 1987). It is a kind of choice reaction task which requires the subject to respond as fast and as accurately as possible to the appearance of a target stimulus at one of several horizontally arranged locations on a screen by pressing a spatially corresponding key. Unknown to the subject, the sequence of target locations follows a set of rules (i.e., a grammar that can be deterministic or probabilistic). With ongoing training on the SRTT, subjects not only acquire simple visuomotor skills leading to generally improved reaction times, but also implicit knowledge about the rules governing the sequence of successive target locations. This is reflected by increased reaction times to sudden violations of the rules. Knowledge as induced by this task is implicit, as subjects typically remain unaware of any structure in the sequence of target locations. In order to probe any awareness of the hidden rules underlying the sequence of target positions, it has been proposed to apply to the subject a generation task after SRTT training. On this task, the subject is asked explicitly to use his/her knowledge about the sequence to correctly predict

the location of each upcoming target on the basis of the preceding target locations (Jimenez et al., 1996; Cleere-mans & McClelland, 1991; Cohen, Ivry, & Keele, 1990). Performance on this task is not uninfluenced by implicit memory processes such as a sense of familiarity of where the next target stimulus will appear. However, as a direct measure of available sequence knowledge, whereby subjects are explicitly instructed to use all their knowledge about the sequence to optimize their prediction behavior, performance on the generation task is highly sensitive also to the contributions of explicit knowledge, especially if compared with the indirect measure of reaction time difference between grammatically correct and incorrect trials during SRTT performance (Reingold & Merikle, 1988).

There is compelling evidence that both explicit and implicit memories can be substantially enhanced by sleep. Thus, sleep has been demonstrated to benefit memory consolidation in explicit tasks (when declarative) such as the learning of word-pairs as well as in tasks considered to induce implicit memory processes such as the learning of motor and sensory motor skills (Maquet, Schwartz, Passingham, & Frith, 2003; Peigneux, Laureys, et al., 2003; Plihal & Born, 1997). For the SRTT, apart from generally decreasing reaction times at retesting, sleep after practice has also been shown to induce specific learning of a probabilistic grammar underlying the sequence (Peigneux, Laureys, et al., 2003). This was indicated by an increased difference between reaction times to grammatically correct as compared to incorrect target locations at retrieval testing after retention sleep. Moreover, brain regions activated during initial performance of the SRTT were reactivated during subsequent rapid eye movement (REM) sleep, with the strength of reactivation being correlated to the level of implicit acquisition of the sequence grammar (Peigneux, Laureys, et al., 2003; Maquet, Laureys, et al., 2000). However, a recent study by Robertson, Pascual-Leone, and Press (2004) challenged the view of the crucial function of sleep for this type of implicit learning. They found implicit sequence learning to be improved after a 12-hour retention interval regardless of whether or not it did contain a period of sleep. However, when the same sequence was trained under explicit conditions, with the subject being aware of the sequence structure, an improved sequence performance was observed only when training was followed by sleep. These data suggest that an interaction of explicit with implicit memory eventually determines what is learned during sleep, and this interaction may take place during sleep as well (Born & Wagner, 2004).

On this background, the present study aimed at characterizing the interaction of explicit with implicit processes during sleep-dependent memory consolidation. Specifically, we examined the question whether sleep facilitates the development of explicit knowledge of the hidden rules implicitly acquired during prior

training on the SRTT. For this purpose, the subjects were trained before periods of sleep and wakefulness on an SRTT, which was based on a probabilistic sequence grammar. Implicit sequence knowledge at the end of this training was determined by the difference in reaction times between grammatically correct and incorrect target locations in two test blocks. Immediately thereafter and after retention periods of sleep and wakefulness, explicit sequence knowledge was assessed using the generation task. The session ended with another testing of implicit sequence knowledge.

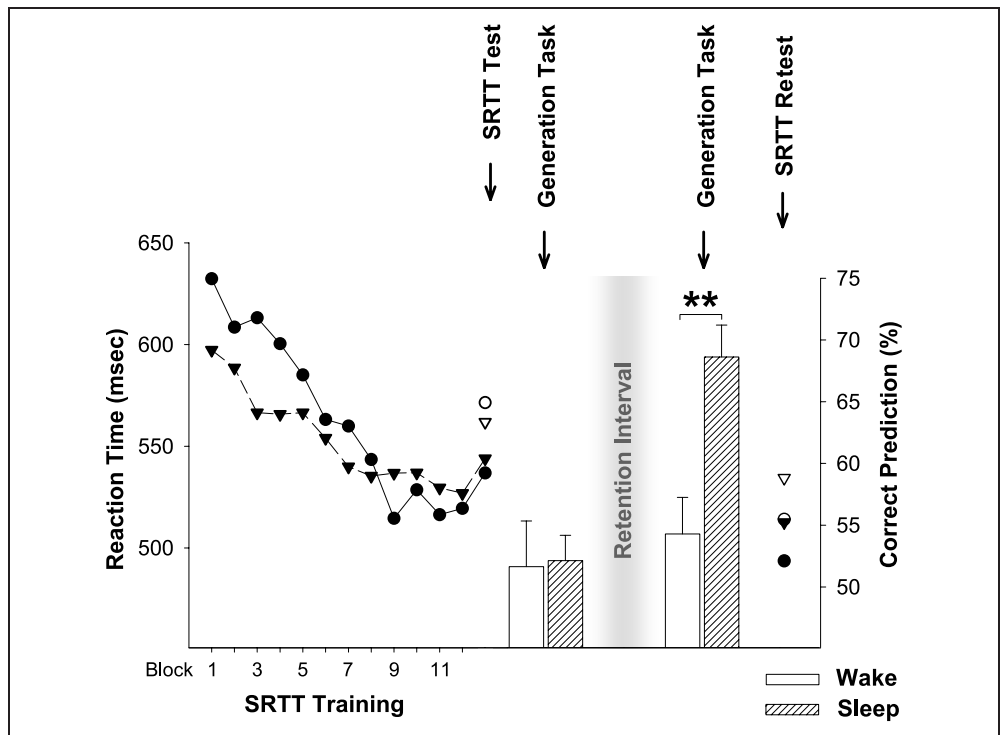
RESULTS

Serial Reaction Time Task

At learning, training on the first 12 blocks of the SRTT prior to the introduction of grammatically incorrect trials was closely comparable in both the sleep and the wake group ($p > .3$, for respective comparisons; Figure 1). In the sleep group, reaction times on average improved from 597.25 ± 20.81 in Block 1 to 526.84 ± 18.58 msec in Block 12 [$11.56 \pm 2.02\%$; 5.87 ± 1.14 msec per block; $F(1,9) = 26.71$; $p < .001$]. In the wake group, reaction times improved from 632.35 ± 50.66 in Block 1 to 519.49 ± 31.99 msec in Block 12 [$16.38 \pm 3.02\%$; 9.41 ± 2.21 msec per block; $F(1,9) = 18.04$; $p < .002$].

Table 1 summarizes mean reaction times on the two test blocks of the SRTT performed before and after the retention intervals of sleep and wakefulness. The introduction of grammatically incorrect trials slightly slowed down general performance speed in both the sleep [from 526.84 ± 18.58 to 543.88 ± 20.59 ; $F(1,9) = 9.75$; $p < .05$] and the wake group [from 519.49 ± 31.99 to 536.83 ± 34.21 ; $F(1,9) = 10.0$; $p < .05$]. Reaction times were distinctly greater to grammatically incorrect than correct target locations [$F(1,18) = 45.63$; $p < .001$ for main effect of Grammatical correctness; Figure 1]. Across the retention interval, global reaction times improved on average by 31.70 ± 6.73 msec ($5.74 \pm 1.11\%$) irrespectively of whether subjects slept or stayed awake during this time [$F(1,18) = 12.26$; $p < .005$ for main effect Before/after]. However, the difference in reaction times between grammatically correct and incorrect trials indicating implicit learning of the sequence grammar did not significantly increase across the retention interval. In the sleep group, this difference remained essentially unchanged [average difference before: 17.97 ± 4.80 msec; average difference after: 21.85 ± 5.80 msec; $F(1,9) = 0.39$, $p > .5$]. In the wake group it tended to decrease, however, not reaching any significance [before: 34.62 ± 9.15 msec and after: 20.47 ± 5.10 msec; $F(1,9) = 2.08$, $p > .2$; $F(1,18) = 2.50$; $p > .2$ for the Grammatical correctness \times Before/after \times Sleep/wake interaction; Figure 1]. Because the general reduction in response times might have masked the effect of sleep on implicit learning, differences between

Figure 1. SRTT and generation task performance at learning before and at retesting after retention intervals of sleep and wakefulness (sleep group: triangles; wake group: circles). SRTT performance at learning included 12 blocks of training followed by two test blocks (containing 15% grammatically incorrect target positions). Performance is indicated in terms of reaction time (left ordinate) and for test blocks, separately for grammatically correct (filled symbols) and incorrect target positions (empty symbols). Generation task performance (bars) is expressed in terms of percentages of correctly predicted target locations (right ordinate). Note: Implicit knowledge of the sequence grammar as indicated by the difference between reaction time to grammatically correct and incorrect trials on the test



blocks did not improve across retention intervals of sleep or wakefulness, although after the retention intervals reactions were generally faster. On the generation task, subjects' performance did not differ from chance at learning. At retesting, however, subjects who had slept during the retention interval were distinctly superior in correctly predicting the respective next target location, indicating a significant gain in explicit sequence grammar knowledge. Performance in subjects who stayed awake again did not differ from chance at retesting. $**p < .002$.

grammatically correct and incorrect trials were additionally calculated as percentages of individual average reaction times. Analyses based on these values also did not provide any hint that implicit learning occurred across the wake ($p > .3$) or sleep retention interval ($p > .5$). Post hoc between-group comparisons likewise failed to reveal any differences in reaction times between the sleep group and the wake group ($p > .6$, for all comparisons). During SRTT testing before and after the retention interval, subjects on average made 6.19 ± 1.15 ($3.19 \pm 0.59\%$) errors per block. The number of errors did not differ between any of the two conditions ($p > .3$, for all comparisons).

Generation Task

Immediately before and after the retention interval, subjects performed the generation task (see Table 1 for a summary of results). At learning prior to the retention interval, generation performance was closely comparable in both the sleep and the wake groups [$52.14 \pm 2.05\%$ and $51.64 \pm 3.70\%$, respectively; $F(1,18) = 0.01$; $p > .9$; Figure 1] and also did not differ from chance ($p > .3$, respectively). Interestingly, in the sleep group, generation performance strikingly improved to $68.62 \pm 2.59\%$ correctly predicted target locations corresponding to an average gain of explicit knowledge of $34.12 \pm 8.89\%$ [$F(1,9) = 19.55$; $p < .005$].

In contrast, generation performance in the wake group only slightly and nonsignificantly improved across the retention interval on $54.30 \pm 2.95\%$ correct predictions, that is, a gain of $7.22 \pm 4.01\%$ [$F(1,9) = 2.08$; $p > .1$]. Generation performance of the wake group in fact remained at chance level also at retesting ($p > .2$). The selective gain in explicit sequence grammar knowledge in the sleep group in comparison with the wake group was statistically confirmed by significance for the Before/after \times Sleep/wake interaction in the overall analysis of variance [ANOVA; $F(1,18) = 11.06$; $p < .005$]. Comparing percentages of correctly predicted target locations between the sleep and wake groups at retesting revealed a highly significant difference of $30.38 \pm 9.60\%$ in favor of the sleep group [$F(1,18) = 13.30$; $p < .002$]. Separate analyses revealed that the two blocks of generation task performance after sleep were closely comparable with respect to the number of correctly predicted target locations ($p > .6$).

Sleep

Subjects in the sleep group displayed a sleep architecture quite typical for nocturnal sleep in the laboratory. Total sleep time (TST) averaged 470.50 ± 5.76 min, with a sleep efficiency of $98.02 \pm 1.20\%$ (% of the 8-hour retention interval not spent awake). Slow wave sleep (SWS) latency averaged 17.20 ± 1.55 , and REM sleep

Table 1. Performance on the SRTT Test Blocks and the Generation Task at Learning before Retention Intervals of Sleep and Wakefulness and at Retesting, thereafter

		Wake	Sleep
<i>SRTT—Test Blocks (msec)</i>			
Learning	Correct	536.83 ± 34.21	543.88 ± 20.59
	Incorrect	571.45 ± 37.78 ^a	561.85 ± 19.52 ^a
	Difference	34.62 ± 9.15	17.97 ± 4.80
Retest	Correct	501.77 ± 38.72	520.67 ± 20.56
	Incorrect	522.24 ± 39.64 ^a	542.52 ± 21.18 ^a
	Difference	20.47 ± 5.10	21.85 ± 5.80
<i>Generation Task (%)</i>			
Learning		51.64 ± 3.70	52.14 ± 2.05
Retest		54.30 ± 2.95	68.62 ± 2.59 ^{b,c}

Values are means ± SEM. On the SRTT, implicit knowledge of the sequence grammar was measured in the test blocks with reference to the difference in reaction times between grammatically correct and incorrect target positions. On the generation task, explicit knowledge of the sequence grammar was quantified by the percent of correctly predicted target positions on two blocks each containing 194 trials. A value of 50% corresponds to chance level (i.e., guessing). Significant pairwise comparisons: $p < .005$ with reference to ^agrammatically correct target locations, ^bperformance at learning, and ^cwake condition.

latency was 89.15 ± 8.78 min (with reference to sleep onset, respectively). On average, after sleep onset, subjects spent 0.90 ± 0.43 min awake ($0.19 \pm 0.09\%$), 36.65 ± 4.78 min in sleep stage 1 ($7.84 \pm 1.02\%$), 246.30 ± 12.31 min in sleep stage 2 ($52.39 \pm 2.63\%$), 99.65 ± 12.40 min in SWS ($21.13 \pm 2.56\%$), and 86.80 ± 6.34 min in REM sleep ($18.46 \pm 1.31\%$). The amount of REM sleep appeared to be marginally lower than in comparable studies which may have been due to the early (7:00 a.m.) awakening of the subjects who were used to get up somewhat later. Explorative analyses did not reveal a consistent correlation between the various sleep parameters and the improvement on the generation task ($r < .2$; $p > .5$). Self-reported mood and feelings of activation as revealed by an adjective checklist did not indicate differences between the wake and sleep groups at learning ($p > .3$) or at retesting ($p > .5$).

DISCUSSION

The central finding of our study is that explicit sequence knowledge on the implicitly acquired rules was achieved only after subjects had slept during the retention interval between learning and retesting. In contrast, after wakefulness, generation task performance at retesting was still at the same level as at learning and did not differ from chance. A final retesting of the subjects on the

SRTT did not reveal an increase of implicit sequence knowledge, that is, reaction time differences between grammatically correct and incorrect trials in the test blocks were unchanged at this retesting, irrespectively of whether retention intervals were filled with sleep or wakefulness. Taken together, these findings indicate a sleep-dependent interaction of explicit and implicit memory mechanisms that leads to a selective gain in explicit sequence knowledge on the basis of implicitly acquired rules.

Differences during learning between the sleep and wake conditions cannot account for these results. During the initial 12 blocks of training, the time course of SRTT performance was quite comparable between both the sleep and the wake groups with reaction times improving on average by about 14%. Indeed, the two subsequent test blocks revealed a selective facilitation of responses to grammatically correct as compared to incorrect trials (i.e., during prior training subjects became gradually sensitive to, and thus, implicitly learned the sequential constraints of the sequence grammar). Nevertheless, when thereafter the subject's knowledge of the underlying probabilistic sequence grammar was directly probed on the generation task, this testing did not reveal any hint that conscious learning had occurred. In fact, on both conditions, generation task performance did not differ from chance during the learning period.

Because the retention interval in the wake group was placed during daytime and in the sleep group during the night, possible influences of circadian rhythm were not controlled. Such effects, however, for several reasons are not likely to have substantially confounded our results. First, initial performance on the SRTT and the generation task did not differ depending on whether learning took place in the evening (sleep group) or in the morning (wake group). Subjective ratings of mood and concentration also did not reveal any differences between groups and experimental sessions. Previous experiments likewise indicated that SRTT performance and associated implicit or explicit off-line learning in no way depended on whether learning and retesting took place in the morning or in the evening (Robertson et al., 2004). Even if there were circadian influences on reaction time, these should equally affect both grammatically correct and incorrect trials, hence, leave the difference measure of implicit learning unaffected. One study (Fischer, Hallschmid, Elsner, & Born, 2002) indicated that off-line learning of a finger motor skill benefits even more from retention intervals placed during daytime than nighttime. However, the circadian effect was much smaller than that of sleep in that study.

Although performance on the generation task is not purely explicit but includes some implicit inputs as well, the distinct gain in performance on this task in the absence of changes in signs of implicit learning of the sequence constraints speaks for a preferential formation

of explicit knowledge during the sleep period. In this regard, our findings fit remarkably well with results of a recent study by Robertson et al. (2004), who showed that off-line learning of an SRTT sequence only requires sleep when subjects performed the task under “explicit” conditions (i.e., when they were informed about an underlying structure in the sequence of target locations at learning prior to sleep). According to those findings, the subject’s knowledge on the generation task about the existence of some underlying structure in the sequence prior to the retention interval may have tagged respective sequence representations in memory so that they become accessible by explicit mechanisms that enable sleep-dependent off-line learning. In fact, the overnight improvement in explicit sequence learning in our study (averaging 34%) appeared to be also quite comparable with the improvement of 38% observed by Robertson et al., although the different response measures in the studies limit any direct comparison. In addition, both of these studies failed to show any off-line learning improvements with respect to retention intervals of wakefulness when participants were informed about an underlying sequence. Nevertheless, it remains unresolved here whether explicit sequence encoding (as in Robertson et al.’s study) triggers processes of off-line learning comparable with those induced here by simply informing a subject about the existence of an underlying sequence *after* implicit encoding has finished.

It is to be noted that during generation task performance at learning, subjects did not develop any substantial explicit sequence knowledge and subjects of the wake group even at retesting performed at chance level. Thus, in the absence of any hint that there was some explicit knowledge at learning, it cannot be decided whether SRTT training alone, or in combination with prior generation task performance, is necessary for the explicit memory effect of subsequent sleep. In any case, the sleep-dependent gain in explicit knowledge does not appear to depend on an awareness of the specific associations encoded at learning, but only the awareness could suffice that there is some structure in the sequence to be learned. The fact that explicit sequence knowledge is enhanced selectively after sleep suggests that off-line learning during sleep not only strengthens newly acquired associations, but also reorganizes respective memory representations to allow explicit access. In this regard, our findings complement previous research indicating that sleep facilitates the gain of explicit knowledge of a hidden rule in a number reduction task performed under “implicit” conditions before sleep (Wagner, Gais, Haider, Verleger, & Born, 2004). Whether this gain of explicit knowledge depends on specific sleep processes and stages is unclear. Here, none of the sleep parameters was found to be correlated with improved generation task performance. Results from previous studies suggest that the reorganizing influence of sleep

on sequence representation in memory might originate from covert reactivations of the newly acquired representations which have been observed during REM sleep after extensive training on the SRTT as well as during SWS following the training of other skills and explicit tasks (Huber, Ghilardi, Massimini, & Tononi, 2004; Peigneux, Laureys, et al., 2003; Maquet, Laureys, et al., 2000). The specific characteristics of such reactivations clearly need to be further explored. However, they probably differ essentially in quality from mentations during the wake state. This is suggested by the negative findings from the wake condition, where no gain in explicit knowledge occurred, although subjects were informed about being retested at learning, and thus, had the chance to actively think about the task sequence.

Retesting on the SRTT test blocks did not provide evidence for an implicit off-line learning of the probabilistic sequence grammar neither after sleep nor after the wake retention interval. This result differs from previous studies reliably demonstrating that sleep supports implicit memory formation in various tasks including implicit knowledge in SRTTs with probabilistic sequence grammar (Fenn, Nusbaum, & Margoliash, 2003; Peigneux, Laureys, et al., 2003; Gais, Plihal, Wagner, & Born, 2000). Robertson et al. (2004) using an SRTT with deterministic sequence grammar found gains in implicit sequence knowledge across both sleep and wake retention periods. Here, there was only a general improvement in reaction times across both the sleep and wake retention periods that might reflect implicit off-line learning processes depending on the passage of time rather than requiring sleep, as reported by others (Fischer et al., 2002; Brashers-Krug, Shadmehr, & Bizzi, 1996). Because it has been shown previously that the skill achieved at learning influences the magnitude of subsequent off-line learning (Krakauer, Ghez, & Ghilardi, 2005; Hauptmann & Karni, 2002), the amount of training in the present experiments may have not been sufficient to induce a process of implicit off-line learning that expresses an increased difference between reaction times to grammatically correct versus incorrect trials at retesting after the retention interval. However, this view would not explain the general decrease in reaction times in the SRTT in our subjects at retrieval testing as well as the significant increase in sequence awareness selectively in the sleep group. Hence, a more plausible explanation for the lack of signs of implicit off-line learning at retrieval testing after sleep refers to the fact that SRTT performance was tested after the generation task, and subjects were informed about the presence of some structure in the sequence. Thus, given the structural similarity of the SRTT and the generation task, using the same stimuli, the generation task may have created some interference, thereby masking any signs of implicit learning during subsequent SRTT testing. Additionally, the contextual differences between learning and later retesting might have contributed to the absence of off-

line learning on the SRTT, as subjects encoded the sequence implicitly, whereas during SRTT retesting explicit sequence knowledge had been developed, at least for the sleep group (Robertson, 2004; Willingham, Salidis, & Gabrieli, 2002). However, a previous study showed that merely instructing the subjects about the presence of hidden rules does not change performance on the SRTT (Jiménez, Méndez, & Cleermans, 1996), which is also consistent with other findings from artificial grammar learning (Dienes, Broadbent, & Berry, 1991; Perruchet & Pacteau, 1990; Mathews et al., 1989; Dulany, Carlson, & Dewey, 1984). Nevertheless, once explicit sequence knowledge has been acquired, as after retention intervals of sleep here, this possibly could interfere with subsequent SRTT performance, although the direction of this effect is difficult to predict. The subjects could continue engaging in explicit processes of hypothesis testing, thus slowing reaction times. On the other hand, reaction times could also be speeded up due to the subject's enhanced ability to predict the upcoming target location (Born & Wagner, 2004; Poldrack et al., 2001; Willingham, 2001; Jimenez et al., 1996). In the present study, subjects of the sleep group, however, were obviously not able to translate their explicit sequence knowledge at retesting into improved SRTT performance. It should also be noted that whether the information about an underlying sequence does or does not affect the subject's SRTT performance is an issue different from that of the potential impact this information may have on the consolidation process itself. Because our subjects were informed already before the retention interval that there was some underlying structure in the sequences at generation task performance, it cannot be excluded that this information induced processes that directly blocked any implicit off-line improvements during the retention interval.

Although this issue of an acute (proactive) interference of generation task performance on SRTT performance at retesting remains unresolved here, there is increasing evidence that explicit and implicit memory processes interact during retention, specifically during sleep-associated off-line learning. A foregoing study (Wagner et al., 2004) used a number reduction task consisting of strings of numbers which subjects before retention intervals of sleep and wakefulness processed implicitly without awareness of an underlying structure in the digit strings. At retesting, sleep led to a facilitated gain of explicit knowledge of the digit sequence structure. Notably, subjects who gained explicit knowledge of the structure at retesting showed slower rather than faster reaction times on the task as compared to those subjects who did not gain explicit knowledge, suggesting that the sleep-dependent formation of explicit memories is linked to diminished signs of implicit memory formation (Born & Wagner, 2004). In combination with those data, the present finding of a selective increase in generation task performance across sleep supports the

notion that during sleep explicit knowledge can be gained on the basis of rules acquired implicitly before sleep. The gain in explicit knowledge implies that newly acquired memory representation become reorganized during sleep presumably as a result of an interaction between explicit and implicit memory processes. In the present experiments, the preferential formation of explicit memory may have been further supported by the reward that was announced to the subjects before the retention interval for good generation task performance at retesting. However, the dependence of off-line learning on reward is an issue which is clearly in the need of further examination.

METHODS

Participants

Subjects were 20 right-handed healthy volunteers between the age of 17 and 30 years (mean age \pm SD: 22.79 ± 3.90 years; 12 women). None had a history of neurological or psychiatric diseases or of sleep disorders. Participants had a regular sleep-wake cycle with an average sleep time of about 8 hours during the 6 weeks prior to the experiment. During the study period, subjects had to abstain from caffeine and alcohol and were instructed to get up before 7:00 a.m. and not to take any nap during the day. All subjects gave written informed consent and were paid for participation. The experiments were approved by the Ethics Committee of the University of Lübeck.

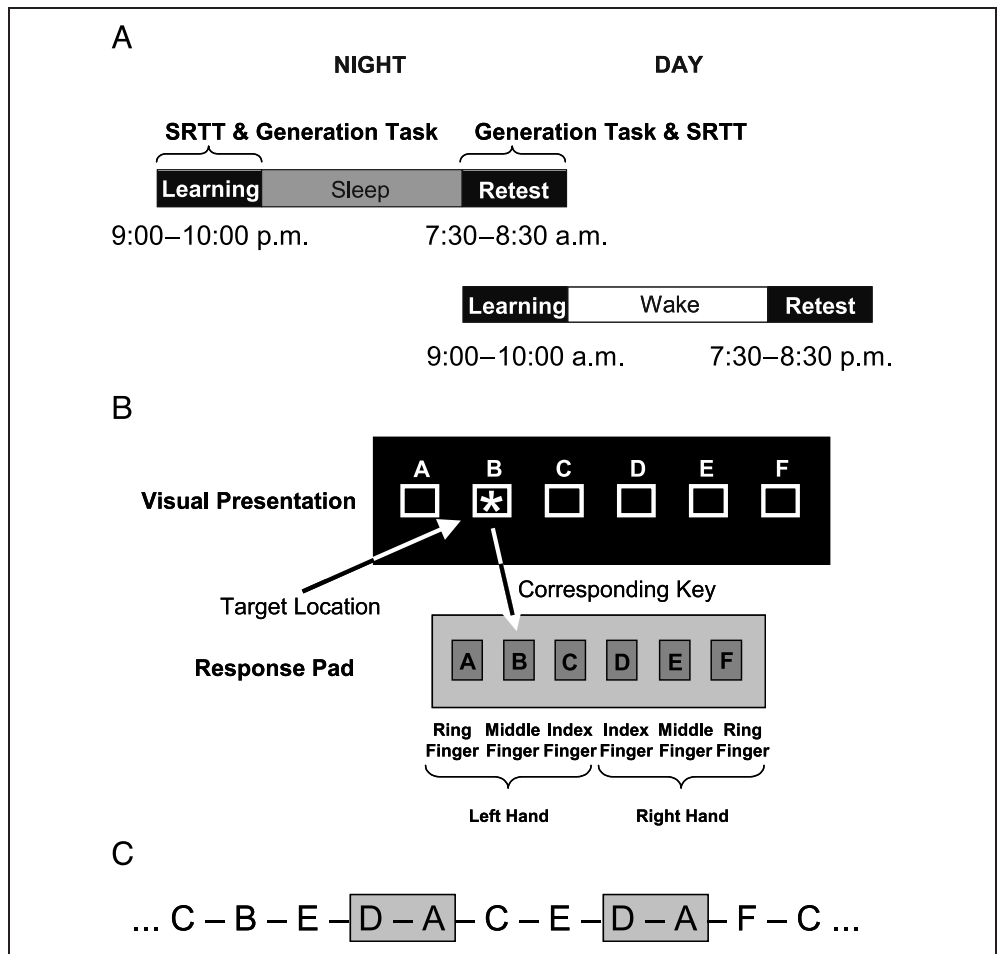
Design and Procedure

Half of the 20 subjects were randomly assigned to the sleep and the wake group (Figure 2A). Subjects of the wake group performed the tasks between 9:00 and 10:00 a.m. (learning) and were retested after a 9.5-hour retention interval of wakefulness between 7:30 and 8:30 p.m. on the same day. During the day they were not allowed to take any naps and to physically strain themselves. Subjects of the sleep group arrived in the sleep laboratory at 8:00 p.m. and were first prepared for polysomnographic recordings. Initial training on the tasks took place between 9:00 and 10:00 p.m. (learning). Bedtime was from 11:00 p.m. to 7:00 a.m. Retesting took place between 7:30 and 8:30 a.m. on the following morning. All subjects of the sleep group had spent an adaptation night in the sleep laboratory before beginning the experiments.

Experimental Tasks

At learning and retesting, subjects performed on the SRTT and a "generation task" taking place in a darkened room with the subject sitting in front of a 17-in. computer screen. Immediately after task performance, sub-

Figure 2. Study design, SRTT, and an example sequence of target locations. (A) In the sleep group, initial SRTT and generation task performance took place between 9:00 and 10:00 p.m. At 11:00 p.m. bedtime started and lights were turned off to allow sleep. Retesting took place on the next morning between 7:30 and 8:30 a.m. Subjects were awakened always 30 min before retesting started. In the wake group, both tasks were initially performed between 9:00 and 10:00 a.m. Retesting took place on the same day between 7:30 and 8:30 p.m. (B) During SRTT performance subjects were presented six horizontally arranged target locations on a computer screen (white boxes). They were instructed to react as fast and as accurately as possible to the occurrence of a target stimulus (white star) at one of these locations by pressing a spatially corresponding key on a response pad. (C) The sequence of target locations in the SRTT and the generation task was based upon a set of probabilistic rules, such that each of two successive trials constituted the temporal context that legally could be followed by one of two possible target locations, each occurring with a probability of 50%. In the present example sequence the temporal context “D,” “A” (gray fields) in one case was legally followed by Position “C,” in the other case by Position “F.”



jects rated their feelings of activation, concentration, tiredness, and mood on an adjective checklist (Janke & Debus, 1978).

Serial Reaction Time Task

The SRTT consisted of the permanent presentation of six white boxes on the computer screen, which were arranged horizontally on a black background, separated by intervals of 3 cm. Each screen position corresponded to a key on a six-key response pad below the screen (Figure 2B). Subjects were instructed to place the index finger, middle finger, and ring finger of the left hand on the left three response keys and those of the right hand on the right three response keys. The target cue consisted of a white star which successively appeared in the center of one of the boxes. On each trial, the subject had to react as fast and as accurately as possible by pressing the spatially corresponding response key, as soon as the cue was presented. Incorrect responses were indicated by a short beep. The next target cue was displayed after a fixed interval of 120 msec. Target cues were presented

in blocks of 194 trials, separated by short breaks of 30 sec. Unknown to the subjects, the sequence of target locations was generated on the basis of a probabilistic finite state grammar providing a set of rules that defined legal transitions between successive trials (Figure 2C; see below). At learning, subjects performed 12 blocks of the SRTT task after having been told that the task would measure vigilance. The blocks were followed by two test blocks in which in 15% of the trials the grammatically correct target locations were replaced by noncorrect locations violating the rules of the underlying grammar. The amount of implicit learning of the grammar was then determined by the increase of reaction times to grammatically incorrect target locations with reference to reaction times to correct target locations in these two blocks.

Generation Task

After completion of the 14 blocks of the SRTT at learning, subjects were informed that “there was a set of rules that determined the succession of target locations,

and that next it is tested whether they had acquired any knowledge about the constraints in the sequence.” For this purpose, subjects were instructed to not longer react on the current stimulus but to generate respective next target cue position by pressing the corresponding response key. There was no time limit for these responses. The correct stimulus was displayed following each response, regardless whether the response was correct or not. The generation task consisted of two blocks of 194 trials which followed exactly the same sequence of target positions as in the last two blocks of SRTT training (i.e., they did not contain any grammatically incorrect trials). The number of correct predictions of the target position was used as an estimate of explicit knowledge. At the end of the learning session, subjects were informed that after the retention interval they were again tested on the generation task and that they could improve their payment by the number of accurately predicted target locations.

Retesting

At retesting, subjects first performed two blocks of the generation task and were then retested on the two test blocks of the SRTT task. Both the generation task and the SRTT task consisted of exactly the same stimulus material as used of the generation task and the two test blocks of the SRTT task at learning.

Sequence Characteristics

The sequence of target positions in the SRTT was generated on the basis of a probabilistic finite state grammar. According to this grammar, each of two successive trials can be followed by two possible target locations, each appearing with a probability of 50%. For example, the successive appearance of Positions D and A is equally often followed by Positions C and F (Figure 2C). Thus, the grammar defines a second-order sequential context in which each target position can occur. Immediate repetitions (e.g., “A,” “A”) as well as alternations (e.g., “A,” “C,” “A”) were not possible. Further sequence constraints were that each single target position and each of the 48 triple combinations occurred at the same rate in each block. To control for motor effects and for serial and spatial order effects, the labels “A,” “B,” “C,” and so forth of the grammar as illustrated in Figure 2B and C were randomly mapped on the different target positions, and then systematically modified by shifting the screen position one step to the right per subject.

In the last two test blocks of the SRTT, in 15% of the trials, the target stimulus was presented at a grammatically incorrect position. These incorrect trials exclusively violated second-order contingencies. Incorrect target positions also never led to alternations in the sequence. Moreover, these grammatically incorrect tar-

get stimuli equally often occurred at each of the six screen positions.

Dependent Variables and Statistical Analyses

Individual SRTT performance was determined by calculating the median reaction times for all correct responses per block. For the two test blocks presented at learning and retrieval, this was done separately for grammatically correct and incorrect trials. Then, grand means were formed across all subjects in each group. Implicit learning of the (second order) rules was expressed in terms of difference between mean reaction times of grammatically correct and incorrect trials. Explicit knowledge of the sequence constraints in the generation task was determined by the number of correctly predicted target positions. The number of correct responses was averaged for the two blocks and transformed to percent values. Explicit knowledge was given when the rate of correctly predicted target positions was significantly above chance level, which was 50%.

Polysomnographic recordings were digitally stored and visually scored off-line according to standard criteria (Rechtschaffen & Kales, 1967). Time spent in the different sleep stages (wake, sleep stages 1–4, REM sleep) was also transformed into percentages of TST. In additional explorative analyses, sleep parameters were correlated with improvements in generation task performance using Pearson’s correlation coefficients.

Dependent variables were analyzed according to the general linear model using ANOVAs, including a between-subject factor (sleep vs. wake) and two within-subject factors (before/after, grammatical correctness). A p value $<.05$ was considered significant.

Acknowledgments

This research was supported by a grant from the Deutsche Forschungsgemeinschaft (DFG) to J. B.

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REFERENCES

- Born, J., & Wagner, U. (2004). Awareness in memory: Being explicit about the role of sleep. *Trends in Cognitive Sciences*, 8, 242–244.
- Boyd, L. A., & Winstein, C. J. (2001). Implicit motor-sequence learning in humans following unilateral stroke: The impact of practice and explicit knowledge. *Neuroscience Letters*, 298, 65–69.
- Brashers-Krug, T., Shadmehr, R., & Bizzi, E. (1996). Consolidation in human motor memory. *Nature*, 382, 252–255.
- Cleeremans, A., & McClelland, J. L. (1991). Learning the

- structure of event sequences. *Journal of Experimental Psychology: General*, *120*, 235–253.
- Cohen, A., Ivry, R. I., & Keele, S. W. (1990). Attention and structure in sequence learning. *Journal of Experimental Psychology: Learning, Memory, and Cognition*, *16*, 17–30.
- Destrebecqz, A., & Cleeremans, A. (2001). Can sequence learning be implicit? New evidence with the process dissociation procedure. *Psychonomic Bulletin and Review*, *8*, 343–350.
- Dienes, Z., Broadbent, D., & Berry, D. (1991). Implicit and explicit knowledge bases in artificial grammar learning. *Journal of Experimental Psychology: Learning, Memory, and Cognition*, *17*, 875–887.
- Dulany, D. E., Carlson, R. C., & Dewey, G. I. (1984). A case of syntactical learning and judgment: How conscious and how abstract? *Journal of Experimental Psychology: General*, *113*, 541–555.
- Fenn, K. M., Nusbaum, H. C., & Margoliash, D. (2003). Consolidation during sleep of perceptual learning of spoken language. *Nature*, *425*, 614–616.
- Fischer, S., Hallschmid, M., Elsner, A. L., & Born, J. (2002). Sleep forms memory for finger skills. *Proceedings of the National Academy of Sciences, U.S.A.*, *99*, 11987–11991.
- Gais, S., Plihal, W., Wagner, U., & Born, J. (2000). Early sleep triggers memory for early visual discrimination skills. *Nature Neuroscience*, *3*, 1335–1339.
- Hauptmann, B., & Karni, A. (2002). From primed to learn: The saturation of repetition priming and the induction of long-term memory. *Brain Research, Cognitive Brain Research*, *13*, 313–322.
- Huber, R., Ghilardi, M. F., Massimini, M., & Tononi, G. (2004). Local sleep and learning. *Nature*, *430*, 78–81.
- Janke, W., & Debus, G. (1978). *Die Eigenschaftswörterliste EWL*. Göttingen: Hogrefe.
- Jimenez, L., Mendez, C., & Cleeremans, A. (1996). Comparing direct and indirect measures of sequence learning. *Journal of Experimental Psychology: Learning, Memory, and Cognition*, *22*, 948–969.
- Knopman, D. S., & Nissen, M. J. (1987). Implicit learning in patients with probable Alzheimer's disease. *Neurology*, *37*, 784–788.
- Krakauer, J. W., Ghez, C., & Ghilardi, M. F. (2005). Adaptation to visuomotor transformations: Consolidation, interference, and forgetting. *Journal of Neuroscience*, *25*, 473–478.
- Maquet, P., Laureys, S., Peigneux, P., Fuchs, S., Petiau, C., Phillips, C., Aerts, J., Del Fiore, G., Degueldre, C., Meulemans, T., Luxen, A., Franck, G., Van Der, L. M., Smith, C., & Cleeremans, A. (2000). Experience-dependent changes in cerebral activation during human REM sleep. *Nature Neuroscience*, *3*, 831–836.
- Maquet, P., Schwartz, S., Passingham, R., & Frith, C. (2003). Sleep-related consolidation of a visuomotor skill: Brain mechanisms as assessed by functional magnetic resonance imaging. *Journal of Neuroscience*, *23*, 1432–1440.
- Mathews, R. C., Buss, R. R., Stanley, W. B., Blanchard-Fields, F., Cho, J. R., & Druhan, B. (1989). Role of implicit and explicit processes in learning from expamles: A synergistic effect. *Journal of Experimental Psychology: Learning, Memory, and Cognition*, *15*, 1083–1100.
- Nissen, M. J., & Bullemer, P. (1987). Attentional requirements of learning: Evidence from performance measures. *Cognitive Psychology*, *19*, 1–32.
- Peigneux, P., Laureys, S., Fuchs, S., Destrebecqz, A., Collette, F., Delbeuck, X., Phillips, C., Aerts, J., Del Fiore, G., Degueldre, C., Luxen, A., Cleeremans, A., & Maquet, P. (2003). Learned material content and acquisition level modulate cerebral reactivation during posttraining rapid-eye-movements sleep. *Neuroimage*, *20*, 125–134.
- Peigneux, P., Maquet, P., Meulemans, T., Destrebecqz, A., Laureys, S., Degueldre, C., Delfiore, G., Aerts, J., Luxen, A., Franck, G., Van Der, L. M., & Cleeremans, A. (2000). Striatum forever, despite sequence learning variability: A random effect analysis of PET data. *Human Brain Mapping*, *10*, 179–194.
- Perruchet, P., & Pacteau, C. (1990). Synthetic grammar learning: Implicit rule abstraction or explicit fragmentary knowledge. *Journal of Experimental Psychology: General*, *119*, 264–275.
- Plihal, W., & Born, J. (1997). Effects of early and late nocturnal sleep on declarative and procedural memory. *Journal of Cognitive Neuroscience*, *9*, 534–547.
- Poldrack, R. A., Clark, J., Pare-Blagoev, E. J., Shohamy, D., Creso, M. J., Myers, C., & Gluck, M. A. (2001). Interactive memory systems in the human brain. *Nature*, *414*, 546–550.
- Reber, A. S. (1989). Implicit learning and tacit knowledge. *Journal of Experimental Psychology: General*, *120*, 112–116.
- Reber, P. J., & Squire, L. R. (1998). Encapsulation of implicit and explicit memory in sequence learning. *Journal of Cognitive Neuroscience*, *10*, 248–263.
- Rechtschaffen, A., & Kales, A. (1967). *A manual of standardized terminology, techniques and scoring system for sleep stages of human subjects*. Bethesda, MD: NIH Publication No. 204.
- Reingold, E. M., & Merikle, P. M. (1988). Using direct and indirect measures to study perception without awareness. *Perception & Psychophysics*, *44*, 563–575.
- Robertson, E. M. (2004). Skill learning: Putting procedural consolidation in context. *Current Biology*, *14*, R1061–R1063.
- Robertson, E. M., Pascual-Leone, A., & Press, D. Z. (2004). Awareness modifies the skill-learning benefits of sleep. *Current Biology*, *14*, 208–212.
- Seger, C. A. (1994). Implicit learning. *Psychological Bulletin*, *115*, 163–196.
- Shanks, D. R., & John, M. F. (1994). Characteristics of dissociable learning systems. *Behavioral and Brain Sciences*, *17*, 367–395.
- Squire, L. R. (1992). Declarative and nondeclarative memory: Multiple brain systems supporting learning and memory. *Journal of Cognitive Neuroscience*, *4*, 232–243.
- Wagner, U., Gais, S., Haider, H., Verleger, R., & Born, J. (2004). Sleep inspires insight. *Nature*, *427*, 352–355.
- Willingham, D., & Goedert-Eschmann, K. (1999). The relation between implicit and explicit learning: Evidence for parallel development. *Psychological Science*, *10*, 531–534.
- Willingham, D. B. (2001). Becoming aware of motor skill. *Trends in Cognitive Sciences*, *5*, 181–182.
- Willingham, D. B., Nissen, M. J., & Bullemer, P. (1989). On the development of procedural knowledge. *Journal of Experimental Psychology: Learning, Memory, and Cognition*, *15*, 1047–1060.
- Willingham, D. B., Salidis, J., & Gabrieli, J. D. (2002). Direct comparison of neural systems mediating conscious and unconscious skill learning. *Journal of Neurophysiology*, *88*, 1451–1460.