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ORIGINAL ARTICLE

Day versus night consolidation of implicit sequence learning using manual and oculomotor activation versions of the serial reaction time task: reaction time and anticipation measures

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Abstract
This study presents two experiments that explored consolidation of implicit sequence learning based on two dependent variables—reaction time (RT) and correct anticipations to clarify the role of sleep, and whether the manual component is necessary for consolidation processes. Experiment 1 ($n = 37$) explored the performance of adults using an ocular variant of the serial reaction time task (O-SRT) with manual activation (MA), and Experiment 2 ($n = 37$) used the ocular activation (OA) version of the task. Each experiment consisted of a Day and a Night group that performed two sessions of the O-SRT with an intervening 12-h offline period (morning/evening in Day group, evening/following morning in Night group). Night offline had an advantage only when manual response was required and when correct anticipations (i.e., accuracy) but not RT (i.e., speed) were measured. We associated this finding with the dual-learning processes required in the MA O-SRT that led to increased sequence specific learning overnight. When using the OA O-SRT, both groups demonstrated similar rates after offline in RT and correct anticipations. We interpreted this finding to reflect stabilization, which confirmed our hypothesis. As expected, all the groups demonstrated reduced performance when another sequence was introduced, thus reflecting sequence-specific learning. This study used a powerful procedure that allows measurement of implicit sequence learning in several ways: by evaluating two different measures (RT, correct anticipations) and by isolating different aspects of the task (i.e., with/without the manual learning component, more/less general skill learning), which are known to affect learning and consolidation.

Introduction

Learning processes have been shown to occur both during repeated training intervals (online) and between practice sessions (offline) that could last a few hours, 12-h or even 24 h. Offline learning can occur during different states of consciousness such as active waking, quiet rest or sleep. Offline learning is referred to as consolidation, and can result in increased resistance to interference, stabilization or even improvement in performance following an offline period (Csabi et al., 2014; Krakauer & Shadmehr, 2006; Robertson, 2009; Song, 2009; Yotsumoto et al., 2013). It is assumed that these processes taking place during the offline period between training sessions take place without additional training and without awareness. It is important to note that some recent theories have emerged which challenge the consolidation approach as an explanation for the stabilization effect of sleep (or delay interval). Yonelinas et al. (2019) proposed the contextual binding theory as an alternative explanation to the beneficial effect of sleep. They attribute this effect to the fact that encoding of interfering information is significantly reduced during sleep. Note that this theory focuses primarily on episodic, rather than procedural, memory.

Sleep was shown to contribute to consolidation of memory traces by enhancing neuronal plasticity (Diekelmann et al., 2009). However, the beneficial effect of sleep on consolidation of implicit sequence learning has remained controversial. In their review, Janacsek and Nemeth (2012) point to several factors affecting consolidation of implicit sequence learning, namely awareness, length of offline period, perceptual/motor sequence, and age. Thus, ignoring these factors might lead to inconsistent findings. Nemeth

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et al. (Preprint, 2019) suggest that these inconsistencies could also stem from several methodological pitfalls characteristic of these studies. In recent years, increasing reports indicate offline memory improvement even without post-training sleep, meaning that consolidation is not sleep-dependent, but rather time-dependent (Doyon et al., 2009; Nemeth et al., 2010; Song et al., 2007; Urbain et al., 2013). In their literature review, Janacsek and Nemeth (2012) concluded that consolidation of sequence learning is not a unitary process, but rather a set of multiple mechanisms, including sequence-specific learning and general motor learning, which are influenced differentially by the various task components.

The serial reaction time (SRT) task (Nissen & Bullemer, 1987) is one of the tasks used most frequently to study implicit motor sequence learning. Implicit skill learning occurs when information is acquired from an environment of complex stimuli without conscious access either to what was learned or even to the fact that learning occurred (Fischer et al., 2006; Reber, 1989). More specifically, in this task participants are presented with four squares on the screen that appear in a repeated spatial sequence. Participants are asked to respond as fast as possible by pressing a key whose location corresponds to the position of the square on the screen. Following several learning blocks, a block with a new or random sequence is presented. Despite vast research conducted using the SRT task, it is not yet clear what exactly is learned during this task. Recent studies suggest that there are two separate aspects of learning that occur during the SRT task. In addition to the sequence-specific learning which refers to acquisition of implicit knowledge about the specific pattern of the stimuli's location in a sequence, a general aspect of the task is learned as well (Fischer et al., 2006; Helmuth et al., 2000). Besides becoming more familiar with the task, this general aspect also consists of an association between manual and perceptual learning components, i.e., *stimulus–response* (S–R) learning, namely, the stimulus that appears on the screen, and its corresponding manual response. Thus, reduced RT as a function of training stems from both the general factor and learning the specific sequence. This explains the typical finding when the interference sequence is introduced using the SRT task: although there is a cost in RT (i.e., slower RT), it does not revert to baseline performance. The interpretation of such a finding is that general learning is carried over to the new sequence as well (Ferraro et al., 1993; Vakil et al., 2002).

Meier and Cock (2014) showed that only the general motor learning aspect of the SRT task was enhanced during an offline period, regardless of whether the interval lasted 24 h or one week. Song et al. (2007) found offline improvement of general skill learning only in the no-sleep condition. Nemeth et al. (2010) used a probabilistic version of the SRT task and found offline improvement of general skill (overall RT) for young adults after a 12-h interval, regardless of whether or not it included sleep. Other SRT studies found similar consolidation of implicit skill learning during both sleep and wakefulness (Nemeth et al., 2010; Song et al., 2007). It has been suggested that both conscious awareness and attention are necessary components for the development of sleep-dependent memory consolidation effects (Robertson et al., 2004; Song, 2009). These results, among others, indicate the possibility of consolidation of the general motor aspect of the task rather than of implicit sequence learning, as demonstrated by enhanced performance after an offline period, subject to certain conditions and settings (Csabi et al., 2014; Meier & Cock, 2014; Nemeth et al., 2010). Unlike the general motor aspect of sequence learning, no offline improvement in performance was evident for sequence-specific learning (Janacsek & Nemeth, 2012). Nevertheless, performance after an offline period was significantly better than baseline, which may demonstrate stabilization of memory (Nemeth et al., 2010). The researchers concluded that the differences in consolidation processes for the two aspects of implicit sequence learning represent the involvement of different brain regions, specifically, the subcortical structures and cerebellum (Doyon, 2008).

Csábi et al. (2016) tested children with sleep disorder with the Alternating Serial Reaction Time (ASRT) task. Their results showed offline learning in both sequence-specific and general motor skill. Simor et al. (2019) used the ASRT task under three offline conditions awake, quiet rest (i.e., by reducing distractions) or sleep. They found that the acquired sequence as well as the statistical knowledge were preserved, regardless of the offline condition.

By means of eye tracking, Vakil et al. (2017) designed an occular version of the SRT task (O-SRT). The O-SRT task, in addition to the typical RT measure, enables the number of correct anticipations, which will be explained below. In addition, this version of the task generates an RT measure either in the standard Manual Activation (MA) or Ocular Activation (OA) RT measure, in which fixation of 100 miliseconds on target is considered a reaction to target. Their results showed that the extent of sequence learning in these two versions of the task (MA & OA) was identical, suggesting that implicit sequence learning is not necessarily dependent on motor components, as suggested by some researchers (Deroost & Soetens, 2006; Ziessler & Nattkemper, 2001). Furthermore, consistent with previous results in the literature, Vakil et al. (2017) found that RT does not return to baseline when a new sequence is introduced in the standard MA version of the SRT (Ferraro et al., 1993; Vakil et al., 2002). This may indicate that although the sequence has been changed, the S-R mapping learned during the learning phase with the original sequence is carried over to the new sequence. Interestingly, with the OA version of O-SRT, RT returned close to baseline. The researchers concluded that OA may produce a purer measure of implicit sequence learning that eliminates the additional general S-R mapping. That is because the sequence of S-R mappings is learned during MA task training, whereas, oculomotor responses in the OA task (moving the gaze to target) are automatic and exist prior to training. As mentioned above, in addition to measuring RT, the O-SRT task enables generation of a new measure called *correct anticipations*. Eye tracking enables the measurement of correct anticipations by recording whether the eyes move towards the next correct position during the 500-ms interval between targets (see Vakil et al., 2017). Note that this measure is recorded in both the MA and OA versions of the O-SRT task. The advantage of the correct anticipation measure over the RT measure is that the former is a direct measure of correct anticipations, while the latter is an indirect measure of anticipations of the correct locations, which reflects sequence learning. In addition, compared to the RT measure in the MA version, the OA RT measure of the O-SRT task is viewed as a purer measure of sequence learning, because the general learning component of the learning process is significantly reduced.

This study attemped to shed light on the contribution and the interactions between several variables that mediate the consolidation effect in implicit sequence learning in general, and in the SRT task in particular. The primary goal was to compare the consolidation effect of a 12-h offline period, with and without sleep (Night and Day group, respectively). This study included two experiments: in Experiment 1 we applied the MA version of the O-SRT task, and in Experiment 2 we applied the OA version of the O-SRT task. This allowed us to compare the consolidation effect when the task either did or did not involve manual learning (MA versus OA, respectively), thus addressing the question whether consolidation is dependent on manual learning and whether only general skill learning is improved, as has been claimed by some researchers (Csabi et al., 2014; Meier & Cock, 2014; Nemeth et al., 2010).

Furthermore, using the O-SRT test we can compare two measures of sequence learning, the typical manual RT measure or the OA RT measure, and the newer measure of correct anticipations described above. As demonstrated by Vakil et al. (2017), the latter as compared to the former measure is a purer measure of sequence learning, because correct anticipations reflect sequence learning more directly than RT, which is assumed to be the consequence of correct anticipations. Consistent with our previous study (Vakil et al., 2017), we expected that Experiment 1 (MA O-SRT) and Experiment 2 (OA O-SRT) would show a learning effect and an interference effect when a new sequence is introduced, whether RT or correct anticipations measures were used. In addition, we predicted that performance during the interference block would return close to baseline for the OA O-SRT groups, but not in the MA O-SRT groups. That is because unlike the OA O-SRT group, sequence learning in the MA O-SRT group includes general learning, in the form of S-R learning, and the latter component is carried over from the learning sequence (blocks 1–6) to the interference sequence (block 9). In terms of consolidation, consistent with previous studies (Krakauer & Shadmehr, 2006; Nemeth et al., 2010; Robertson, 2009; Song, 2009), for Experiment 1 (MA O-SRT) we predicted stabilization for both Day and Night groups, i.e., the same performance after and before the offline period, and better than baseline performance. We also predicted the latter result for Experiment 2 (OA O-SRT), because we presumed that consolidation would be similar, whether or not performance included the manual factor. This assumption was based on previous reports demonstrating implicit sequence learning without the involvement of manual responses (Albouy et al., 2006; Marcus et al., 2006).

Experiment 1—MA O-SRT

Method

Participants

Study participants included female and male undergraduate or graduate students at Bar-Ilan University with normal sleeping behavior. Sleeping behavior was screened during recruitment according to the following four questions: (1) How many hours do you sleep? (2) How would you rate your sleep quality? (3) How long does it take you to fall asleep? (4) How often do you wake up in the middle of the night or early morning? Only participants that stated that they sleep five or more hours (question 1), rated their sleep quality as good (question 2), explained that it does not take them more than 60 min to fall asleep (question 3) and do not wake up more than twice in the middle of the night (question 4), were invited and randomly assigned into one of the two groups, MA Day or MA Night. In addition to disturbed sleeping behavior, left handedness, neurological or psychiatric disturbances or learning disorders were also used as exclusion criteria. In order to have gender balanced groups, towards the end of data acquisition we pseudo-randomly assigned the participants in reference to their gender to the study groups. Nineteen young adults participated in the MA Day group (10 males, mean age 30.3 years, range 25–35 years), and 18 participated in the MA Night group (9 males, mean age 31.2 years, range 29–37 years). Participants either volunteered for the study or took part in the experiment to fulfill academic requirements. The study received prior approval from the Ethics Committee of the Psychology Department at Bar-Ilan University, and each participant signed an informed consent form.

Tools

The MA version of the O-SRT task was a replication of the task described in the study by Vakil et al. (2017). The task was programmed in E-Prime 2.0. Eye movements were recorded by the SMI iView 120 REDm Eye Tracker, sample rate: 120 Hz. Stimuli were presented on a LCD computer screen (Size 42 × 24 cm; resolution 1600×900 pixels). The recording device was installed beneath the screen. Participants were seated in front of the screen, approximately 60 cm away from it. Calibration was conducted at the beginning of every task using a standard 5-point grid for both eyes. A 4-point grid was used for validation after each calibration trial. If the mean deviation of calibration and validation accuracy exceeded 0.8°, calibration and validation were repeated.

Stimuli

Stimuli consisted of five slides, each with a resolution of 1400×900 pixels. Each stimulus included four white squares arranged in a diamond shape on a grey background. A black dot (indicating the target) appeared in one of the four white squares. The size of each square was 6×6 cm and the diameter of the dot was 1.5×1.5 cm (see Fig. 1). Four slides included a target image, and the fifth slide was a blank slide used to measure anticipation.

Procedure

A black dot (the target) appeared in one of four white squares arranged in a diamond shape (see Fig. 1). Before each slide with a dot appeared on the screen, a blank slide with four empty squares was displayed for 500 ms (i.e., the anticipation slide). Importantly, participants were not aware of this slide, since the experiment was perceived as a continuous flow from one to the next target slide.

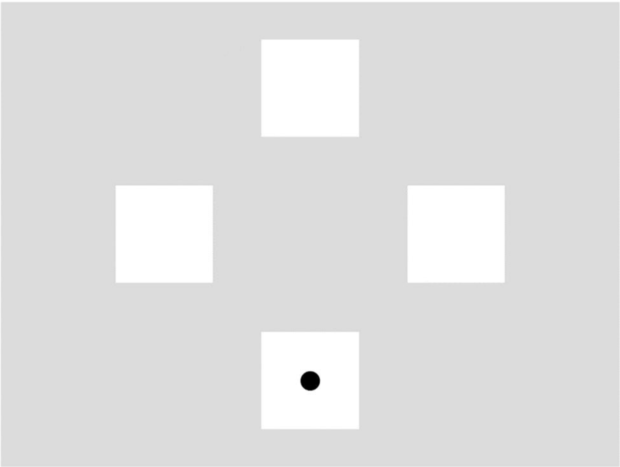

Fig. 1 Illustration of the target slide of the ocular serial reaction time (O-SRT) task designed by Vakil et al. (2017)

Each block consisted of a 12-element sequence repeated nine times (see Fig. 2). The sequence in each block began from a different element of the sequence, i.e., from a different starting point. No first-order predictive information was provided in the sequence (i.e., each location was preceded by the same location only once), but only second order conditional sequences (i.e., dependent upon two previous stimuli). Each element in the sequence was matched with one of the four squares: 1, 2, 3, and 4 to correspond with down, left, right, and up, respectively. Two sequences were designed: ‘’sequence A’ (3–4–2–3–1–2–1–4–3–2–4–1; the repeated sequence) and ‘sequence B’ (3–4–1–2–4–3–1–4–2–1–3–2; the interference sequence). See Fig. 2 for an illustration of ‘sequence A’.

Each group completed two sessions—a learning session and a test session, which were administered 12-h apart (see Fig. 3 for the experimental design). The Day group learning session took place in the morning (between 7 and 11 a.m.) and was followed by the test session 12-h later in the evening. Participants were explicitly instructed not to go to sleep during this period. All participants confirmed that they followed our instructions. Vice versa, the Night group learning session was held in the evening (between 7 and 11 p.m.) and the test session took place the next morning after a 12-h interval that had included a sleep phase. The order of the sequences was the same for all participants.

Participants were instructed to respond as quickly and as accurately as possible to the changing location of the black dot, that appeared on the screen. Response was given by pressing the corresponding button on the keyboard using only the index finger of their right hand. Round black stickers were placed on the 8, 4, 2 and 6 keys on the keyboard number pad, to correspond with the up, left, down and right directions, respectively. The slide containing the stimulus remained visible until one of the four keys was pressed and then the dot disappeared; otherwise it was displayed for 3000 ms. In addition, participants were instructed to follow the dot with their eyes throughout the experiment.

The learning session included six blocks (block 1–block 6) which took approximately 15 min. All blocks included ‘sequence A’ in a repeated pattern as described above. Unlike the classic design of the SRT task, the learning session did not include presentation of the interference sequence (i.e., ‘sequence B’), so that exposure to the new sequence during learning would not interfere with the consolidation process. After each block, a one-minute break was given before starting the next block.

The test session was conducted exactly 12-h after the learning session and included four blocks. The first two blocks (blocks 7 and 8) included the original sequence used

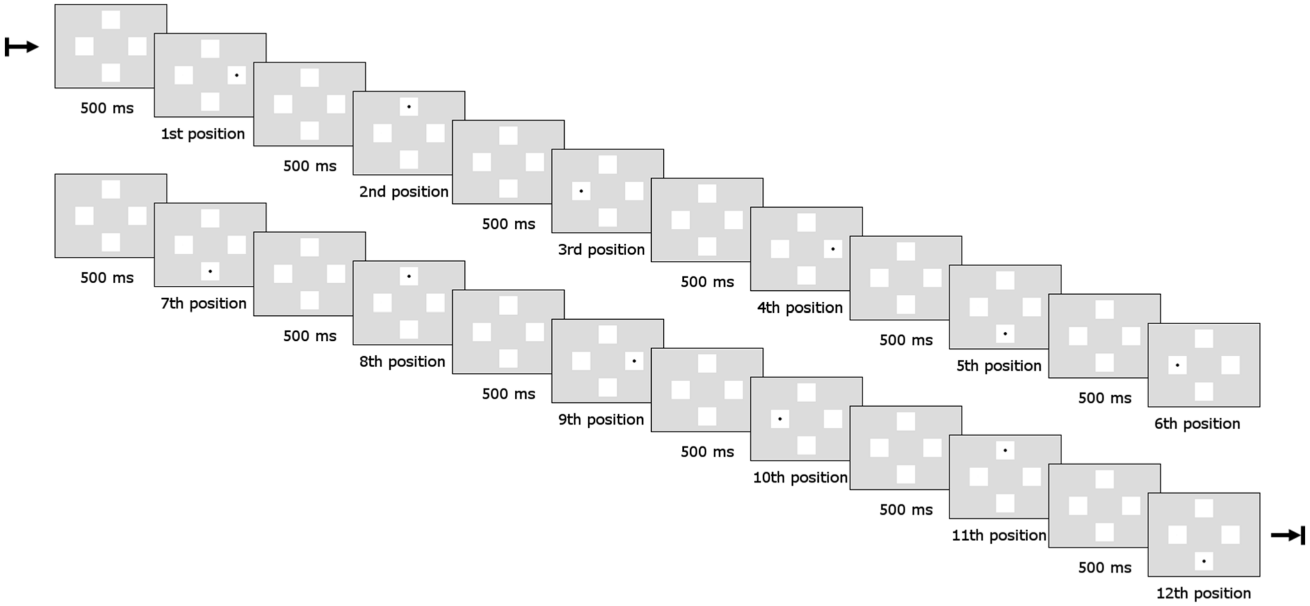

Fig. 2 Illustration of one of the sequences used in the experimental design of the O-SRT. A sequence consisting of 12 elements (=positions) was repeated nine times per block. At the beginning and in between the target slides, a blank slide containing only the squares was presented for 500 ms to measure correct anticipations

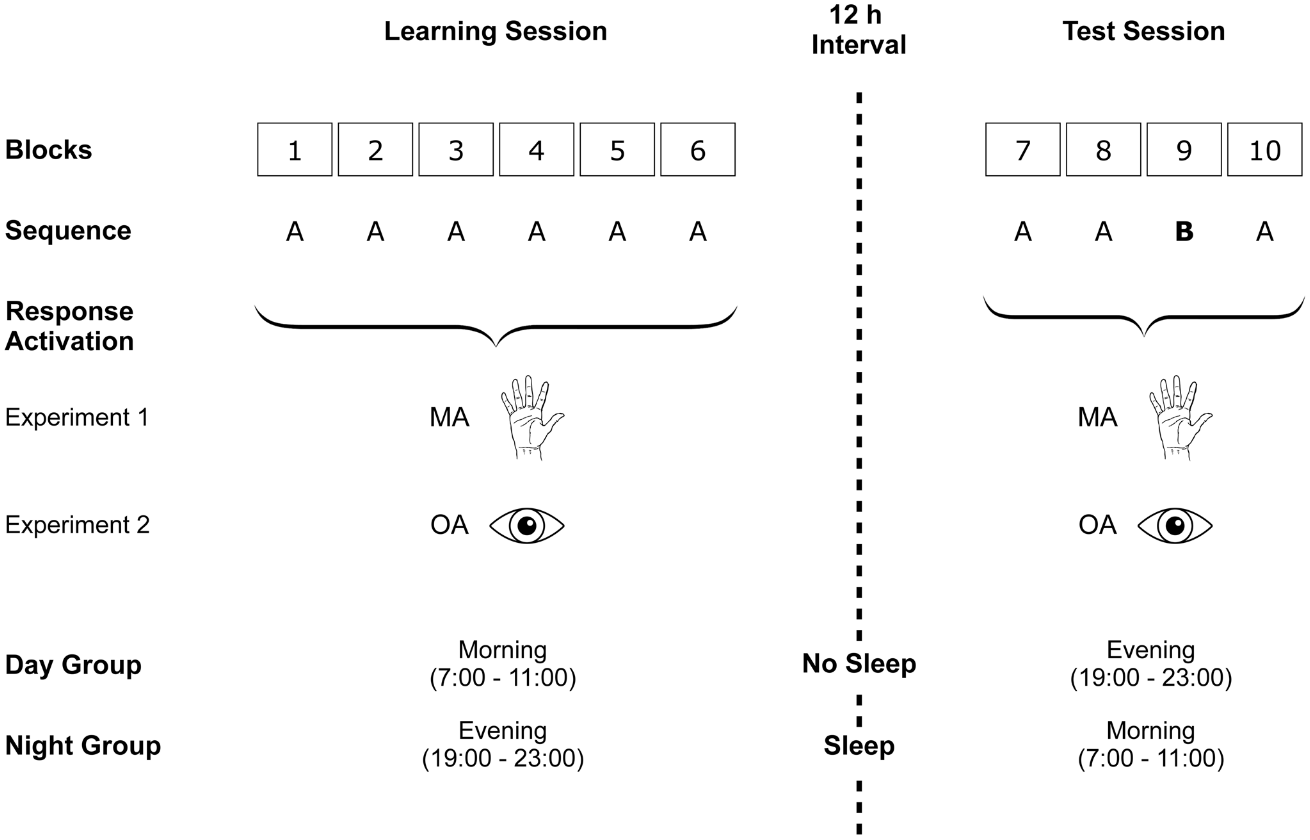

Fig. 3 Illustration of the experimental procedure

in the learning session (‘sequence A’). These blocks were followed by the interference block (block 9) containing a different sequence (‘sequence B’). This was followed by the recovery block (block 10) which used the original sequence (’sequence A’).

Data analysis

Data were registered using BeGaze™ (SensoMotoric Instruments, Teltow, Germany) to generate eye movement measures. Manual responses were registered using E-prime. Two dependent measures were used: speed (MA RT to target) and accuracy (correct anticipations in the anticipation slide).

For the speed measure, we calculated the median MA RT for each 12-item sequence (i.e., for each 12 target trials). Then, the mean of medians of MA RT per block (i.e., 9 sequences of 12-items each; 108 trials) was computed.

Correct anticipations were evaluated by tracking the transition of the participant’s gaze to the correct subsequent position while the blank slide between target slides was presented for 500 ms. We used the function "area of interest" in the BeGaze program and turned the squares into larger triangles, so that four triangles covered the four squares and the center point of the screen (see Fig. 4).

Our analysis showed that across all blocks, participants’ gaze moved to only one location in most (79.7%) of the trials. In 19.5% of the trials, their gaze remained in the same location, and only in a small percentage of trials (0.8%) did participants’ gaze shift to more than one location. These percentages are similar to those reported by Vakil et al. (2017).

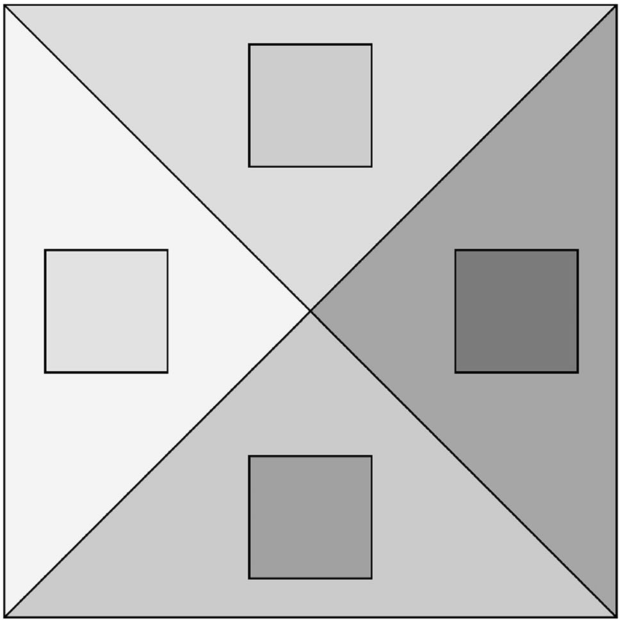

Fig. 4 The AOIs (areas of interest) used for calculating the percentage of correct anticipations

The measure *correct anticipations* included only the trials in which participants moved their gaze towards one different location. An anticipation score of "1" was set for the slides in which there was at least one fixation on only the correct location (where the next target was going to appear), and a "0" score for fixations on one of the incorrect locations. Then, the number of correct anticipations per sequence (average anticipation score range 0–12) was counted and averaged for nine sequences per block. This established the percentage of correct anticipations score for each block for all participants.

Five phases of performance were analyzed separately: (1) *Learning:* blocks 1 to 6 in the learning session. (2) *Offline:* block 6 in the learning session vs. block 7, 12-h later in the test session. (3) *Saving:* baseline (block 1) vs. offline (block 7). (4) *Interference:* original sequence (block 8) vs. new sequence (block 9) both in the test session, and (5) *Recovery:* new sequence (block 9) vs. original sequence (block 10) both in the test session. A separate Mixed-design ANOVA was used to test the between-subjects factor of Group (Day vs. Night) and each one of the above five independent measures as within-subjects factors. These analyses were conducted twice: first for the RT measure, and second for the correct anticipations measure. Furthermore, to display interaction effects of the 2×2 Mixed design ANOVAs, we computed the difference scores of the independent measures (e.g., for Saving we computed block 1 minus block 7, and in an analogous way for the remaining independent measures) per Group, and then from those difference scores we computed the difference scores between the Groups.

Results

Reaction Time–time elapsed from stimulus presentation to manual response (reflects speed)

See Fig. 5 that presents the mean of median MA RT as a function of MA O-SRT blocks 1–10 for both groups. See also Table 1 that presents the difference scores analyzed.

Learning (change in RT across the six learning blocks) As shown in Fig. 5, there was a significant reduction in RT over blocks 1–6, $F(2.10, 73.39) = 50.01, p < 0.001, \eta_p^2 = 0.59$, and the main effect of Group was significant, $F(1, 35) = 10.44, p < 0.01, \eta_p^2 = 0.23$. Group by Learning interaction did not reach significance, $F(2.10, 73.39) = 0.86, p = 0.51, \eta_p^2 = 0.02$. This indicates that although the Night group had overall slower performance, participants from both groups improved at a similar rate and learned the sequence during the learning session as reflected in faster RT.

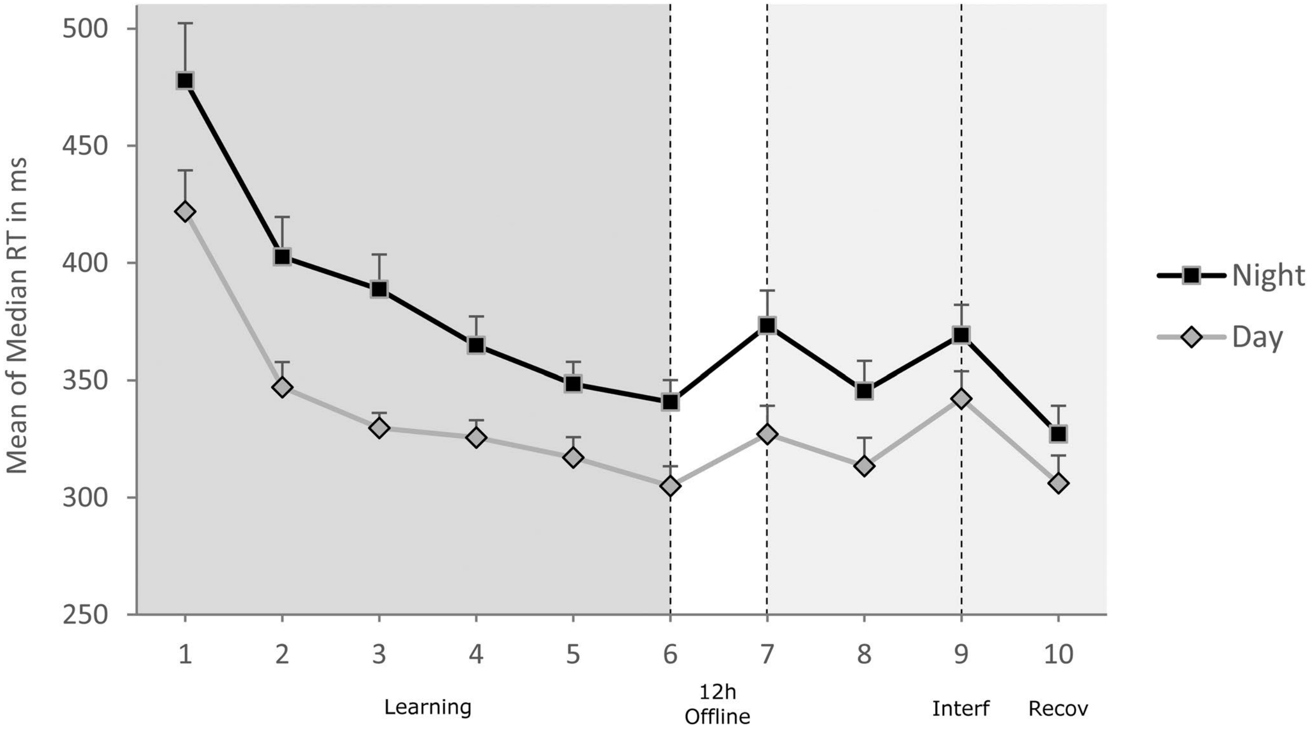

Fig. 5 The mean of the median manual reaction time (RT) of the Day and the Night groups for Experiment 1 (MA O-SRT). Abbreviations: *Interf* interference, *Recov* recovery

Table 1 Mean RT and CA difference scores and interaction effect difference scores of 2×2 ANOVAs

	RT difference scores			CA difference scores		
	Night	Day	Inter-action	Night	Day	Inter-action
Offline (block 6–block 7)	− 32.51	− 22.17	− 10.34	− 3.25	1.90	− 5.15
Saving (block 1–block 9)	104.51	94.88	9.63	− 9.31	− 8.44	− 0.87
Interference (block 8–block 9)	− 23.71	− 28.79	5.08	7.53	9.15	− 1.62
Recovery (block 9–block 10)	42.12	36.13	5.99	− 6.52	− 11.12	4.60

The Interaction difference scores reflect the difference of the mean RT or CA Night and Day difference scores (i.e., Night difference score minus Day difference score) of the relevant difference scores of the dependent measure.

Offline (change in RT before block 6—and after a 12 h offline phase—block 7) As shown in Fig. 5, comparing block 6 (last learning block) with block 7 (first block after 12-h offline) revealed that RTs were significantly increased after a 12-h interval, $F(1, 35) = 17.20$, $p < 0.001$, $\eta_p^2 = 0.33$. The Night group was significantly slower overall compared to the Day group, $F(1, 35) = 7.73$, $p < 0.01$, $\eta_p^2 = 0.18$. The interaction was not significant, $F(1, 35) = 0.62$, $p = 0.44$, $\eta_p^2 = 0.02$. Contrary to our prediction, both Day and Night groups demonstrated higher RTs to the same extent following a 12-h offline interval.

Saving (change in RT between baseline performance—block 1 and performance following offline—block 7) The main effect of Saving reached significance, $F(1, 35) = 63.43$, $p < 0.001$, $\eta_p^2 = 0.64$. As can be seen in Fig. 5, overall RT in block 1 was slower than in block 7. Thus, although performance was slower after offline, it did not return to baseline performance. Group main effect was significant, $F(1, 35) = 5.49$, $p < 0.05$, $\eta_p^2 = 0.14$. Overall, the Night group was slower than the Day group. Group by Saving interaction was not significant, $F(1, 35) = 0.15$,

$p = 0.70$, $\eta_p^2 = 0.004$, indicating that both groups showed saving of the learning to the same extent.

Interference (change in RT from presentation of the original sequence—block 8 to new sequence—block 9) Interference effect reached significance, i.e., RT in block 8 was faster than in block 9, $F(1, 35) = 42.85$, $p < 0.001$, $\eta_p^2 = 0.55$. Main effect for Group was marginally significant, $F(1, 35) = 3.03$, $p = 0.09$, $\eta_p^2 = 0.08$., and the interaction did not reach significance, $F(1, 35) = 0.40$, $p = 0.53$, $\eta_p^2 = 0.01$. These results indicate that although the Night group tended to be slower than the Day group, Interference affected both groups equally, so that both groups demonstrated higher RTs when a different sequence was presented (see Fig. 5).

Recovery (change in RT from the new sequence—block 9 and re-presentation of the original sequence—block 10) Recovery effect reached significance, $F(1, 35) = 44.24$, $p < 0.001$, $\eta_p^2 = 0.56$. As can be seen in Fig. 5, overall performance time in block 9 was slower than in block 10. Group main effect was not significant, $F(1, 35) = 2.21$, $p = 0.15$, $\eta_p^2 = 0.06$. Group by Recovery interaction was not significant either, $F(1, 35) = 0.26$, $p = 0.61$, $\eta_p^2 = 0.01$. The results show that both groups recovered similarly from interference, meaning RTs in both groups decreased when the original sequence was represented.

Correct anticipations—number of times the eyes move towards the next correct position during the 500-ms interval between targets (reflects accuracy)

See Fig. 6 that presents the average percentage of correct anticipations as a function of the MA O-SRT blocks 1–10 for both groups.

Learning (change in correct anticipations across the six learning blocks) Learning main effect reached significance, $F(3.60, 126.15) = 11.62$, $p < 0.001$, $\eta_p^2 = 0.25$, reflecting a significant increase in number of correct anticipations from block 1 to 6. Neither main effect for Group, $F(1, 35) = 0.01$, $p = 0.94$, $\eta_p^2 = 0.0001$, nor Group by Learning interaction were significant, $F(3.60, 126.15) = 0.84$, $p = 0.52$, $\eta_p^2 = 0.02$. Overall, these results (see Fig. 6) indicate that as the session progressed, both groups showed a similar increase in the percentage of correct anticipations of the next target location.

Offline (change in correct anticipations before—block 6—and after a 12 h offline phase—block 7) In this comparison,

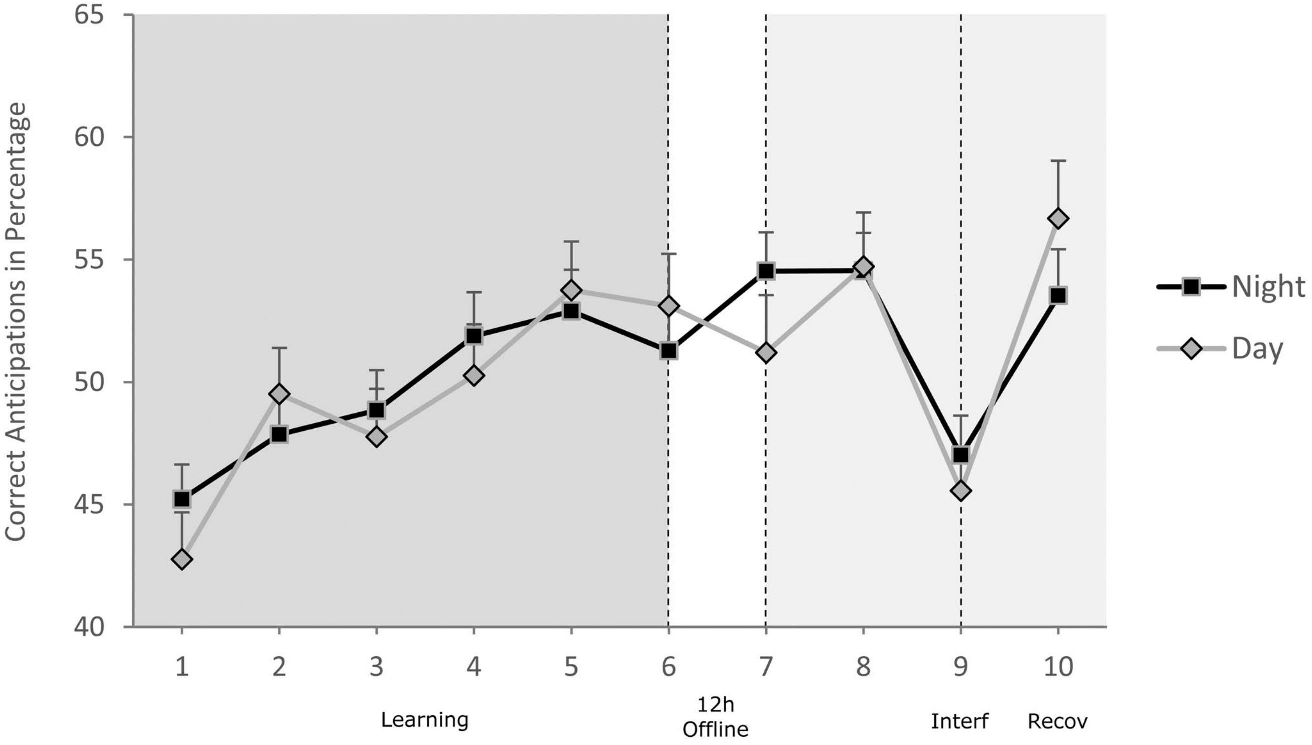

Fig. 6 The mean percentage of correct anticipations for the Day and Night groups in Experiment 1 (MA O-SRT). *Interf* interference, *Recov* recovery

neither Offline main effect, $F(1, 35) = 0.32$, $p = 0.58$, $\eta_{p}^{2} = 0.009$ nor Group main effect were significant, $F(1, 35) = 0.09$, $p = 0.77$, $\eta_{p}^{2} = 0.002$, respectively. However, Group by Offline interaction did reach significance, $F(1, 35) = 4.70$, $p \leq 0.05$, $\eta_{p}^{2} = 0.12$. As can be seen in Fig. 7, the 12-h offline affected the groups differently. While the overnight offline resulted in improved performance (i.e., offline learning block 7 > block 6) expressed in a higher rate of correct anticipations, the offline during the day resulted in decreased performance (block 7 < block 6), i.e., a lower rate of correct anticipations.

Saving (change in correct anticipations between baseline performance—block 1 and performance following offline—block 7) The main effect of Saving reached significance, $F(1, 35) = 22.30$, $p \leq 0.001$, $\eta_{p}^{2} = 0.39$. Overall percent of correct anticipations in block 1 was lower than in block 7. Neither Group main effect nor Group by Saving interaction reached significance, $F(1, 35) = 2.41$, $p = 0.13$, $\eta_{p}^{2} = 0.06$, $F(1, 35) = 0.05$, $p = 0.82$, $\eta_{p}{}^{2} = 0.002$, respectively. As can be seen in Fig. 6, both groups showed the same amount of saving.

Interference (change in correct anticipations from presentation of the original sequence—block 8 to new sequence—block 9) Interference main effect was significant, $F(1, 35) = 46.61$, $p < 0.001$, $\eta_{p}^{2} = 0.57$, where the number of correct anticipations was lower in block 9 compared to block 8. Group main effect and Group by Interference interaction were not significant, $F(1, 35) = 0.08$, $p = 0.78$, $\eta_{p}^{2} = 0.002$, $F(1, 35) = 0.44$, $p = 0.51$, $\eta_{p}^{2} = 0.01$, respectively. As shown in Fig. 6, both groups were similarly affected by the presentation of a new sequence, as expressed by lower percentages of correct anticipations when ‘sequence B’ were presented.

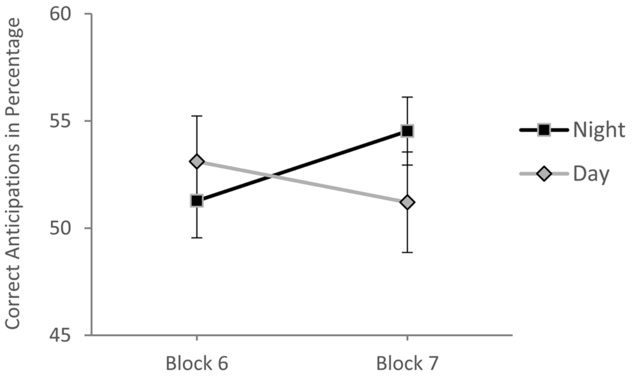

Fig. 7 The mean percentage of correct anticipations for the Day and Night groups in Experiment 1 (MA O-SRT) of the Offline effect, comparing block 6 with block 7

Recovery (change in correct anticipations from the new sequence—block 9 and re-presentation of the original sequence—block 10) Recovery main effect reached significance, $F(1, 35) = 46.29$, $p < 0.001$, $\eta_{p}^{2} = 0.57$, as reflected by the higher number of correct anticipations in block 10 compared to block 9. Group main effect did not reach significance, $F(1, 35) = 0.12$, $p = 0.73$, $\eta_{p}^{2} = 0.004$. Group by Recovery interaction was marginally significant, $F(1, 35) = 3.15$, $p = 0.084$, $\eta_{p}^{2} = 0.08$. As can be seen in Fig. 6, the percentage of correct anticipations was higher in block 10 than in block 9 in both groups, but tended to be more pronounced for the Day group.

Discussion

This experiment attempted to study consolidation of implicit sequence learning and the role of sleep in this process when using the MA version of the O-SRT (Vakil et al., 2017). Two types of intervals were used: with sleep (Night group) and without sleep (Day group). As hypothesized, the results showed that MA RT and correct anticipation rates improved over the course of the learning session. Moreover, the results of correct anticipation rates coincide with the results reported by Vakil et al. (2017), confirming the hypothesis made by Marcus et al. (2006) that repeated training leads to more accurate anticipation of the next position, thus resulting in faster RT.

As hypothesized, sequence-specific learning was also demonstrated through performance in the interference block. Longer MA RTs and lower correct anticipation rates were measured when participants were presented with a new sequence in the ninth block. Eye movement measurements enable refinement of the sequence-specific learning component, thus enabling us to claim that both components of learning did indeed take place in the experiment. As predicted, the subsequent block (block 10) which consisted of the original sequence, produced a decrease in MA RTs and higher anticipation rates, compared to the interference block (block 9). Thus, both measures showed a similar picture in terms of the Learning, Interference and Recovery independent variables.

With regard to the offline effect, we did not expect improvement in performance following the 12-h delay period (block 6 vs. block 7), but we did expect consolidation-based stabilization. Contrary to our prediction, when MA RT was measured, performance declined in both groups following the offline period. MA RT was higher on block 7 after the offline period, compared to block 6 before the offline period. Nevertheless, although performance was slower after the offline period, it did not return to baseline performance (as evaluated in Saving, block 1 > block 7).

However, when measuring correct anticipations, we found a differential offline effect on the groups. As with the MA RT results, the Day group showed a decline in performance, specifically, a decrease in correct anticipation rate following the offline period (block 6 > block 7). By contrast, the Night group showed offline learning, i.e., an increase in the percent of correct anticipations rate after the offline period (block 6 < block 7). We associate these findings with dual-learning processes, which likely occurred when performing on the MA O-SRT. This is because participants, besides learning via manual coding, also learn via oculomotor/perceptual coding. Consolidation processes of sequence specific information overnight may lead to increased performance when this information is dual coded rather than single coded. In contrast, the higher RT after offline may be related to a slight decrease in general skill learning. Our interpretation is rather speculative, and future research is needed to test this hypothesis.

In summary, MA RT and anticipations measures showed the effects of Learning, Saving, Interference and Recovery. None of the interactions was significant for any of the measures, indicating that the Day and Night groups showed a similar pattern of results. The two measures differed, because MA RT showed a decline in performance following the offline period for both groups, while the correct anticipations measure showed a differential effect on the groups.

Experiment 2—OA O-SRT

Method

Participants

The same procedure of recruitment and assignment to the OA Day group or OA Night group was followed as in Experiment 1. The OA Day group consisted of 18 young adults (9 males, mean age 29.8, range 21–36 years) and the OA Night group consisted of 19 young adults (10 males, mean age 28.8, range 18–38 years). Participants were undergraduate or graduate students and either volunteered for the study or took part in the experiment to fulfill academic requirements. The study received prior approval from the Ethics Committee of the Psychology Department at Bar-Ilan University, and each participant signed an informed consent form.

Tools, stimuli and procedure

In this experiment the OA version of the O-SRT was used, which was a replication of the task described in the study by Vakil et al. (2017). The stimuli used and the general task design were identical with the MA O-SRT task of Experiment 1, yet with the key difference that instead of key presses, participants responded with eye fixations on target. The slide containing the stimulus remained visible until the correct location was fixated for a minimum of 100 ms; otherwise, it was displayed for 1000 ms.

Data analysis

Data were registered using BeGaze™ (SensoMotoric Instruments, Teltow, Germany) to generate eye movement measures. As in Experiment 1, two dependent measures were used: speed (i.e., RT) and accuracy (i.e., correct anticipations). Correct anticipations were computed in the same way as in Experiment 1. For the speed measure we used the BeGaze™ generated parameter Entry Time (i.e., elapsed time until first fixation on AOI) of the correct square in which the target appeared. As in Experiment 1, we computed the median RT for each 12-item sequence (i.e., for each 12 target trials). Then, mean of medians of RT per block (i.e., 9 sequences of 12-items each; 108 trials) was computed. As in Experiment 1 Mixed-design ANOVA was used to test the between subjects factor of Group (Day vs. Night) and each one of the above comparisons (Learning, Offline, Saving, Interference and Recovery) as independent measures within subjects factors.

Furthermore, in *Baseline* we evaluated the difference between the MA RT and OA RT groups in the baseline performance on the first block of Sequence A (block 1) and the first block of Sequence B (block 9, interference block). For this purpose we collapsed the Day and Night group of each O-SRT version (MA, OA) to one group and performed a 2 × 2 Mixed-design ANOVA to test the between-subjects factor of Group (MA vs. OA) and the within-subjects factor Baseline (block 1 vs block 9).

Results

Reaction time–time elapses from stimulus presentation to ocular response—100 ms on target (reflects speed)

See Fig. 8 that presents the mean of median OA RT as a function of OA O-SRT blocks 1–10 for both groups**.** See also Table 2 that presents the analyzed difference scores.

Learning (change in RT across the six learning blocks) Analysis of the mean of median RT of the two groups in the first six blocks (see Fig. 8, showed significant reduction in RT over blocks 1–6 for both groups, $F(3.07,\ 101.26) = 13.60$ $p < 0.001$, $\eta_p^2 = 0.29$. Neither the main effect of Group $F(1,\ 33) = 2.60$, $p = 0.12$, $\eta_p^2 = 0.07$, nor Group by Learning interaction were significant, $F(3.07,\ 101.26) = 0.91$, $p = 0.48$, $\eta_p^2 = 0.03$. These results indicate that participants from both groups improved at a similar rate, as reflected by decreasing

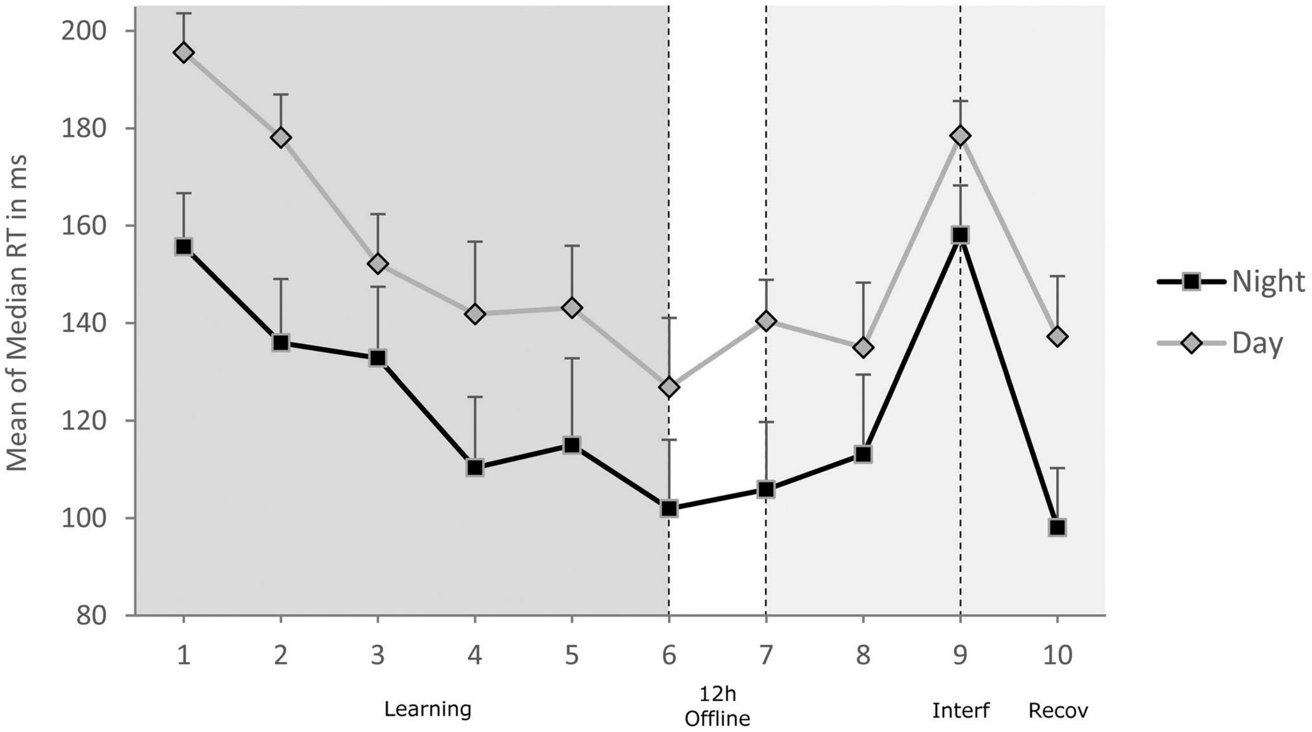

Fig. 8 The mean of the median ocular reaction time (RT) of the Day and the Night groups of Experiment 1 (OA O-SRT). *Interf* interference, *Recov* recovery

Table 2 Mean RT and CA difference scores and interaction effect difference scores of 2 × 2 ANOVAs of Experiment 2

	RT difference scores			CA difference scores		
Dependent measures	Night	Day	Inter-action	Night	Day	Inter-action
Offline (block 6–block 7)	– 3.95	– 13.54	9.50	– 0.19	1.27	– 1.46
Saving block 1–block 9)	45.54	56.04	– 10.50	– 6.05	– 11.00	4.95
Interference (block 8–block 9)	– 45.02	– 43.50	– 1.52	12.25	6.66	5.59
Recovery (block 9–block 10)	60.04	41.27	18.77	– 15.77	– 10.07	– 0.5.70

The Interaction difference scores reflect the difference of the mean RT or CA Night and Day difference scores (i.e., Night difference score minus Day difference score) of the relevant difference scores of the dependent measure.

RT across the first six blocks that comprised the learning session.

Offline (change in RT before—block 6—and after a 12 h offline phase—block 7) Offline main effect did not reach significance, $F(1, 35) = 1.30$, $p = 0.26$, $\eta_p^2 = 0.04$, i.e., overall RT was not significantly different before (block 6) or after the 12-h offline (block 7). Group main effect was marginally significant, $F(1, 35) = 3.20$, $p = 0.08$, $\eta_p^2 = 0.08$, where the Night group tended to be faster overall than the Day group. The interaction between Offline and Group did not reach significance, $F(1, 35) = 0.38$, $p = 0.54$, $\eta_p^2 = 0.01$ (see Fig. 8). Thus, these results did not point to an improvement of performance after a sleep or time interval. At the same time, the results did indicate that both groups could maintain their level of performance.

Saving (change in RT between baseline performance—block 1 and performance following offline—block 7) The main effects of Saving and Group reached significance, $F(1, 33) = 28.54$, $p < 0.001$, $\eta_p^2 = 0.46$, $F(1, 33) = 8.31$, $p = 0.01$, $\eta_p^2 = 0.20$, respectively. As can be seen in Fig. 8, overall RT in block 1 was slower than in block 7. In addition, the RT of the Night group was faster overall than that of the Day group. Group by Saving interaction was not significant, $F(1, 33) = 0.31$, $p = 0.59$, $\eta_p^2 = 0.01$, indicating that both groups showed saving to the same extent.

Interference (change in RT from presentation of the original sequence—block 8 to new sequence—block 9) Interference effect reached significance, $F(1, 35) = 19.11$, $p < 0.001$, $\eta_p^2 = 0.36$. Main effect for Group and Group by Interference interaction did not reach significance, $F(1, 35) = 2.24$, $p = 0.14$, $\eta_p^2 = 0.06$, $F(1, 35) = 0.01$, $p = 0.94$, $\eta_p^2 = 0.0001$, respectively. These results indicate that the interference affected both groups equally, so that both groups demonstrated higher RTs for a new sequence (block 9), compared to the RT for the original sequence (block 8), see Fig. 8.

Recovery (change in RT from the new sequence—block 9 and re-presentation of the original sequence—block 10) Recovery effect as well as Group main effect reached significance, $F(1, 35) = 32.91$, $p < 0.001$, $\eta_p^2 = 0.49$, $F(1, 35) = 5.82$, $p < 0.05$, $\eta_p^2 = 0.14$, respectively. As can be seen in Fig. 8, the overall performance time in block 9 was slower than in block 10, and the Night group was faster than the Day group. Group by Recovery interaction was not significant, $F(1, 35) = 1.13$, $p = 0.30$ $\eta_p^2 = 0.03$. The results showed that both groups recovered from interference, meaning that RTs in both groups were faster when the original sequence was presented again.

Correct anticipations—number of times the eyes move towards the next correct position during the 500-ms interval between targets (reflects accuracy)

Participants' gaze moved to only one location in most (77.2%) of the trials. In 22.3% of the trials, their gaze remained in the same location, and only in a negligible percent of trials (0.5%) did participants' gaze switch to more than one location. These numbers are similar to those in Experiment 1 (MA O-SRT).

See Fig. 9 that presents the average percentage of correct anticipations as a function of the OA O-SRT blocks 1–10 for both groups**.**

Learning (change in correct anticipations across the six learning blocks) Main effect for Learning was significant, $F(3.14, 103.46) = 7.59$, $p < 0.001$, $\eta_p^2 = 0.19$. Main effect for Group was marginally significant, $F(1, 33) = 3.39$, $p = 0.08$, $\eta_p^2 = 0.09$. The Group by Learning interaction did not reach significance, $F(3.14, 103.46) = 1.41$, $p = 0.23$, $\eta_p^2 = 0.04$. Thus, as can be seen in Fig. 9, both groups showed the same extent of increase in the correct anticipation rate across the

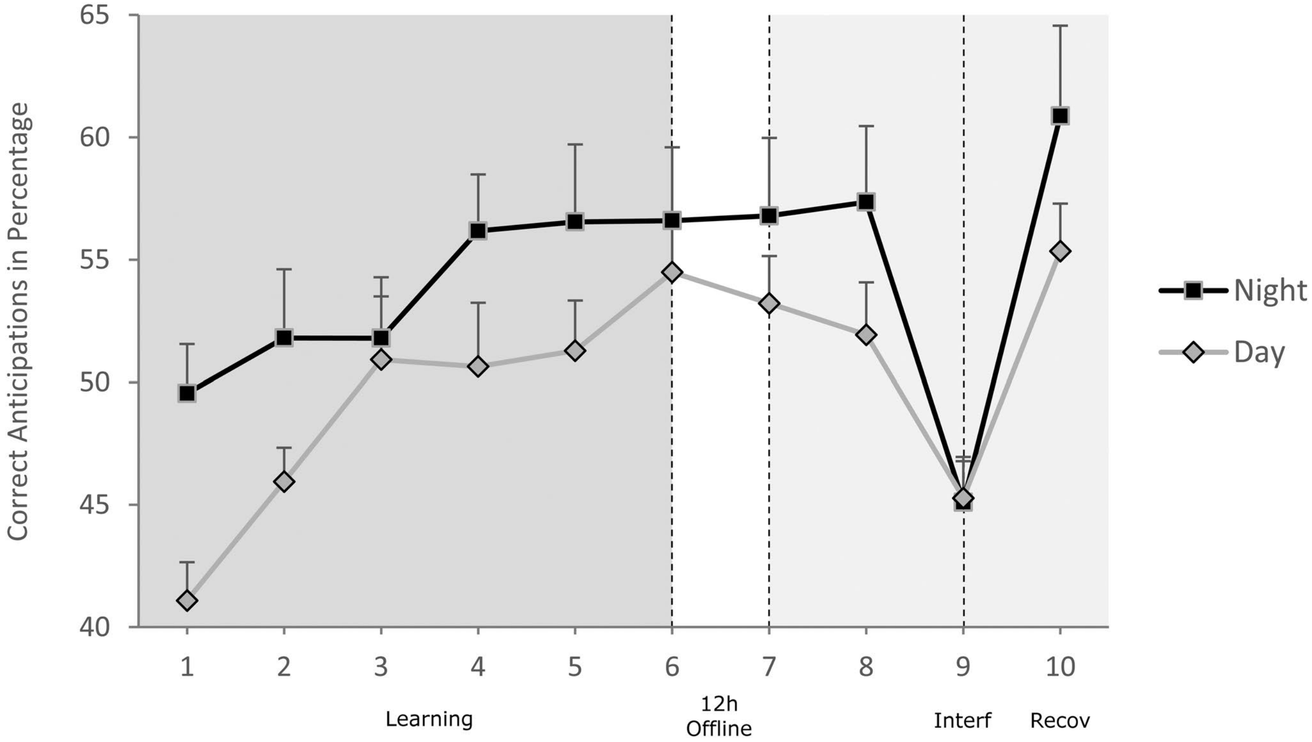

Fig. 9 The mean percentage of correct anticipations for the Day and Night groups in Experiment 2 (OA O-SRT). *Interf* interference, *Recov* recovery

six learning trials. Overall, the Night group tended to have a higher rate of correct anticipations than the Day group.

Offline (change in correct anticipations before—block 6—and after a 12 h offline phase—block 7) None of the effects reached significance; Offline, $F(1, 35) = 0.43$, $p = 0.52$, $\eta_p^2 = 0.01$, Group, $F(1, 35) = 0.61$, $p = 0.44$, $\eta_p^2 = 0.02$, Group by Offline interaction, $F(1, 35) = 0.79$, $p = 0.38$, $\eta_p^2 = 0.02$. As shown in Fig. 9, the percentage of correct anticipations was similar before (block 6) and after the 12-h offline interval (block 7) for both groups.

Saving (change in correct anticipations between baseline performance—block 1 and performance following offline—block 7) Saving main effect, $F(1, 33) = 13.15$, $p < 0.001$, $\eta_p^2 = 0.29$, as well as Group main effect $F(1, 33) = 8.5$, $p \leq 0.01$, $\eta_p^2 = 0.20$ were both significant. As can be seen in Fig. 9, overall percent of correct anticipations in block 1 was lower than in block 7. Overall, the Night group had a higher correct anticipations rate than the Day group. Group by Saving interaction did not reach significance, $F(1, 33) = 1.11$, $p = 0.30$, $\eta_p^2 = 0.03$, indicating that both groups demonstrated similar amounts of saving.

Interference (change in correct anticipations from presentation of the original sequence—block 8 to new sequence—block 9 The main effect of Interference was significant, $F(1, 35) = 19.87$, $p < 0.001$, $\eta_p^2 = 0.36$., as reflected by the decrease in overall amount of correct responses when the new sequence was presented (block 9), compared to the previous block, see Fig. 9. Neither the Group main effect, $F(1, 35) = 1.23$, $p = 0.28$, $\eta_p^2 = 0.03$, nor the Group by Interference Interaction reached significance, $F(1, 35) = 1.74$, $p = 0.20$, $\eta_p^2 = 0.05$.

Recovery (change in correct anticipations from the new sequence—block 9 and re-presentation of the original sequence—block 10) Recovery effect reached significance, $F(1, 35) = 35.69$, $p < 0.001$, $\eta_p^2 = 0.51$. Neither Group main effect, $F(1, 35) = 1.00$, $p = 0.32$, $\eta_p^2 = 0.03$, nor Group by Recovery interaction reached significance, $F(1, 35) = 1.73$, $p = 0.20$, $\eta_p^2 = 0.05$, respectively. As can be seen in Fig. 9, the percentage of correct anticipations for both groups was higher in block 10 than in block 9.

Baseline reaction time: Experiment 1 (MA O-SRT) vs. Experiment 2 (OA O-SRT)

To compare RT baseline performance on sequence A (block 1) versus baseline performance on sequence B (block 9) of MA and OA O-SRT (Day and Night combined), we conducted a mixed design ANOVA with repeated measures: Group (MA vs. OA) as a between subjects factor, and baseline (block 1 vs. block 9) as a within subjects factor.

All effects were significant: the main effect of Baseline, $F(1, 70) = 39.39$, $p < 0.001$, $\eta_p^2 = 0.36$, Group by Baseline interaction, $F(1, 70) = 28.85$, $p < 0.001$, $\eta_p^2 = 0.29$, and the main effect of Group, $F(1, 70) = 353.47$, $p < 0.001$, $\eta_p^2 = 0.84$. Follow up analyses conducting paired sample *t*-tests separately per group of RT block 1 vs RT block 9 revealed that this comparison was significant in the MA group (block 1: $M = 449.1$, $\mathrm{SD} = 94.1$, block 9: $M = 355.3$, $\mathrm{SD} = 53.6$), $t(36) = 6.77$, $p < 0.001$), but not in the OA group (block 1: $M = 175.1$, $\mathrm{SD} = 44.9$, block 9: $M = 167.8$, $\mathrm{SD} = 39.0$), $t(34) = 0.94$, $p = 0.35$). These results indicate that only in the MA group was baseline RT significantly lower in sequence B (block 9) compared to baseline RT of sequence A (block 1). Furthermore, the MA had higher RTs overall (see Fig. 10). To test whether there was no meaningful difference between block 1 and block 9 of the OA version, we conducted the two one-sided tests (TOST) procedure to test equivalence (Lakens et al., 2018). Based on the mean difference between the first blocks of a previous study using the OA O-SRT (Vakil et al., 2017), we set the equivalence bounds to ± 16 ms. The TOST procedure (based on paired sample *t*-tests) revealed that equivalence did not reach significance, $t(34) = 1.136$, $p = 0.132$. This result needs to be viewed with caution, since statistical power for this analysis was low (e.g., given a power of 0.8 and an alpha level of 0.05 at least 68 participants were needed). When all results are taken into account, it is evident that RT Baseline differed between the MA and OA versions. Whereas in MA the RT

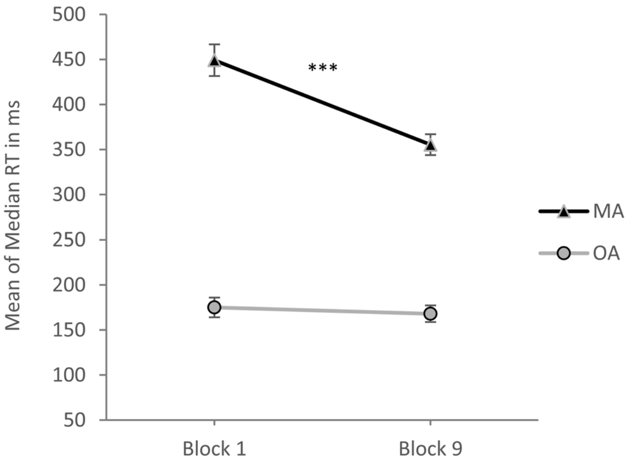

Fig. 10 The mean of median RT for the MA O-SRT (Experiment 1) and OA O-SRT (Experiment 2) groups) of Baseline comparing block 1 with block 9

of block 9 was significantly lower compared to block 1, this difference did not deviate significantly in OA. However, based on the equivalence tests' results, we cannot assert that there was no meaningful difference. Thus, the only claim we can make based on the interaction reflected in Fig. 10, is that the difference between baselines of sequence A and sequence B (block 1 vs block 9) is greater under the MA condition than under the OA condition.

Discussion

As in the previous experiment, this experiment examined the role of sleep in consolidation of implicit skill learning, in order to shed light on the underlying cognitive processes that enable implicit sequence learning. This experiment used the OA version of the O-SRT task, introduced by Vakil et al. (2017). One of the major advantages of the OA O-SRT version is the absence of manual responses while performing the task, making it possible to differentiate between general manual learning (i.e., S-R mapping) and specific sequence learning. In addition to measuring ocular RT, the use of an eye tracker allows for providing a measure of correct anticipation of the subsequent target.

Consistent with previous reports that implicit sequence learning occurred without the involvement of manual responses (Albouy et al., 2006; Marcus et al., 2006; Song et al., 2008), we hypothesized that sequence learning would occur during the OA O-SRT task. We assumed that this would be reflected by a gradual decrease in RTs and an increase in correct anticipations rates in both groups throughout the learning sessions. As expected, we found that OA RTs decreased significantly along the first six blocks of the learning session, and correct anticipations gradually increased in both groups. The learning pattern that was identified also resembled the learning patterns found in traditional MA SRT tasks (Schwarb & Schumacher, 2012), proving that implicit sequence learning is not dependent on manual responses during the learning process. This is consistent with previous studies using verbal responses or other methods that demonstrated sequence learning to be independent of sequence motor responses (Kühnel et al., 2019; Schwizer Ashkenazi, et al., 2020; Vakil et al., 2000; Zirngibl, & Koch, 2002). The learning rate of both groups was similar, whether measured with RT or correct anticipations.

Previous studies have claimed that implicit sequence learning that occurs in MA versions of SRT involves two components: specific sequence learning and general motor learning (Csabi et al., 2014; Nemeth et al., 2010; Vakil et al., 2017), which are thought to be represented together in RT. However, the OA version of the O-SRT task is thought to reflect implicit sequence learning more purely without the general manual aspect of learning, since there is no manual response, so that there is no S-R learning in this version. We therefore expected higher RTs and less correct anticipations in the interference block in both groups; lower RTs and higher percentages of correct anticipations after the interference block when the familiar sequence is reintroduced; and RTs and correct anticipations rates in the interference block that approach baseline performance. As expected, we found a pattern of decreased performance (higher RTs and lower percentage of correct anticipations) in both groups, during the interference block (block 9) compared to block 8. In addition, and as expected, participants in both groups showed a pattern of improved performance (lower RTs and higher percentage of correct anticipations) in block 10. This represents recovery from interference (Vakil et al., 2002), and further reinforces the presence of implicit sequence learning. In addition, as predicted, we found that in OA RT the group (i.e., Baseline) returned nearly to baseline level performance during the interference block. This differs from our findings with the MA RT and previous findings using the MA SRT, in which performance (as measured using RT) decreased during the interference block, but was still significantly better than baseline (Savic & Meier, 2016). This is interpreted as evidence of a general motor component (i.e., S-R) that was learned in addition to the specific sequence and produced better than baseline performance (Vakil et al., 2017). The observation that in this experiment OA RT returned nearly to baseline during the interference block, coincides with the notion that OA RT reflects implicit sequence learning more purely than the standard MA RT, because it is dependent on eye movements and not on a manual response.

As in Experiment 1, we did not expect improvement in performance following the offline period (block 6 vs. block 7), but we did expect consolidation-based stabilization. As expected, both OA RT and correct anticipations measures showed stable performance before, versus after, the 12-h offline for both Day and Night groups. Thus, consolidation of implicit sequence learning as measured with the OA O-SRT is time- rather than sleep-dependent.

In summary, as in Experiment 1, RT and correct anticipations measures showed the effects of Learning, Saving, Interference and Recovery. Furthermore, as expected, neither RT nor correct anticipations measures before and after the offline period differed, indicating consolidation-based stabilization. None of the interactions reached significance, indicating that the Day and Night groups showed a similar pattern of results, thus suggesting that learning and consolidation are time-dependent and not sleep-dependent.

General discussion

Although there is a continuous increase of interest in the consolidation process of sequence learning and the role of sleep, findings are still mixed. Some researchers have pointed to the fact that several factors have an effect on the results, such as awareness and length of the offline period (Janacsek & Nemeth, 2012). Others have referred to the methodological pitfalls that contribute to the inconsistent findings (Nemeth et al., 2019). We conducted two experiments in order to address several controversial issues related to consolidation of implicit sequence learning. First, is consolidation sleep- or just time-dependent? To address this question, we tested two groups in each experiment: a Day group and a Night group. We tested each group twice 12-h apart, the Day group in the morning and evening and the night group in the evening and the following morning. The second question was, is consolidation dependent on manual activation of the sequence? To address this question, we applied in Experiment 1 the MA version of the O-SRT and the OA version of the O-SRT in Experiment 2. In addition, we used two dependent measures of the test: one was RT and the other was correct anticipations. These reflect two expressions of sequence learning, where the former is dependent on speed and the latter on accuracy. Based on previous studies (Krakauer & Shadmehr, 2006; Nemeth et al., 2010; Robertson, 2009; Song, 2009) we hypothesized that in implicit sequence learning following 12-h offline we would find consolidation-based stabilization, but not necessarily improved performance.

Several findings emerged consistently. In both experiments, RT and correct anticipations measures showed the effects on four variables, Learning, Saving, Interference and Recovery. In the latter four variables, for both measures none of the interactions reached significance, indicating that the Day and Night groups showed a similar pattern of results. With regard to the offline effect, our hypothesis of consolidation-based stabilization was confirmed in Experiment 2, regardless of whether OA RT or correct anticipations were used. Our findings in Experiment 1 were more complicated. Contrary to our prediction, when MA RT was used, both groups showed an overall higher MA RT following the offline period (block 7) compared to before the offline period (block 6); although performance was still better than baseline (i.e., Saving), learning was stabilized to some extent. The other interesting finding that emerged in the first experiment using the correct anticipations measure, was that the interaction between Group and Offline was significant. While the Night group showed offline learning as reflected in a higher rate of correct anticipations after the offline period (block 7) than before the offline period (block 6), the Day group showed the opposite pattern: a decreased rate of correct anticipations after the offline period (block 7) compared to before the offline period (block 6). This too, was not predicted, and requires further research to validate these findings. One possible, although speculative, explanation for the Night group's offline learning, is that this condition is unique, because under this condition the sequence is learned via dual coding. It is performed manually, and in addition participants are asked to follow the sequence with their eyes, so that it is learned via the oculomotor system as well. It is possible then, that these optimal learning conditions (i.e., for the Night group) enabled offline learning of this unique procedure. Further research is required to test this hypothesis.

The notion that sleep leads to increased general skill but not sequence specific learning, as suggested in previous studies (Csabi et al., 2014; Meier & Cock, 2014; Nemeth et al., 2010) was not supported by our findings. The finding that sequence-specific learning increased after offline in the night MA O-SRT group (i.e., higher correct anticipations rate) stands in contrast to this assumption. However, this finding may indicate that implicit sequence learning consolidation is task-dependent, as has been previously suggested (Nemeth et al., 2010). This task dependency may further be associated with differences in the type (e.g., manual, oculomotor, auditive, etc.) of information and number of different types (e.g., single coding, dual coding, triple coding, etc.) of information learned during implicit sequence learning.

In summary, there are two major advantages of the O-SRT task (Vakil et al., 2017) used in the present study that enabled us to directly address several controversial issues in the field. One advantage is the possibility of conducting two versions of the task, thereby comparing MA with OA. This allowed testing of whether the manual component is necessary for consolidation to occur. Our results demonstrate that the manual component per se is not an essential factor, since in the OA SRT consolidation occurred in the form of stabilization. Testing speed in the MA O-SRT consolidation also demonstrated a form of stabilization. In terms of accuracy, sleep led to a post-offline improvement which we associated with dual learning. Thus, the manual component alone does not seem superior to other components, in our case the oculomotor component. Furthermore, as explained above, while the standard MA version, in addition to sequence learning, involves general learning of S-R mapping, the OA version is considered to provide purer measures of sequence learning. This assertion was confirmed again in the present study in the findings of Experiment 1 (MA O-SRT), that upon presentation of the interference sequence (sequence B, block 9) speed performance did not revert back to baseline (sequence A block 1). This indicates that despite the change of sequence, the general aspect of learning (i.e., S-R mapping) was carried over to the new sequence. In contrast, when the OA version was used, performance of the interference sequence was similar to baseline performance in the first sequence. Therefore, the fact that the findings of both versions (i.e., MA and OA) were similar, clearly indicates that learning and consolidation are not dependent on the manual component or the general learning aspect of implicit sequence learning as claimed by some researchers (Csabi et al., 2014; Meier & Cock, 2014; Nemeth et al., 2010).

The second advantage in using the O-SRT task is that it enables the generation of two measures of implicit sequence learning: RT as a measure of speed and correct anticipations reflecting accuracy. The fact that in most of our findings RT and correct anticipations yielded similar results strengthens the reliability of the findings. On the other hand, the O-SRT measures are sensitive to demonstrating differential aspects of implicit sequence learning. This was the case in Experiment 1, where the offline phase had a differential effect on RT and on correct anticipations measures (i.e., Offline, block 6 vs. block 7). The results based on RT showed that following a 12-h offline interval, Day and Night groups demonstrated higher RTs to the same extent. However, when correct anticipations were analyzed, the 12-h offline affected the groups differently. While the Night group showed offline learning expressed by an increase in correct anticipations, the Day group showed a decrease in correct anticipations. These results show that although RT and correct anticipations usually yield similar findings, nevertheless in some situations these measures generate different results, indicating that they are not redundant. These results suggest that under the MA condition accuracy, but not speed, reflects an offline improvement. Further research is required to clarify the effect of training on speed versus accuracy.

Limitations and future directions

Nemeth et al. (Preprints 2019) listed four methodological pitfalls with respect to experimental designs frequently found in studies on the effect of sleep on memory. We would like to point out the limitations of our study in light of this paper. First, an additional Day quiet rest condition control group would have helped in determining whether sleep in particular is a necessary condition for offline effect, or whether a quiet period limiting interferences to the learned information would be as effective. In the current paradigm with an active Day group we cannot definitely exclude the possibility that under a quiet rest condition, the same results would have occurred as in the sleep condition. A second possible pitfall mentioned by Nemeth et al. (Preprints 2019) is the need to control for the time of the day on performance. In other words, weaker performance in the evening as compared to the morning in the Night group may be due to fatigue, rather than the effect of sleep. A solution offered by the researchers is to add two control groups with a 24-h delay between sessions, where one is PM-PM and the other is AM-AM, so that the testing time is matched, which has not been done in our study. One aspect that may argue against the possibility that fatigue effects took place in the learning sessions of our Night groups, is the absence of group main effects of the correct anticipations measure (i.e., accuracy). Further limitations of our study are the lack of systematically assessed sleep quality and failure to record the time elapsed between the learning session the actual bed time (i.e., for the Night groups) and activity data (i.e., for the Day groups). Moreover, our sample size was rather small, and this may have led to overlooking additional effects. In future research, larger sample sizes should be tested and standardized sleeping questionnaires and/or sleep detectors may be used to measure sleep quality. Moreover, it may be interesting to evaluate whether a more extensive learning protocol might have a differential outcome on consolidation.

Final conclusions

This study demonstrates that not only the nature of the skill-learning task (i.e., manual and/or oculomotor performance) may reveal different consolidation processes, but also task-generated measures (i.e., RT and correct anticipations) may show different aspects of implicit sequence learning consolidation. In sum, our results indicate that implicit sequence learning and its consolidation is not dependent on the manual component or the general learning aspect. Furthermore, time rather than sleep is the essential factor in consolidation. Our study should be viewed as preliminary research, using a powerful procedure that measures implicit sequence learning in several ways, enabling isolation of various aspects of the task (i.e., with and without the manual component of learning, more or less general skill learning) known to affect learning and consolidation.

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Declarations

Conflicts of interest/competing interests The authors report no conflicts of interest or competing interests.

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