ONLINE AND OFFLINE MODULATORS OF MOTOR LEARNING

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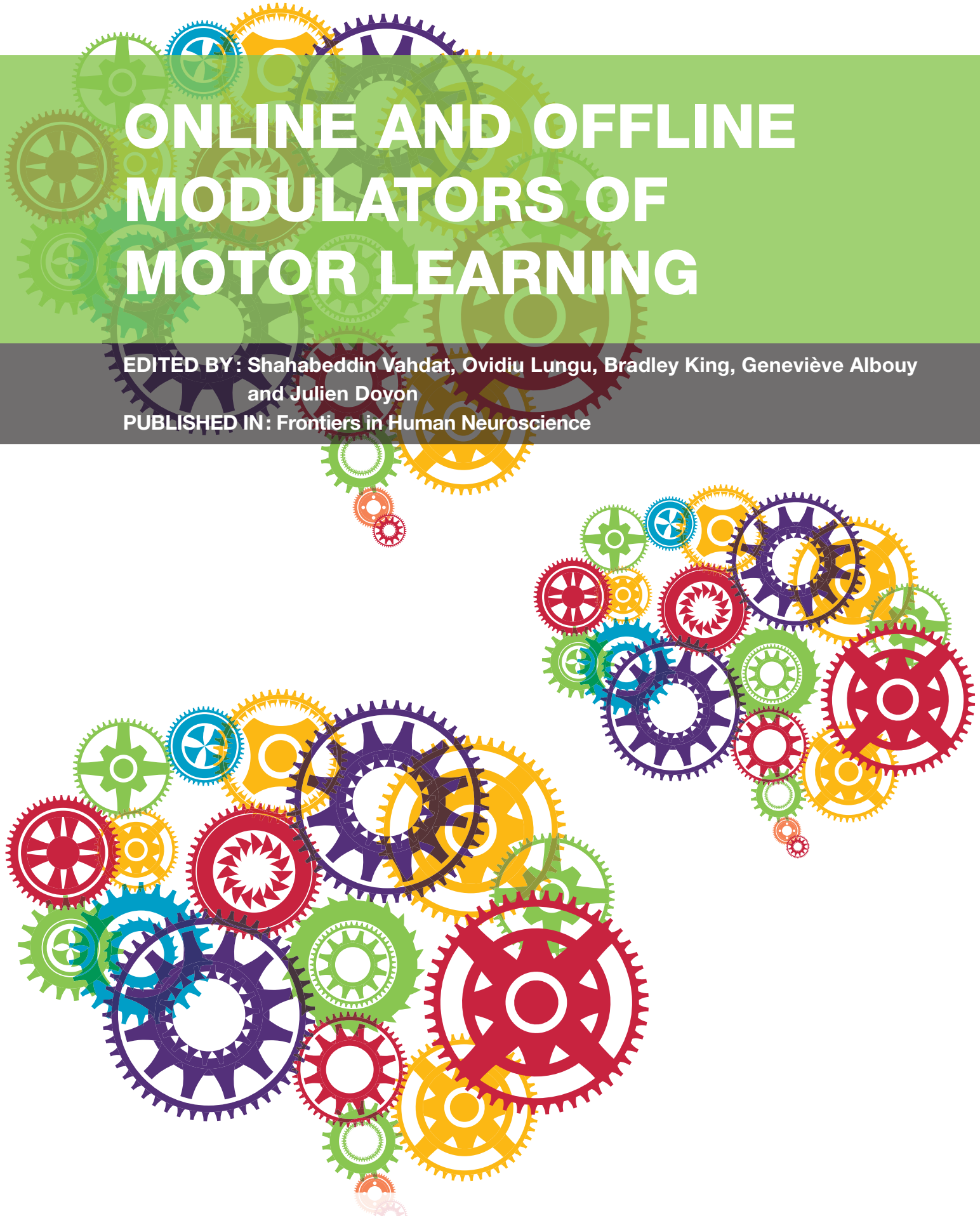

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ONLINE AND OFFLINE MODULATORS OF MOTOR LEARNING

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Both the acquisition of new and the modification of previously acquired motor skills are necessary to achieve optimal levels of motor performance in everyday functioning as well as to attain expert performance levels that are evident in sports and arts. A multitude of factors have been shown to influence the various stages of the learning process, from the acquisition (i.e., motor memory encoding) to the consolidation and subsequent retention of a skill. These factors, or modulators, can affect learning through online processes taking place during practice of a new motor skill or through offline processes occurring in the absence of task performance (i.e., after training sessions). Although much of the recent research from various disciplines has placed an increased emphasis on identifying factors that can influence the motor learning process, we lack an integrated understanding of online and offline determinants of motor skill behaviours.

Potential motor learning modulators include, but are certainly not limited to, stress, anxiety, attention, executive functioning, social interaction, stimulus-response mapping, training schedule/regimen, learning environment, vigilance/consciousness states including sleep, wakefulness or meditation, brain stimulation, interference as well as resting state brain connectivity. Pathological and non-pathological (i.e., development or aging) changes in the brain can also be conceptualized as potential modulators.

The aim of this Research Topic is to bridge research from the cognitive, sensory, motor and psychological domains using various behavioural paradigms and neuroimaging techniques in order to provide a comprehensive view of the online and offline modulators of motor learning, and how they interact to influence motor performance. Critically, the overarching goal is to gain a better understanding of how motor behaviour can be optimized. We believe that merging research from diverse neuroscientific communities would contribute to fulfilling this goal and potentially highlight possible shared neurophysiological mechanisms influencing motor learning.

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EDITORIAL
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Editorial: Online and Offline Modulators of Motor Learning

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Keywords: motor learning, memory, consolidation, sleep, brain stimulation, rehabilitation

Editorial on the Research Topic

Online and Offline Modulators of Motor Learning

What are the multitude of factors and processes that shape the acquisition and stabilization of a new motor skill? This is an important question that needs to be meticulously considered in order to design efficient paradigms for sports training programs as well as new rehabilitative protocols for restoring motor function following trauma or disease. Although the motor learning literature is abundant with research investigating the behavioral and neuronal determinants of online and offline motor learning (i.e., occurring during and after motor practice, respectively), an integrated view of the various factors influencing these determinants is not available in the literature. The aim of this Research Topic is therefore to address this gap and to bring together a set of articles that document how different factors modulate online and offline motor learning.

Critically, this special issue presents a wide range of modulators targeting both online and offline motor learning processes that can potentially be translated into clinical applications. Specifically, interventions including brain stimulation (Savic and Meier), exercise (Taubert et al.), the manipulation of the nature of motor practice (actual vs. imagination, Di Rienzo et al.), the timing of motor practice (de Beukelaar et al.), the training schedule (Müssgens and Ullén), the nature of the learned material (Du et al.), the cognitive load (Borragan et al.), the psychosocial context (Zemankova et al.), the availablity of visual (Rjosk et al.), or sensory (van Polanen and Davare) feedback all represent promising modulators of online motor learning processes. This special issue also reports interventions directly targeting offline processes, including the manipulation of post-practice vigilance and activity states, with the introduction of post-training sleep (Csabi et al.; Di Rienzo et al.; Malangre and Blischke) and exercise (Taubert et al.), but also the manipulation of the number and timing of the practice sessions after initial practice (triggering reactivation and reconsolidation processes, de Beukelaar et al.). Last, what makes this special issue unique is not only the variety of motor learning tasks investigated (from finer e.g., de Beukelaar et al. to grosser e.g., Malangre and Blischke), but also the diversity of populations studied [from children (e.g., Julius and Adi-Japha) to elderly (e.g., Zemankova et al.); in healthy but also pathological conditions (e.g., Csabi et al.; Zemankova et al.)].

With respect to the variety of tasks investigated, we would like to highlight two papers in particular that examined motor tasks that are highly relevant in clinical settings. Specifically, Rjosk et al. investigated whether mirror visual feedback can modulate a ball rotation task performed with the dominant or non-dominant hand; critically, these results have important implications in neurorehabilitation. Likewise, Malangré and Blischke investigated whether sleep facilitates the consolidation of gross motor sequence learning with a task in which subjects were required to fit a small peg into different target-holes. Consistent with previous literature using more laboratory-specific sequence learning tasks, they demonstrated offline performance improvements, which only occurred after an off-line period including sleep, but not wake.

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From a lifespan perspective, two studies demonstrated that modulation of motor learning via developmental factors. Julius and Adi-Japha studied children in different age ranges and showed that successful motor learning depends on encapsulation of an initial, relatively accurate motor performance that could be improved throughout training. Csábi et al. investigated the effect of sleep-disordered breathing (SDB) on memory consolidation in children. Children with SDB exhibited intact motor sequence memory consolidation following sleep compared to healthy children, in contrast to previous reports in sleep-disordered adults.

As an example of intervention with important translational value, Savic and Meier reviewed current evidence supporting the modulatory effects of transcranial direct current stimulation (tDCS) on implicit motor learning. They outlined different parameters and mechanisms for tDCS to attain improved motor learning and consolidation. Likewise, Taubert et al. delineated the role of exercise, or more specifically endurance training, in optimizing motor learning. They provide a mechanistic link between exercise and motor learning-induced neuroplasticity at the systems, cellular, and molecular levels of brain organization. Furthermore, Di Rienzo et al. proposed in their review on the effects of motor imagery practice on motor learning that, as an execution-free training protocol can be a useful tool especially for the rehabilitation of patients with severe motor impairments.

Manipulation of the timing and schedule of motor practice, as well as of the nature of the learned information can also be introduced in clinical practice in order to modulate motor learning. Specifically, time spent awake before learning a new motor sequence task is believed to modulate motor learning. Using transcranial magnetic stimulation, De Beukelaar et al. measured changes in the excitability of the primary motor cortex following sequence learning either in the morning or in the evening. Their results support the view that during the day synaptic weights become saturated, which decreases the capacity for learning-induced changes in synaptic strength, as measured by corticomotor excitability. Using a similar motor task, Müssgens and Ullén investigated the effects of constant vs. variable training schedule on the extent of skill transfer to different conditions. Their results indicated that variable training led to greater general transfer to a new sequence of movements, likely attributed to more interference associated with constant training. Du et al. showed that learning a motor task governed by probabilistic rules is mainly driven by offline, rather than online, improvements in performance. This study highlights the importance of offline learning processes in more natural environments, where the desired sequence of movements depends on several unaccounted parameters.

Non-motor neural processes can also exert a substantial influence on motor learning processes and a subset of papers in this review have provided critical insights into this issue (Rjosk et al.; van Polanen and Davare). It has been proposed that cognitive control and motor learning compete for limited brain resources. In order to test this hypothesis, Borragán et al. instructed subjects to perform working-memory updating tasks with either high or low cognitive load before motor sequence learning. Their results indicated that high cognitive fatigue enhanced motor sequence learning. Zemankova et al. reviewed various psychosocial factors that might influence motor learning, and hence motor rehabilitation interventions, in Parkinson's disease. They elaborated on the contributions of social interaction, mindset and self-regulatory mechanisms and emotions on the effectiveness of motor training in more ecological environments, and highlighted their interactions with Parkinson's disease.

Modulation of offline processes supporting motor learning is also an interesting avenue in the quest of the optimization of motor behavior. Sleep has been shown to play an essential role in this offline process as it facilitates memory consolidation and stabilization of sequential motor skills. Several papers in this issue have documented the characteristics of sleep-dependent motor memory consolidation in relation to developmental factors (Csábi et al.), motor imagery (Di Rienzo et al.), and in gross motor tasks (Malangré and Blischke). Moreover, the study by de Beukelaar et al. showed that that reconsolidation of motor sequence memories, taking place post-training after a short re-exposure to the task, depends on both additional motor practice as well as the passage of time. Longer time delays, as opposed to a short delay, between re-exposure (reactivation) and interference practice were unable to de-stabilize the consolidated motor sequence memory trace. Hence, their findings support a time-dependent offline process in motor memory reconsolidation.

We believe that this special issue will increase our understanding of the physiological processes underlying motor learning and offers a more comprehensive view of how these processes can be modulated at different time points during the online and the offline learning periods. The integrated approach offered by this Research Topic may serve as a stepping-stone from which optimized neurorehabilitative approaches can be developed to help recovery of motor functions following injuries or neurodegenerative processes.

AUTHOR CONTRIBUTIONS

SV, GA, BK, OL, and JD wrote the editorial article.

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ORIGINAL RESEARCH
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Transfer in Motor Sequence Learning: Effects of Practice Schedule and Sequence Context

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Transfer (i.e., the application of a learned skill in a novel context) is an important and desirable outcome of motor skill learning. While much research has been devoted to understanding transfer of explicit skills the mechanisms of skill transfer after incidental learning remain poorly understood. The aim of this study was to (1) examine the effect of practice schedule on transfer and (2) investigate whether sequence-specific knowledge can transfer to an unfamiliar sequence context. We trained two groups of participants on an implicit serial response time task under a Constant (one sequence for 10 blocks) or Variable (alternating between two sequences for a total of 10 blocks) practice schedule. We evaluated response times for three types of transfer: task-general transfer to a structurally non-overlapping sequence, inter-manual transfer to a perceptually identical sequence, and sequence-specific transfer to a partially overlapping (three shared triplets) sequence. Results showed partial skill transfer to all three sequences and an advantage of Variable practice only for task-general transfer. Further, we found expression of sequence-specific knowledge for familiar sub-sequences in the overlapping sequence. These findings suggest that (1) constant practice may create interference for task-general transfer and (2) sequence-specific knowledge can transfer to a new sequential context.

Keywords: skill learning, movement sequence, serial reaction time, practice schedule, skill transfer, contextual interference

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INTRODUCTION

Learning new motor skills can take a considerable amount of time and effort. Therefore, it is often desirable that a newly learned skill can also be applied outside the specific context within which it was acquired. Motor skill transfer (i.e., the application of a learned skill in a new task or context) is thus an important aspect of motor learning. Transfer can be described along several dimensions, such as positive vs. negative or broad vs. narrow (Adams, 1987; Schmidt and Lee, 2005). Positive transfer is seen when training of one skill facilitates performance in another, novel situation. Negative transfer is the opposite phenomenon, where earlier training interferes with performance on a new task. In narrow transfer, such influences are seen between similar tasks, while in broad transfer, training effects are seen on a wide range of tasks. Finally, certain skills may, or may not, transfer between effectors (i.e., they may be more or less specific to the effector with which they were trained). In most training scenarios one would thus want to achieve broad positive skill transfer, potentially also between effectors.

One factor that influences the amount of skill transfer is the training schedule. Variable training schedules (e.g., randomizing or alternating between different tasks) typically lead to greater performance retention and transfer than blocked schedules (Shea and Morgan, 1979; Magill and Hall, 1990). This phenomenon, termed contextual interference (CI), has been observed for a variety of motor tasks such as explicit visuo-motor sequence learning (e.g., Shea and Morgan, 1979; Wymbs and Grafton, 2009; Tanaka et al., 2010), handwriting (Ste-Marie et al., 2004), simple drawing tasks (Albaret and Thon, 1998), various sports skills (Wrisberg and Liu, 1991; Hall et al., 1994; Douvis, 2005, but see: Brady, 2008), and certain other complex tasks such as bimanual coordination (Pauwels et al., 2014) and rotatory pursuit skills (Heitman et al., 2005). One prominent theory on the mechanisms of CI argues that variable practice is advantageous because each switch between tasks requires the effortful reconstruction of motor plans in working memory (Lee and Magill, 1983; Immink and Wright, 1998; Cross et al., 2007). This repeated planning and updating of movement parameters is more attention demanding (Li and Wright, 2000), which is thought to eventually lead to more persistent skill representations in long-term memory. However, this explanation does not account for more recent findings from studies demonstrating superior skill retention after variable practice also for more implicit tasks such as incidental motor sequence learning (Song et al., 2012, 2015; Lin et al., 2013).

Transfer in implicit motor learning seems to be rather narrow and inflexible, with no transfer being observed, e.g., after changes in response locations (Willingham et al., 2000) or stimulus–response associations (Schwarb and Schumacher, 2010). Even changes in task-irrelevant aspects of the visual context in which a motor sequence is presented can be detrimental for implicit skill transfer (Jiménez et al., 2006; Abrahamse and Verwey, 2008). Jiménez et al. (2006) showed that changing superficial task parameters by adding task-irrelevant distractor stimuli abolished implicit skill transfer. Yet, despite of being rather inflexible with regards to superficial changes in stimulus presentation, implicit skills seem to be more robust than explicit skills if the sequential context of a task is abruptly changed. When Jiménez et al. (2006) trained participants on a sequential motor task and then changed the stimulus presentation to a random order (experiments 3 and 4), they observed an expected worsening of participants' performance. However, at certain points the random sequence was interspersed with the previously trained sequence. Participants who had learned the sequence implicitly, but not those who had learned it explicitly, showed evidence of sequence transfer as their performance recovered on those sections that contained the familiar sequence. This suggests that expression of implicit sequence knowledge might be triggered by the immediately preceding (familiar) sequence context, even if the familiar sequence itself is embedded within an unfamiliar (random) sequence context. However, given that the interspersed familiar segments in that study consisted of the entire training sequence it is not clear whether the context of the entire trained sequence is necessary or if sequence-specific knowledge can also transfer to familiar subsequences that are embedded within an unfamiliar sequence context.

Based on the previously mentioned findings of a CI effect on implicit motor sequence learning (Song et al., 2012, 2015; Lin et al., 2013) one might expect a similar benefit of variable practice for motor sequence transfer. To understand how different training schedules could affect skill transfer it is important to distinguish between different types of transfer. Generally, transfer in motor sequence learning tasks can be divided into sequence-specific and sequence non-specific components. Transfer is considered to be sequence-specific if performance improvements on a transfer task can be attributed to knowledge of the sequential order of the task elements. Sequence-specific knowledge can, depending on the exact task parameters, be represented in various formats such as stimulus-based coordinates (e.g., Remillard, 2003; Clegg, 2005), effector-based coordinates (e.g., Kami et al., 1995; Bischoff-Grethe et al., 2004; Park and Shea, 2005; Verwey and Clegg, 2005), response location based coordinates (e.g., Willingham et al., 2000; Witt and Willingham, 2006), or in terms of response effects (e.g., Ziessler and Nattkemper, 2001; Stocker et al., 2003; Stocker and Hoffmann, 2004) or the relationship between consecutive responses (e.g., Koch and Hoffmann, 2000; Hoffmann et al., 2001). Transfer of sequence non-specific (task-general) skills refers to performance improvements in task components that are not dependent on knowledge of the sequential structure, such as improvements in visual stimulus processing, stimulus–response mapping, or motor command generation. To distinguish between sequence-specific and task-general contributions to skill transfer it is thus necessary to compare transfer in tasks that contain familiar sequence information with transfer in similar tasks that do not contain familiar sequence structures.

Another dimension of skill transfer is effector specificity (e.g., whether a sequential skill transfers between hands). Although inter-manual skill transfer can again be divided into sequence-specific and non-specific transfer a number of studies have shown rather large inter-manual transfer effects for sequential knowledge (Willingham et al., 2000; Grafton et al., 2002; Verwey and Clegg, 2005; Berner and Hoffman, 2009). Inter-manual transfer is likely to benefit from both increased sequence-specific transfer and from improvements in certain task-general aspects such as stimulus processing or the mapping between stimuli and their relative response locations. Thus, if CI affects any of these components one would expect to see an advantage of variable practice also for inter-manual transfer. Yet, the effects of practice schedule onto implicit inter-manual transfer have – to our knowledge – not been studied so far.

One task that is commonly used to investigate sequence learning and transfer is the serial response time (SRT) task (Nissen and Bullemer, 1987). In an SRT task stimuli appear at different spatial locations and participants respond by pressing a button corresponding to the location of the stimulus. Unbeknownst to the participants, the stimuli follow a sequential order during training and performance improvements are quantified as reductions in response time (RT). After several training blocks a random sequence is introduced and the RT difference between the last training (sequential) block and the random block is typically attributed to sequence-specific learning.

RT decreases in the random block, relative to the first block, are considered sequence non-specific improvements.

When quantifying transfer it is important to carefully choose the training and transfer sequences to avoid confounds due to differences in sequence difficulty or saliency. A methodological challenge is that RT differences between different sequences could reflect learning of both complex sequential structures and simpler statistical regularities (e.g., frequencies of elements and transitions) of the training sequence. In one influential study, Reed and Johnson (1994) used second-order conditional sequences (i.e., sequences where the identity of a given element is determined by the two preceding elements, but not by one element alone) to specifically test whether complex sequence structures can be learned implicitly. Importantly, training and control sequences were matched on a variety of properties, so that knowledge of sequence structure could be disentangled from knowledge of statistical regularities.

Here, we used a similar approach as Reed and Johnson (1994) by employing sequences that were carefully matched in terms of salient structural properties (see Materials and Methods) to investigate different types of transfer effects after constant and variable training. Participants were divided into two groups which received either constant training of a single sequence, or variable (alternating) training of two sequences. Transfer effects were evaluated by comparing performance on three different test sequences before and after training. One test sequence (T0) had no structural overlap with either of the training sequences and thus served to quantify sequence non-specific transfer effects. A second sequence (T3) had partial structural overlap (three shared triplets) with each of the training sequences. This sequence was used to investigate sequence-specific transfer for familiar sub-sequences embedded into an unfamiliar sequence context. A third sequence (TrL) was perceptually identical to the trained sequence but was performed with the opposite (untrained) hand. This sequence was used to investigate inter-manual transfer in extrinsic coordinates. Contrary to transfer sequence T3, where familiar sub-sequences were embedded into novel sequence context, the sequence context for transfer sequence TrL was thus entirely familiar.

We investigated two hypotheses. First, we tested whether variable sequence training leads to greater skill transfer than constant training. As outlined above, variable practice has been found to be advantageous for a variety of motor learning tasks including relatively simple explicit tasks, more complex sports and real-life tasks, and implicit sequence learning tasks. We thus predicted that the Variable practice group would show larger performance improvements on the non-overlapping sequence (T0, sequence-unspecific transfer), as well as on the trained sequence performed with the left hand (TrL, inter-manual transfer) and on the structurally overlapping sequence (T3, sequence-specific transfer). Secondly, we hypothesized that structure-specific knowledge partly transfers to new sequences that contain fragments of the trained sequence. Specifically, we expected that (i) transfer effects would be larger for the partially overlapping sequence (T3) than for the non-overlapping sequence (T0) and (ii) that within the partially overlapping sequence transfer would be specifically larger for predictable elements than for corresponding unpredictable elements.

MATERIALS AND METHODS

Ethical Statement

All participants gave written, informed consent to participate and the study was approved by the Regional Ethical Review Board in Stockholm, Sweden (Dnr. 2012/198-32/4).

Participants

Participants were recruited through posters displayed at the Karolinska Institutet campus and through the website Studentkaninen (www.studentkaninen.se), a Swedish website for research volunteers. Sixty individuals initially participated in the study. Due to technical problems, we did not obtain data from the left hand task in two participants. These participants were excluded from all analyses involving the left hand task, but their data was included in all other analyses. Further, one individual was excluded from the analyses, because of exceptionally slow RTs (2.5–3.8 SD above the sample mean in all tasks). Three additional participants were excluded, because they showed no learning of the experimental sequence Tr1 in the Training session (i.e., the linear regression of RT on trial number had a positive slope). The final analyses thus included 56 participants in all tasks involving the right hand and 54 participants in all left-hand tasks. The age of these participants ranged from 19 to 43 years (mean = 27.8, $SD = 5.5$); 28 participants were male. All participants were right-handed and reported to be free of any neurological or psychiatric conditions.

Participants were randomly assigned to either the Constant group ($n = 28$, mean age = 27.9), which practiced a single sequence during the training session, or to the Variable group ($n = 28$, mean age = 27.7), which practiced two different sequences alternatingly (**Figure 1A**).

Sequential Tasks

Stimulus presentation and data collection were performed on a PC, using a script written in the E-Prime software package (Psychological Software Tools, Inc.). Stimuli were presented on the computer monitor and responses were collected from the computer keyboard. Participants stayed seated in front of the computer during the whole experiment and were allowed to adjust the position of the keyboard and chair. The identity and timing of all stimuli and responses were saved to a log file.

The experiment consisted of a number of SRT tasks (Nissen and Bullemer, 1987; i.e., series of four-choice RT trials). Four empty squares – corresponding to sequence elements 1, 2, 3, and 4 – were presented in a horizontal arrangement along the middle of the computer monitor. On each trial, one of the squares turned yellow and remained yellow until the participant pressed the correct key. Four different response keys (H, U, I, L for the index to little finger of the right hand and G, R, E, A for the index to little finger of the left hand) were used, corresponding to the four stimulus locations. As soon as the participant gave the

correct response the next stimulus appeared; thus, the response-to-stimulus interval was 0 ms. We chose this interval because the absence of a response-to-stimulus delay has been previously shown to reduce explicit sequence awareness (Destrebecqz and Cleeremans, 2001). If no correct response was registered within 2 s the program continued automatically with the next stimulus. The experiment was described as a "reaction time task" and participants were instructed to respond to the stimuli as quickly and as accurately as possible. Participants were not told that the stimuli would appear in a sequential order.

Stimuli always followed a repeating, deterministic sequence of 12 elements. The tasks were administered in blocks consisting of 10 uninterrupted repetitions of the same sequence, (i.e., 120 RT trials per block). Four different sequences were used in different tasks: Tr1, Tr2, T0, and T3 (**Figure 1B**). Sequence Tr1 was also used in a left-hand task, TrL. When comparing performance or transfer between different sequences, it is essential that the sequences are matched on various properties that are likely to influence learning (Reed and Johnson, 1994). The sequence structure of the employed sequences is shown in **Figure 1B**. All sequences were second-order conditional sequences (i.e., each element is uniquely predicted by the preceding bigram of two consecutive elements, but never by one preceding element alone). There were no immediate repetitions of elements. The frequencies of all individual elements (1, 2, 3, 4) were the same (0.25) in all sequences, as were the frequencies (0.083) of all of the 12 allowed bigrams (12, 13, 14, 21, 23, 24, 31, 32, 34, 41, 42, 43). The sequences were also matched on other putatively salient properties that could influence performance (Reed and Johnson, 1994): reversal frequency (0.25; i.e., the frequency of palindromic triplets with a 'back-and-forth' structure, such as in 121), rate of full coverage (5.08; i.e., the average number of elements encountered before each element has occurred at least once), and rate of full transition usage (13; i.e., the average number of elements encountered before each possible transition has occurred once).

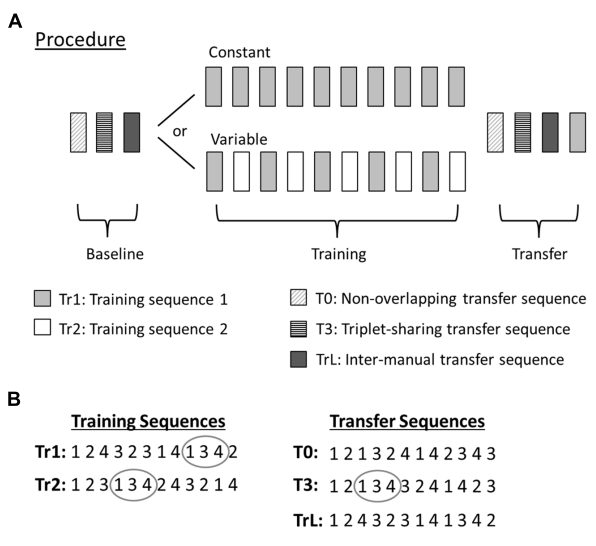

FIGURE 1 | Experimental procedure and sequential stimuli. (A) The experimental procedure consisted of three sessions: Baseline, Training, and Transfer. The Baseline session consisted of one block of each of the three transfer sequences (T0, T3, TrL) and the Transfer session consisted of the same three sequences (presented in the same order as during Baseline) plus one additional block of Tr1 at the end of the session. Block order during Baseline was randomized across participants and counterbalanced between groups. In the Training session the Constant group performed 10 blocks of the Tr1 sequence and the Variable group alternated between Tr1 and Tr2 blocks. All blocks were separated by 20 s of rest, both within and between sessions. Each block contained 10 uninterrupted sequence repetitions, thus requiring a total of 120 responses (12 sequence elements × 10 repetitions) per block. **(B)** Sequence structure of the training (left) and transfer (right) sequences. The training sequences (Tr1 and Tr2) shared six triplets with each other, three of which were also shared with sequence T3 (one example of a shared triplet encircled). None of the training sequences shared any triplets with sequence T0.

Furthermore, the sequences were constructed such that comparisons between the different tasks would be informative about the nature of possible transfer effects. Sequences Tr1 and Tr2 were used as training sequences. The Constant group trained only Tr1 and the Variable group trained alternatingly on Tr1 and Tr2. Sequence T0 shared no triplets with Tr1 or Tr2. Performance on this sequence could thus provide information about sequence non-specific transfer effects. We chose a deterministic, rather than random control sequence, so that we could exclude the possibility of any accidental structural overlap between T0 and the training sequence. Moreover, this enabled us to control the sequence for all of the above mentioned statistical sequence regularities. Sequence T3 shared the same three triplets (134, 231, and 432) with both Tr1 and Tr2 and was used to investigate sequence-specific transfer effects. Since the shared triplets appeared at different ordinal positions in T3 than in Tr1 and Tr2 sequence-specific transfer should only be observed if sequence knowledge for the smallest unique sub-parts (i.e., triplets) is still preserved if triplets are isolated from their sequence context and embedded into an unfamiliar sequence context.

Finally, to investigate inter-manual transfer, a task TrL was included, where participants performed the Tr1 sequence using their left hand. Inter-manual transfer was evaluated in extrinsic coordinates, meaning that both the order of visual stimulus locations and the mapping between visual stimuli and their relative response locations (i.e., leftmost stimulus to leftmost response location, rightmost stimulus to rightmost response location) were the same as for the training sequence.

Experimental Procedure

All experiments were performed in a quiet room. Before the start of the experiment each participant made 12 practice responses with each hand to become familiar with the task. The order of these responses was not related to any of the sequences. The experiment consisted of three sessions: baseline, training, and transfer (**Figure 1A**). The baseline session included one block of each of the TrL, T0, and T3 tasks. The order of these three tasks within the baseline session was randomized across participants and counterbalanced between groups to prevent any possible task-order effects.

Participants then performed the training session, which was organized differently for the two groups. The Constant training group performed 10 blocks of the Tr1 task. The Variable group also performed a total of 10 blocks, but alternated between blocks of Tr1 and Tr2, thus yielding a total of five blocks per task.

The final transfer session included one block each of the TrL, T0, T3, and Tr1 tasks. Task order within the transfer session was the same as during baseline, except for the additional block of Tr1 which was always presented at the end of the session. Participants were not informed that the experiment consisted of three sessions. All blocks were separated by 20 s of rest, both within and between sessions to avoid any noticeable distinction between sessions.

Questions on Explicit Sequence Knowledge

After completion of the three sessions participants filled out a paper-and-pencil questionnaire related to their sequence awareness. First, they were asked a two-choice question whether they had perceived any pattern in the presented stimuli: "Did you notice any regularity in the presentation of the yellow squares?", with response alternatives "Yes" and "No". In the second question they were asked to rate how sure they were that there was a pattern or sequence in the stimuli: "On a scale from 1 (not sure at all) to 10 (very sure) can you indicate how sure you were that there was a pattern or sequence in the presentation of the yellow squares?"

Statistical Analyses

Data were pre-processed using custom-written scripts in MATLAB (version R2013b, The MathWorks, Inc., Natick, MA, USA) and analyzed in SPSS (version 21.0 for Windows, IBM Corp., Armonk, NY, USA). For each participant we excluded wrong responses and calculated the median RT per block. The average percentage of excluded (i.e., wrong) responses varied between 3.0 and 7.7% per block (**Figure 2**). For each sequence we calculated an Improvement Score, defined as RT at baseline – RT at transfer, to quantify RT changes across sessions. Since RTs were approximately normally distributed in each group, except for Tr1 Improvement Scores in the Constant group, which followed a slightly skewed (skewness = –1.04) and non-normal distribution (Shapiro–Wilk test: $p = 0.03$), we did not apply any data-transformation before hypothesis testing.

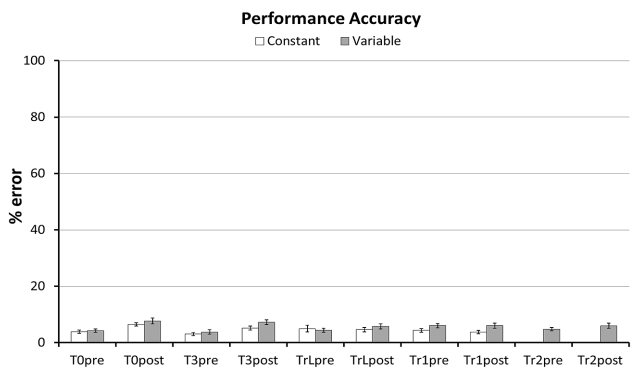

FIGURE 2 | Performance errors per task. Average percent of incorrect responses per task and Group. Error bars represent standard error of the mean. Incorrect responses varied between 3.0 and 7.7% (3.6–9.2 errors out of 120 responses) per block and were removed before further analysis.

We tested the first hypothesis that variable training leads to larger structure-independent, inter-manual, and sequence-specific transfer using three repeated-measures general linear model (GLM) analyses. In each model we regressed the mean RT for the corresponding sequence on the within-subject factor Session (Baseline, Transfer), the between-subjects factor Group (Constant, Variable), and the Session × Group interaction term. We further tested whether the amount of transfer was related to improvements in the training sequence by correlating – in each training group – the Improvement Scores of the transfer sequences (T0, TrL, and T3) with the Improvement Scores of Tr1.

To test the second hypothesis that sequence-specific knowledge transfers to a novel but structurally overlapping sequence we first compared the amount of RT improvement in the triplet-sharing sequence (T3) with RT improvements in the non-overlapping sequence (T0). We used a repeated-measures GLM for Improvement Scores with Transfer Sequence (T0 or T3) as within-subject factor, Group (Constant or Variable) as between-subject factor, and the Transfer Sequence × Group interaction term. To investigate whether sequence knowledge was expressed specifically for overlapping triplets we directly compared Improvement Scores for familiar and unfamiliar sequence transitions. Given that all sequences were second-order conditional sequences the identity of the third triplet element of shared triplets is predictable because it is always preceded by the same two elements, independent of the triplet's ordinal position within the sequence. Comparing RT improvements for such predictable elements with RT improvements for the same, but non-predictable elements (i.e., same key presses but within an unfamiliar triplet) provides a specific estimate of sequence transfer. **Table 1** shows the familiar triplets (with predictable third elements) in T3 and the corresponding unfamiliar triplets (with non-predictable elements) in T3 and T0. For example, element "4" in T3 is predictable when it is preceded by "1-3" (because the triplet "1-3-4" is shared with Tr1), but not when it is preceded by "3-2" or by "4-1". We thus calculated three

TABLE 1 | Familiar and corresponding unfamiliar triplets.

Familiar triplets (in Tr1 and T3)	Unfamiliar triplets (in T3 and T0)
1-3-4	3-2-4, 4-1-4
4-3-2	3-1-2, 1-4-2
2-3-1	1-2-1, 2-4-1

Familiar triplets were shared between Tr1 and T3, unfamiliar triplets were shared between T3 and T0. The last elements of familiar triplets were predictable and each of them had two corresponding unpredictable elements in the unfamiliar triplets of T3 and T0. Three different averages were computed from the RTs of the last (underlined) elements of these triplets: (1) average RTs of predictable elements in T3, (2) average RTs of corresponding unpredictable elements in T3, and (3) average RTs of corresponding unpredictable elements in T0.

separate averages of Improvement Scores for each participant, one for predictable elements in T3, one for corresponding non-predictable elements in T3, and one for the corresponding non-predictable elements in T0. These averages were entered into a repeated-measures GLM with within-subject factor Element type (T3-Predictable, T3-Non-predictable, T0-Non-predictable), between-subjects factor Group (Constant or Variable), and the Transition type × Group interaction. Subsequent pairwise comparisons between the different levels of Transition type were corrected for multiple comparisons using Bonferroni correction.

For hypotheses where we predicted an effect in a particular direction we used one-tailed levels of significance at $\alpha = 0.05$ to maximize power. Where applied, the usage of one-tailed tests is stated in the results. All other tests were performed using two-tailed levels of significance at $\alpha = 0.05$.

RESULTS

Performance Improvements on Trained Sequences

First, to confirm that sequence-learning was successful we performed a repeated-measures GLM on the RTs for each training sequence and Group. **Figure 3** shows a continuous RT decrease across training blocks for each sequence. This was confirmed by linear within-subjects contrasts: Tr1, Constant group [$F(1,27) = 62.6$, $p < 0.001$], Tr1, Variable group [$F(1,27) = 93.1$, $p < 0.001$], and Tr2, Variable group [$F(1,27) = 67.1$, $p < 0.001$]. Furthermore, a repeated-measures GLM with factors Session (Baseline/Transfer) and Group (Constant/Variable) confirmed that in both groups RTs for Tr1 were significantly reduced at Transfer [main effect of Session: $F(1,54) = 127.3$, $p < 0.001$]. Additionally, there was an interaction between Group and Session [$F(1,54) = 10.4$, $p = 0.002$], with greater post-training RT improvements in the Constant training group. Improvement Scores (i.e., Baseline RTs – Transfer RTs) for Tr1 and for the three transfer sequences, T0, T3, and TrL are shown in **Figure 4** and mean RTs per Group and Session (Baseline and Transfer) are shown in **Table 2**.

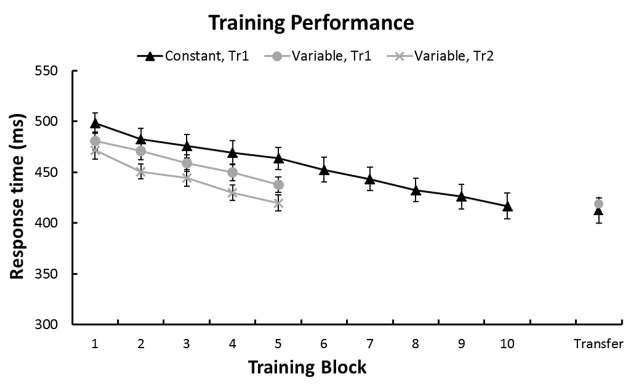

FIGURE 3 | Performance during training. In both groups RTs decreased linearly during training. Error bars represent standard error of the mean. Note that in the Variable group, blocks of Tr1 and Tr2 training were interleaved.

Variable Training and Transfer

Our first hypothesis was that the different types of transfer (i.e., sequence non-specific, inter-manual, and sequence-specific transfer) are larger after variable than after constant training. First, we predicted that the Variable group would have larger transfer to T0 than the Constant group. In line with this prediction the GLM for RTs in T0 revealed a greater RT reduction between sessions in the Variable than in the Constant group, as evident by a significant Session × Group interaction in the predicted direction [$F(1,54) = 3.7$, $p = 0.03$, one-tailed]. Further, there was a significant RT improvement across sessions [main effect of Session: $F(1,54) = 39.7$, $p < 0.001$], but no significant difference between groups [main effect of Group: $F(1,54) = 2.98$, $p = 0.09$]. **Figure 5** shows mean RTs per Group and Session for the T0 sequence. To test whether these differences in RT improvement could be influenced by differences in accuracy we performed the same GLM with factors Session, Group and the Group × Session interaction on the number of errors in T0. The amount of errors did not differ between groups [main effect of Group: $F(1,54) = 0.73$,

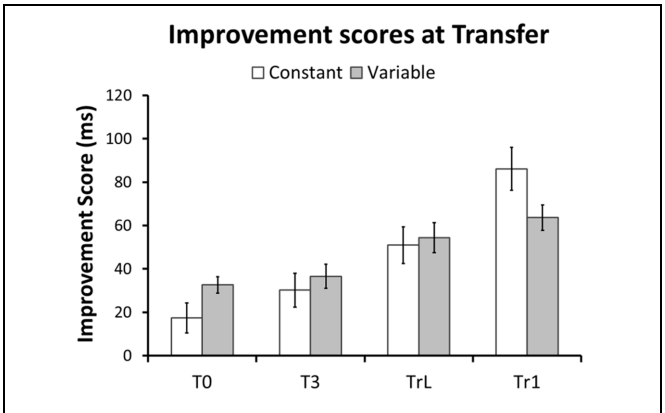

FIGURE 4 | Performance improvements at Transfer. Improvements in RT are shown as between-participant means of the within-participant difference between median RT at Baseline and Transfer. Note that in both groups and for all sequences RTs improved after training. Error bars represent standard error of the mean.

TABLE 2 | Response times per group during Baseline and Transfer sessions.

	Constant		Variable	
Task	**Baseline**	**Transfer**	**Baseline**	**Transfer**
Tr1	498 ± 53	412 ± 66	482 ± 46	419 ± 38
TrL	534 ± 65	483 ± 59	519 ± 50	464 ± 43
T0	507 ± 60	490 ± 57	494 ± 35	461 ± 37
T3	493 ± 57	463 ± 59	483 ± 45	446 ± 39

Response times (in ms ± SD) show the group means of the median RTs of each participant. In both groups and for all sequences, RTs decreased significantly from Baseline (i.e., first block) to Transfer (i.e., last block) sessions (all $p < 0.01$, except for T0 in the Constant group, where $p = 0.019$).

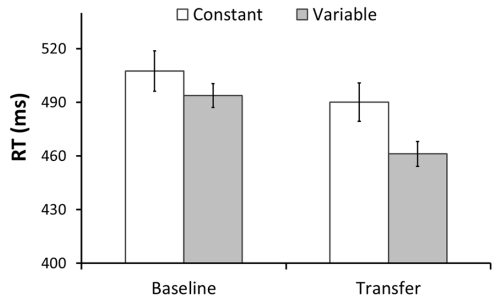

FIGURE 5 | Transfer effects on sequence T0 after constant and variable training. Mean response times at Baseline and Transfer for sequence T0 are shown separately for the each group. The Variable group showed a significantly larger RT reduction at Transfer than the Constant group. There were no significant group differences in RT at Baseline. Error bars represent standard error of the mean.

$p = 0.40$], nor was there an interaction between Group and Session [$F(1,54) = 0.60$, $p = 0.44$]. However, a significant main effect of Session [$F(1,54) = 31.6$, $p < 0.001$] revealed that both groups committed more errors at Transfer than at Baseline (see **Figure 2**). Further, we tested whether, on the between-participant level, performance improvements in the transfer sequence T0 were related to the magnitude of improvement in the trained sequence Tr1. Improvement scores for T0 and Tr1 were significantly correlated in the Variable group ($r = 0.47$, $p = 0.01$; **Figure 6A**) but not in the Constant group ($r = 0.07$, $p = 0.72$; **Figure 6B**). One participant in the Constant group had a somewhat extreme RT improvement in Tr1 of 246.5 ms and might be considered an outlier (see **Figure 6B**). To make sure that the correlation in the Constant group was not distorted by this single value we repeated the same analysis under exclusion of this data point. Removing this value did not change the result, as the correlation remained non-significant ($r = 0.32$, $p = 0.11$). Further, since the Constant group received five more blocks of Tr1 training than the Variable group, one might argue that for the Constant group, a correlation with T0 improvements might rather be present in the first five blocks of Tr1 training. However, no correlation with T0 improvements was found also when using the Improvement Scores of only the first five Tr1 blocks ($r = 0.01$, $p = 0.96$).

Effects of variable training on inter-manual transfer were tested analogously. Contrary to our expectation there was no significant Session × Group interaction [$F(1,52) = 0.10$, $p = 0.38$, one-tailed]. RTs decreased significantly between sessions [main effect of Session: $F(1,52) = 94.31$, $p < 0.001$] and there was no significant difference between groups [$F(1,52) = 1.55$, $p = 0.22$]. The number of errors did not differ between Groups [$F(1,52) = 0.12$, $p = 0.73$] or Sessions [$F(1,52) = 0.42$, $p = 0.52$], nor was there an interaction [$F(1,52) = 1.13$, $p = 0.30$]. Improvement Scores for TrL were not correlated with improvements inTr1, neither in the Variable ($r = 0.31$, $p = 0.11$), nor in the Constant ($r = 0.15$, $p = 0.46$) group.

Finally, we used the same approach to test effects of variable training on sequence-specific transfer in T3. Contrary to our expectation there was no significant Session × Group interaction [$F(1,52) = 0.45$, $p = 0.25$, one-tailed]. Again, RTs decreased significantly across sessions [main effect of Session: $F(1,54) = 48.56$, $p < 0.001$] and there was no significant difference between groups [$F(1,52) = 1.14$, $p = 0.29$]. Similar to the other transfer sequences the number of errors did not differ between groups [$F(1,54) = 2.45$, $p = 0.12$], nor did it show a Group × Session interaction [$F(1,54) = 2.44$, $p = 0.12$]. However, participants made more errors at the transfer than at baseline session [main effect of session: $F(1,54) = 44.48$, $p < 0.001$] (see **Figure 2**). Finally, we tested whether Improvement Scores for T3 were correlated with improvements in Tr1 at the between-participant level. The Variable group showed a strong trend toward a positive correlation ($r = 0.37$, $p = 0.055$), but there was no correlation in the Constant group ($r = -0.23$, $p = 0.24$).

Sequence-specific Transfer

The second hypothesis was that sequence-specific knowledge acquired during training can be used in the context of a novel sequence. To test this, we first investigated whether transfer effects were larger for the sequence that shared triplets with the trained sequence (T3) than for the sequence that had no structural overlap with the trained sequence (T0). We examined this using a repeated-measures GLM for Improvement Scores with the factors Transfer Sequence (T0/T3), Group (Constant/Variable), and the Transfer Sequence × Group interaction term. In line with the hypothesis, we found an effect of Transfer Sequence in the predicted direction (i.e., larger improvement for T3 than for T0) [$F(1,54) = 4.06$, $p = 0.025$, one-tailed]. There was no effect of Group [$F(1,54) = 1.95$, $p = 0.17$] nor a Transfer Sequence × Group interaction [$F(1,54) = 1.12$, $p = 0.30$].

As a more precise test for sequence-specific transfer, we investigated whether predictable sequence elements (last element of familiar triplets) showed greater RT improvements after training than corresponding non-predictable elements (last element of unfamiliar triplets). A GLM analysis of the Improvement Scores with within-subject factor Element Type (T3-Predictable, T3-Non-predictable, T0-Non-predictable), between-subjects factor Group (Constant, Variable), and the Element × Group interaction revealed a significant main effect of Element Type [$F(2,107) = 10.0$, $p < 0.001$, one-tailed], but no effect of Group [$F(1,54) = 2.1$, $p = 0.15$], or of the Element Type × Group interaction [$F(2,107) = 0.09$, $p = 0.91$]. Bonferroni-corrected pairwise comparisons further confirmed that predictable elements in T3 showed greater RT improvements than corresponding non-predictable elements in both T3 [$F(1,54) = 11.8$, $p = 0.004$] and T0 [$F(1,54) = 16.2$, $p = 0.001$]. RT improvements for non-predictable elements in T3 and T0 did not differ from each other [$F(1,54) = 0.68$, $p = 1.0$]. There were no differences between or interactions with Group in any of the comparisons (T3-Predictable vs. T3-Non-predictable Group effect: [$F(1,54) = 1.32$, $p = 0.78$], interaction: [$F(1,54) = 0.08$, $p = 1.0$]; T3-Predictable vs. T0-Non-predictable Group effect: [$F(1,54) = 2.0$, $p = 0.48$],

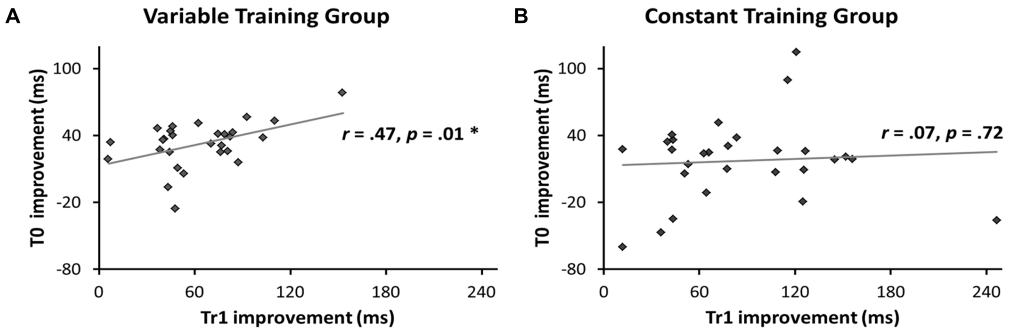

FIGURE 6 | Correlations between improvement scores on the training sequence Tr1 and the transfer sequence T0. Improvement scores were based on RT differences between the first block and the Transfer block of each sequence. Correlations between improvement scores for Tr1 and T0 were calculated separately for the Variable **(A)** and the Constant **(B)** group. Only the Variable group showed a significant correlation.

interaction: [$F(1,54) = 0.02$, $p = 1.0$]; T3-Non-predictable vs. T0-Non-predictable Group effect: [$F(1,54) = 2.7$, $p = 0.33$], interaction: [$F(1,54) = 0.20$, $p = 1.0$]). **Figure 7** shows that for both groups Improvement Scores for predictable elements are larger than those for non-predictable elements.

Sequence Awareness

The two groups did not differ on either of the two sequence awareness measures. In both the Constant and the Variable group, 19 out of 28 participants answered "Yes" to the first question on whether they had noticed any pattern in the stimulus presentation. Further, there was no group difference in participants' response to the second question on how sure (1 = "not sure at all"; 10 = "very sure") they were of the presence of a sequential pattern [Constant: mean = 6.89, $SD = 2.81$; Variable: mean = 6.75, $SD = 3.04$; $t(54) = 0.18$, $p = 0.86$].

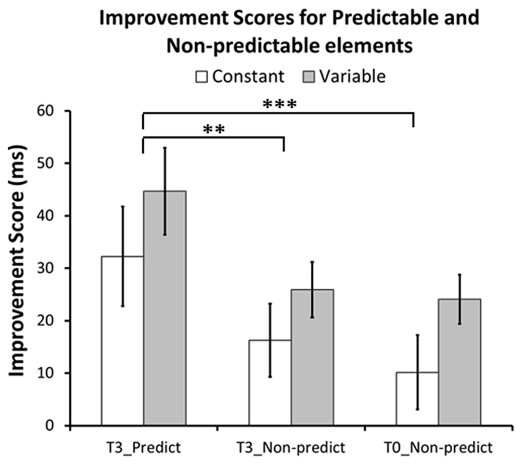

FIGURE 7 | Performance improvements for predictable and non-predictable elements. Improvement scores for predictable elements are derived from the average improvement scores from the last elements of familiar triplets in T3. Improvement scores for non-predictable elements are derived from the average improvement scores from corresponding elements (i.e., same finger) of unfamiliar triplets in T3 and T0. In both groups, performance increased more for predictable elements (in T3) than for corresponding non-predictable elements (in both T3 and T0). There was no difference between non-predictable elements in T3 and T0. None of the comparisons revealed a significant group or interaction effect. $^{**}p = 0.004$, $^{***}p = 0.001$, Bonferroni corrected

DISCUSSION

We investigated two hypotheses about transfer of motor sequence skills. First, we tested whether variable practice leads to greater skill transfer than constant practice by examining the effect of practice schedule on three different types of transfer: task-general, inter-manual, and sequence-specific transfer. Second, we tested if structure-specific sequence knowledge can transfer to a novel sequence context. In partial support of our first hypothesis, we found greater transfer after variable than after constant practice, but only for the structurally non-overlapping (T0) sequence and not for the structurally identical inter-manual (TrL) and the triplet-sharing (T3) transfer sequences. Variable practice was thus advantageous for task-general transfer but not for inter-manual or sequence-specific transfer. Our second hypothesis that fragments of sequence-specific knowledge can transfer to a novel sequential context was supported by two observations. First, transfer was larger for the triplet-sharing (T3) sequence than for the non-overlapping (T0) sequence. Moreover, within the triplet-sharing sequence transfer was larger for elements that were predictable based on previously acquired sequence knowledge (i.e., the third element of shared triplets) than for elements that were not predictable (i.e., corresponding elements of non-shared triplets).

Practice Schedule and Sequence-unspecific Transfer

The presence of transfer effects to the structurally non-overlapping sequence T0 indicates that task-general skills (i.e., skills that are independent of sequence structure) contributed to skill transfer. Performance of any SRT paradigm requires stimulus perception, response selection and generation, and the formation of correct stimulus–response associations.

Training could improve one or several of these basic task components. This likely explains why even RTs on random SRT sequences improve with practice (Thomas and Nelson, 2001; Robertson, 2007; Song et al., 2015). But why is transfer of task-general skills larger after variable than after constant training, despite of equal amounts of practice with the SRT task?

Generally, this finding is in agreement with transfer benefits of variable practice that have been reported for various real-life skills such as, e.g., tennis (Douvis, 2005), wheelchair driving (Yao et al., 2009), and rotatory pursuit skills (Heitman et al., 2005). However, in the present task both practice schedules provided exactly the same amount and format of task-general practice, making it unlikely that one group would have truly improved more on non-specific skills such as stimulus processing or stimulus–response mapping. This suggests that, somewhat paradoxically, differences in task-general transfer might have resulted from differences in the exposure or learning of sequential structures.

One possible explanation for this could be that the transfer differences between training groups reflect differences in their susceptibility to interference from negative transfer. Negative transfer has been found, e.g., in sequential rule application paradigms where a set of number manipulation rules has to be applied in a specific order to solve a cognitive task (Woltz et al., 2000). The more training participants received in that task, the more errors they made on a transfer task in which the same rules had to be applied in a different order. Participants were often unaware of their errors, suggesting that the errors reflect the involuntary behavioral expression of implicit sequence representations, which are inaccessible to conscious control. A similar phenomenon has been described for SRT paradigms where RTs of random order trials are slowed down by the previous execution of sequential trials (Robertson, 2007). Again, this suggests that also for motor sequences expectations about sequence order can interfere with performance at transfer. In line with these findings, we observed that the Constant practice group showed less skill transfer to the non-overlapping sequence, despite of larger performance improvements during training. Thus, greater sequence knowledge may have caused more interference with a non-overlapping sequence at transfer. The Variable group had the same total amount of SRT practice, but the alternating training schedule may have led to weaker or more flexible sequence expectations. Thus, while both variable and constant practice promote transfer of task-general skills, constant practice may limit the total amount of transfer due to interference from violated sequence expectations. Such differences in the susceptibility to negative transfer would also explain why the amount of transfer correlated with training improvements only in the Variable and not in the Constant practice group. If constant practice increases both task-general skills and sequence-specific expectations, then improvements during training will simultaneously increase positive and negative transfer thereby precluding a direct relation between training improvements and transfer.

The finding that after training, both groups made more errors on the T0 and T3 sequences suggests that negative transfer also affects performance accuracy. This post-training decrease in T0 and T3 accuracy cannot be explained by a general performance drop toward the end of the experiment because accuracy on the perceptually familiar sequences (both Tr1 and TrL) did not decrease at transfer. Further, it is important to note that the interference with accuracy was similar in both groups, making it unlikely that the observed differences in RT transfer were due to differences in accuracy.

Practice Schedule and Inter-manual Transfer

Both groups showed inter-manual transfer, as evident in performance improvements with the untrained (left) hand after training. Hikosaka et al. (2002) suggested that sequence learning may involve the acquisition of multiple sequence representations: a rapidly acquired, effector-independent representation in extrinsic visuo-spatial coordinates and a more gradually acquired effector-specific representation in intrinsic motor coordinates (Hikosaka et al., 2002). According to this model, the relatively short training period in the current task would have promoted predominantly effector-independent representations in extrinsic coordinates (see also: Shea et al., 2011). Inter-manual transfer would thus require the remapping of external response locations to a new set of motor commands for the opposite hand. Similar to the present results, earlier experiments also found large inter-manual transfer effects for sequences of the same perceptual structure (Japikse et al., 2003; Kovacs et al., 2009), suggesting that inter-manual transfer makes use of sequence representations in extrinsic space.

However, contrary to our hypothesis, we observed no difference in inter-manual transfer between the Constant and the Variable group. This suggests that practice schedule has a weak or no influence on inter-manual transfer and that constant and variable practice do not cause different amounts of interference for the untrained hand. In fact, given that the inter-manual transfer sequence was perceptually identical to the training sequence, any involuntary expression of perceptual sequence knowledge would have contributed positively to transfer performance. It would be interesting to investigate in future studies whether negative inter-manual transfer can be seen for sequences that are perceptually different but motorically identical (i.e., require the same sequence of finger movements) or homologous (i.e., require the same finger movements but with the opposite hand). A recent study by Handa et al. (2015) showed that overnight offline gains after sequence practice are reduced when either an entirely novel or a motorically similar (but perceptually different) sequence is practiced immediately after the target sequence. Interestingly, offline gains were not impaired after adding a visuo-spatially identical sequence at the end of training (Handa et al., 2015). These findings are in agreement with the present results in that they suggest that interference – both with immediate transfer and with consolidation – is larger for the perceptual than for the motoric component of motor sequencing tasks.

Practice Schedule and Sequence-specific Transfer

Both practice groups showed transfer to the triplet-sharing sequence T3, but contrary to our expectation the amount of transfer did not differ between groups. Based on a recent study by Song et al. (2015), which showed that memories for movement transitions were improved after variable compared with constant practice, we expected performance on the triplet-sharing sequence to show similar advantages of variable practice. However, there are several differences between the two studies that could explain this discrepancy. First, Song et al. (2015) evaluated sequence performance after 30 min and 1 week retention periods, whereas transfer in the present study was evaluated immediately after sequence training. It may be possible that sequence-specific benefits of variable practice need an, albeit short (30 min), consolidation period to take effect. Another difference is that Song et al. (2015) used random sequences, both for alternation with the training sequence in the Variable condition and for evaluation of transfer performance. In their study, transfer was quantified by comparing performance on triplets in the random transfer sequence that also appeared in the training sequence (i.e., 'familiar' triplets) with triplets in the random sequence that did not appear in the training sequence (i.e., 'unfamiliar' triplets). In the present study both the alternating training sequence and the transfer sequence were repeating sequences that were specifically designed to match the training sequence in terms of salient statistical properties and overlapping triplets. It is possible that interleaving sequence practice with random sequences has different effects on sequence learning and transfer than interleaving sequence practice with another sequence. It would be interesting to directly compare these two methods of creating alternated training schedules (i.e., alternation with a random sequence vs. alternation with another deterministic sequence).

Finally, one might argue that evaluating transfer based on only the overlapping sub-sequences (as in Song et al., 2015) rather than on the entire transfer sequence would yield a more accurate quantification of sequence-specific knowledge. However, also the specific comparison of improvement scores on familiar and unfamiliar element transitions did not reveal any differences between the Constant and the Variable practice group (see further discussion below).

Transfer of Sequence-specific Knowledge

In support of our second hypothesis, we found sequence-specific transfer effects in addition to sequence-unspecific transfer. Sequence-specific transfer was demonstrated by two observations. First, transfer sequence T3, which shared sequence structure with the training sequences (Tr1 and Tr2), showed larger transfer effects than the sequence without structural overlap (T0). Importantly, transfer sequences T0 and T3 were constructed to have identical lower-level statistical properties, with the key difference between them being that T3, but not T0, contained trained subsequences (i.e., triplets). This strongly suggests that the performance advantage for T3 at transfer was mediated by familiar sequence fragments contained in T3, but not in T0.

This interpretation is further supported by the results of the element-specific analysis. Because both training sequences (Tr1 and Tr2) were constructed to share the same three triplets with transfer sequence T3, we were able compare RT improvements of predictable elements with those of corresponding non-predictable elements. As expected, transfer was larger for predictable elements in T3 than for corresponding non-predictable elements in T3 and T0. A control analysis showed that this was not due to non-specific RT differences between T3 and T0 because improvements for non-predictable elements were similar in both sequences. We did not observe any difference between practice groups or interaction between groups and element-specific improvements. This was in line with our previous results where practice schedule did not seem to affect transfer to the entire T3 sequence. In contrast to the study by Song et al. (2015), we considered only the last element and not the entire shared triplet as familiar. This was because in a second order conditional sequence each element is only determined by its two preceding elements. Thus, if a familiar triplet is placed into a novel sequence context the first two triplet elements are necessary (and sufficient) to predict the third element, but the first two elements themselves are not predictable. Further, it is important to note that the non-predictable elements that were used for comparison with predictable elements required the same button presses (i.e., same finger movements). This excludes the possibility that the observed effect was confounded by simple RT differences between fingers (Lachnit and Pieper, 1990).

The sequence-specific transfer effects resemble *part-whole* transfer where serial task performance is facilitated by previous training of the elemental tasks of a sequence (Schmidt and Lee, 2005; Spruit et al., 2014). Such part-task practice has been commonly studied in relation to complex and difficult real life skills, such as industrial tasks (Seymour, 1954; So et al., 2013), surgery (Dankelman et al., 2005; Spruit et al., 2014), and aircraft control (Adams and Hufford, 1962) and is also an important practice strategy in stage arts like music and dance. In motor-sequence learning paradigms part-whole transfer has been demonstrated for spatiotemporal sequences, where knowledge about the ordinal structure of a sequence partially transferred to sequences with the same ordinal, but a different temporal structure (Ullén and Bengtsson, 2003; Sanchez et al., 2015). Finally, the present results extend the previously described findings by Jiménez et al. (2006), in demonstrating sequence-specific skill transfer after incidental learning even when short sequence fragments (triplets) are taken out of their familiar sequence context and embedded within a novel sequence. Although we did not precisely quantify the amounts of explicit and implicit sequence knowledge it seems likely that triplet-specific sequence transfer was largely implicit since it would be very difficult to identify three overlapping triplets at shifted ordinal positions within a single block of a looping transfer sequence (see Limitations for a further discussion of explicit vs. implicit knowledge).

Limitations

One limitation of the present study is that even though an incidental learning paradigm was employed, we cannot distinguish between transfer of explicit and implicit sequence knowledge. Given that the SRT task is considered to be not a purely implicit learning task (Willingham and Goedert-Eschmann, 1999; Moisello et al., 2009; Abrahamse et al., 2010) and that complete absence of explicit knowledge is difficult to demonstrate (Wilkinson and Shanks, 2004; Abrahamse et al., 2010), it was beyond the scope of the present study to differentiate between the contributions of implicit and explicit sequence knowledge. However, more than two thirds of our participants indicated to have noticed some regularity in the stimulus presentation, suggesting that skill transfer may have involved some explicit knowledge. To differentiate between practice schedule effects on more implicit or explicit knowledge transfer it would be necessary to conduct further studies that directly manipulate the amount of explicit sequence knowledge between participants.

One possible confound in the present study might have been if participants in the Constant practice group developed greater explicit sequence knowledge than those in the Variable group. In this case, group differences in skill transfer could have reflected differences in sequence awareness. However, we think that this is unlikely for two reasons: first, the two groups did not differ in their answers to the sequence awareness questions and second, previous studies have generally not found any relations between explicit sequence knowledge and the amount of skill transfer (Song et al., 2012; Sanchez et al., 2015).

For inter-manual transfer the present design does not allow us to distinguish between contributions from sequence-specific and task-general transfer effects. A non-overlapping transfer sequence for the left hand would have been necessary to control for sequence non-specific inter-manual transfer. However, given the strict constraints on the statistical regularities of our second order conditional sequences we were unable to construct a suitable non-overlapping sequence for the left hand. For future studies it would thus be interesting to include such an inter-manual control sequence to test whether practice schedule affects the task-general component of inter-manual transfer in a similar way as it affected task-general transfer in the trained hand.

Another limitation of this study is that transfer performance was evaluated immediately after training, but not at an additional later time point. In a comprehensive review on the distinction between measures of motor skill learning and performance Kantak and Winstein (2012) point out that delayed retention (e.g., performance measured after 24 h) is a better indicator for motor learning than performance measured immediately at the end of training. The authors argue that performance during acquisition/ at the end of training is influenced by various transient factors that are not reflective of the more permanent performance changes that are indicative of motor learning. Furthermore, different training schedules may have different effects on the mechanisms and neural substrates of skill consolidation (see Kantak et al., 2010; Tanaka et al., 2010). Such effects on the consolidation, rather than the encoding stage can only be detected if performance is assessed after a time delay (e.g., 4–6 h) that allows for consolidation to take place (Kantak et al., 2010). Although the aim of the current study was to evaluate transfer of motor skills, rather than motor learning *per se*, our measures of transfer may have been similarly affected by the presence of transient factors or by the absence of a consolidation period. In fact, the decrease in task-general transfer after constant practice was likely due to such a transient factor at the time of practice (i.e., interference due to acquired sequence expectations). An additional transfer evaluation after a delay period would have provided more information about the temporal persistence of these interference effects. Thus, it would be interesting to investigate in future studies if the effect of practice schedule on sequence-specific transfer differs before and after a consolidation period.

CONCLUSION

Using specifically constructed sequences we were able to show differential effects of practice schedule on different types of skill transfer. A constant practice schedule reduced task-general, but not inter-manual or sequence-specific transfer, suggesting that negative transfer may be an important factor to take into account when similar transfer tasks are performed immediately after a blocked training session. Further, we found that structure-specific knowledge can transfer between sequences, even if the transfer sequence contains only short (i.e., three elements-long) sub-sequences that are embedded within a new sequential context. This finding has an important implication for the design of future SRT paradigms, because it suggests that performance comparisons between training and test sequences should take into account that sequence-specific knowledge may transfer even to short segments of structural overlap that are commonly present in random control sequences.

AUTHOR CONTRIBUTIONS

DM and FU designed the study. DM acquired, analyzed, and interpreted the data. The manuscript was written by DM and revised by FU. Both authors approved the final version of the manuscript.

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Probabilistic Motor Sequence Yields Greater Offline and Less Online Learning than Fixed Sequence

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It is well acknowledged that motor sequences can be learned quickly through online learning. Subsequently, the initial acquisition of a motor sequence is boosted or consolidated by offline learning. However, little is known whether offline learning can drive the fast learning of motor sequences (i.e., initial sequence learning in the first training session). To examine offline learning in the fast learning stage, we asked four groups of young adults to perform the serial reaction time (SRT) task with either a fixed or probabilistic sequence and with or without preliminary knowledge (PK) of the presence of a sequence. The sequence and PK were manipulated to emphasize either procedural (probabilistic sequence; no preliminary knowledge (NPK)) or declarative (fixed sequence; with PK) memory that were found to either facilitate or inhibit offline learning. In the SRT task, there were six learning blocks with a 2 min break between each consecutive block. Throughout the session, stimuli followed the same fixed or probabilistic pattern except in Block 5, in which stimuli appeared in a random order. We found that PK facilitated the learning of a fixed sequence, but not a probabilistic sequence. In addition to overall learning measured by the mean reaction time (RT), we examined the progressive changes in RT within and between blocks (i.e., online and offline learning, respectively). It was found that the two groups who performed the fixed sequence, regardless of PK, showed greater online learning than the other two groups who performed the probabilistic sequence. The groups who performed the probabilistic sequence, regardless of PK, did not display online learning, as indicated by a decline in performance within the learning blocks. However, they did demonstrate remarkably greater offline improvement in RT, which suggests that they are learning the probabilistic sequence offline. These results suggest that in the SRT task, the fast acquisition of a motor sequence is driven by concurrent online and offline learning. In addition, as the acquisition of a probabilistic sequence requires greater procedural memory compared to the acquisition of a fixed sequence, our results suggest that offline learning is more likely to take place in a procedural sequence learning task.

Keywords: online learning, offline learning, probabilistic sequence, sequence knowledge, procedural memory, declarative memory, fast motor sequence learning

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INTRODUCTION

In the laboratory, studies employing the serial reaction time (SRT) task (Nissen and Bullemer, 1987) have demonstrated that adults can learn a motor sequence quickly within a single training session (i.e., in 4 to 8 practice blocks; Nissen and Bullemer, 1987; Willingham et al., 1989; Robertson, 2007). This initial stage of motor sequence learning is referred to as fast learning that leads to the initial acquisition of sequences (Honda et al., 1998; Karni et al., 1998; Walker et al., 2002; Dayan and Cohen, 2011; Censor et al., 2012). Fast learning develops over the course of a single training session, where an individual practices a new motor sequence and demonstrates considerable performance improvement. It has been suggested that such improvement in the performance of motor sequences are driven by online learning (Bornstein and Daw, 2012, 2013; Verstynen et al., 2012), where performance progressively improves as the task is practiced. After the fast learning stage, performance is strengthened without further practice (i.e., offline learning) by an early offline boost (Hotermans et al., 2006; Schmitz et al., 2009) or memory consolidation (Robertson et al., 2004b, 2005; Brown and Robertson, 2007a; Nettersheim et al., 2015). To date, it is unclear whether offline learning drives the acquisition of motor sequence in the fast learning stage. The purpose of this study, therefore, is to examine whether fast learning of a motor sequence arises from offline learning. Furthermore, given that offline learning in the SRT task has been found to be associated with procedural memory (Robertson et al., 2004a; Brown and Robertson, 2007a,b), we further investigate whether a bias towards procedural or declarative memory in the SRT task modulates offline and online sequence learning.

Learning motor sequences in the SRT tasks typically involves both procedural and declarative memory (Willingham et al., 1989; Curran and Keele, 1993; Reber and Squire, 1994; Willingham and Goedert-Eschmann, 1999; Destrebecqz and Cleeremans, 2001; Brown and Robertson, 2007a; Robertson, 2007). In this task, participants press keys on the keyboard to respond to sequential visual stimuli that are presented in a pattern (e.g., a fixed order). Since participants are not informed of the presence of the sequence, learning in the SRT task requires procedural memory. However, participants may recognize the presence of the sequence after they perform the task and thus form a declarative memory of the sequence (Perruchet et al., 1997; Willingham and Goedert-Eschmann, 1999). This entanglement of procedural and declarative learning suggests the infeasibility of eliminating or isolating either of them from the SRT task. Nonetheless, manipulating the sequence type and the preliminary knowledge (PK) of the sequence can modulate procedural or declarative learning. Particularly, it has been shown that learning a probabilistic sequence favors more procedural memory compared to learning a fixed sequence (Jiménez et al., 1996; Song et al., 2007). In contrast, PK of the sequence facilitates declarative learning (Curran and Keele, 1993; Curran, 1997; Destrebecqz, 2004).

In this study, we bias the involvement of procedural/declarative memory by manipulating the sequence type and PK of the sequence in the SRT task to examine whether offline or online learning mediate the acquisition of motor sequences in the fast learning stage. Before the experiment, we informed half of the participants that the visual stimuli followed a specific pattern, but no further information was provided about the sequence. No information about the presence of a sequence was provided to the other participants. The participants were further divided into two groups. In one group, the visual stimuli followed a fixed sequence (i.e., 10 repetitions of a 12-trial sequence) while in the other group; the visual stimuli followed a probabilistic sequence that was generated by a first-order Markov process. We found that a motor sequence is learned quickly through concurrent online and offline learning. However, the involvement of procedural or declarative memory mediated the use of online and offline learning. Particularly, learning of a fixed sequence arose from greater online learning. In contrast, acquisition of a probabilistic sequence resulted from significant offline learning, regardless of PK. These results suggest that the involvement of procedural and declarative memory modulates how a motor sequence is learned in the fast learning stage.

MATERIALS AND METHODS

This study was carried out in accordance with the recommendations and approval of the Institutional Review Board at the University of Maryland, College Park. All participants signed consent forms prior to their participation. Each participant received $10 after the completion of the experiment.

Participants

Forty-eight right-handed adults (24 males, see **Table 1**) were randomly assigned to one of four groups: fixed sequence with PK of the sequence (PK_Fixed; mean age: 21.8 ± 1.91), fixed sequence without PK of the sequence (NPK_Fixed; mean age: 21.5 ± 1.41), probabilistic sequence with PK of the sequence (PK_Prob; mean age: 21.2 ± 0.893), and probabilistic sequence without PK of the sequence (NPK_Prob; mean age; 21.3 ± 0.830). All participants completed a health questionnaire to exclude those with any neurological and motor impairments, the Edinburgh Handedness Inventory (Oldfield, 1971) to assess that participants were right-handed, and the Global Physical Activity Questionnaire (Armstrong and Bull, 2006) to insure that groups did not differ in their level of physical activity.

TABLE 1 | Participant demographic information.

Group	Age (years)[#]	Sex
PK_Fixed	21.8 ± 1.91	6 females; 6 males
NPK_Fixed	21.5 ± 1.41	6 females; 6 males
PK_Prob	21.2 ± 0.893	6 females; 6 males
NPK_Prob	21.3 ± 0.830	6 females; 6 males

[#]*There were no significant differences between the groups in age,* $F_{(3,47)} = 0.564$, $p = 0.642$.

Serial Reaction Time Task

Participants were seated in front of a computer monitor (19″) and keyboard. Participants placed the middle finger of their left hand on the keyboard's "D" key, the index finger of their left hand on the "F" key, the index finger of their right hand on the "J" key, and the middle finger of their right hand on the "K" key (see **Figure 1A**). At the beginning of each trial, a mouse appeared in one of four squares on the screen and the participant pressed the key that corresponded to the location of the stimulus. After the participant pressed a key, the next stimulus appeared after an interval of 300 ms. No visual feedback was provided to participants and a wooden board blocked vision of their finger position. Participants were first randomly assigned to either the PK group or no preliminary knowledge (NPK) group and were further randomly assigned to either the fixed or probabilistic sequence. The probabilistic sequence was created based on a Markov chain transitional matrix with probabilities associated with each stimulus (**Figures 1C,D**). The probabilistic sequence was constrained such that the same stimuli were not repeated one after the other and that each stimulus appeared an equal number of times in each block.

There were a total of six blocks for all groups (see **Figure 1B**), each consisting of 120 trials. Prior to the first block, participants practiced a random sequence. These initial trials were included to ensure that participants were able to accurately associate each finger with a corresponding key before the experimental practice blocks commenced. That is, we observed that participants did not produce reaction times (RTs: amount of time taken to press the corresponding button after the stimulus was presented) that were slower than 2000 m because of incorrect key pressing. After the practice block, participants in the PK groups were informed that a sequence would be present in the subsequent blocks and that they should look for the sequence. No other information about the nature of the sequence was provided. The first four blocks (Blocks 1–4) were the learning blocks consisting of the 120-trial probabilistic sequence or the fixed sequence in which the sequence was repeated 10 times in each block. Block 5 consisted of 120 trials of stimuli occurring in a random order and block 6 consisted of the assigned probabilistic or fixed sequence (**Figures 1C,D**). Participants were given a 2 min mandatory break between each block. The participants' RT was recorded for each trial.

All participants completed a posttest after the completion of the six blocks to determine the amount of declarative knowledge of the sequence. Participants were first asked to recall the sequence and attempted to write down the 12 items of the sequence and rated how confident they were that the sequence they wrote was correct. Participants were then asked to complete a recognition task. They were given eight chunks (i.e., four three-element and four four-element chunks where two of each were correct) and were asked to choose the chunks they thought were included in the sequence.

Data Analysis

The RTs were trimmed according to the individual participant's mean and standard deviation. Within each block for an individual participant, any RT greater or less than 2.5 standard deviations was excluded from the analysis (Ratcliff, 1993; Whelan, 2008). Mean RTs were calculated for each block and were averaged across participants in each group. Learning was measured through a decrease in RT from block 1 to 4 (stimuli in assigned sequence) and an increase in RT from block 4 (stimuli in assigned sequence) to block 5 (stimuli in random order). Online learning was defined as the amount of learning within a block and was determined by performing a linear regression on the 120 RTs within a block. Offline learning was computed as the RT change after a short break without performing the task. Given that the fixed sequence consisted of 10 repetitions of a 12-item long sequence, the difference between mean RT of the last 12 taps in one block and that of the first 12 taps in the succeeding block was used to quantify offline learning. In addition, since participants typically acquire the sequence transitions of higher probabilities in probabilistic sequence learning (Hunt and Aslin, 2001; Howard et al., 2004; Bornstein and Daw, 2012), we expect that participants in the two probabilistic sequence groups would only learn sequential stimuli that were associated with transitional probabilities of 0.3 and 0.6 and fail to learn those associated with transitional probability of 0.1. Thus, we computed mean RT, offline- and online-learning of stimuli with transitional probabilities of 0.3 and 0.6 in the two probabilistic sequence groups.

A controversy regarding offline improvement in RT is whether the improvement results from reactive inhibition/fatigue (Rickard et al., 2008; Brawn et al., 2010) or it is driven by active learning mechanisms (i.e., offline learning; Eysenck and Frith, 1977; Robertson et al., 2004a). According to Eysenck and Frith (1977), in the case of reactive inhibition/fatigue-induced offline improvement, post-rest performance should return to the starting performance level before the rest or so called pre-rest performance, but without improvement over that level. In contrast, post-rest performance is superior to the pre-rest performance if offline improvement arises from offline learning. Given that RT increased (i.e., became slower) within blocks in some participants so that the mean RT of the last 12 taps may not reflect the pre-rest performance, we calculated corrected offline learning. Specifically, if RT increased (i.e., became slower) within the previous block, corrected offline learning was calculated by subtracting the amount of RT deterioration (i.e., negative online learning) within the previous block from the amount of offline learning so that the corrected offline learning reflects the difference between the pre-rest and post-rest performance. If RT improved (i.e., became faster) within the previous block, indicating no RT deterioration, corrected offline learning was the same as offline learning, computed as the difference between mean RT of the last 12 taps in the block and that of the first 12 taps in the succeeding block. We expect that all groups should exhibit the same amount of corrected offline learning (none), if offline improvement in RT observed in this study were caused by reactive inhibition or fatigue.

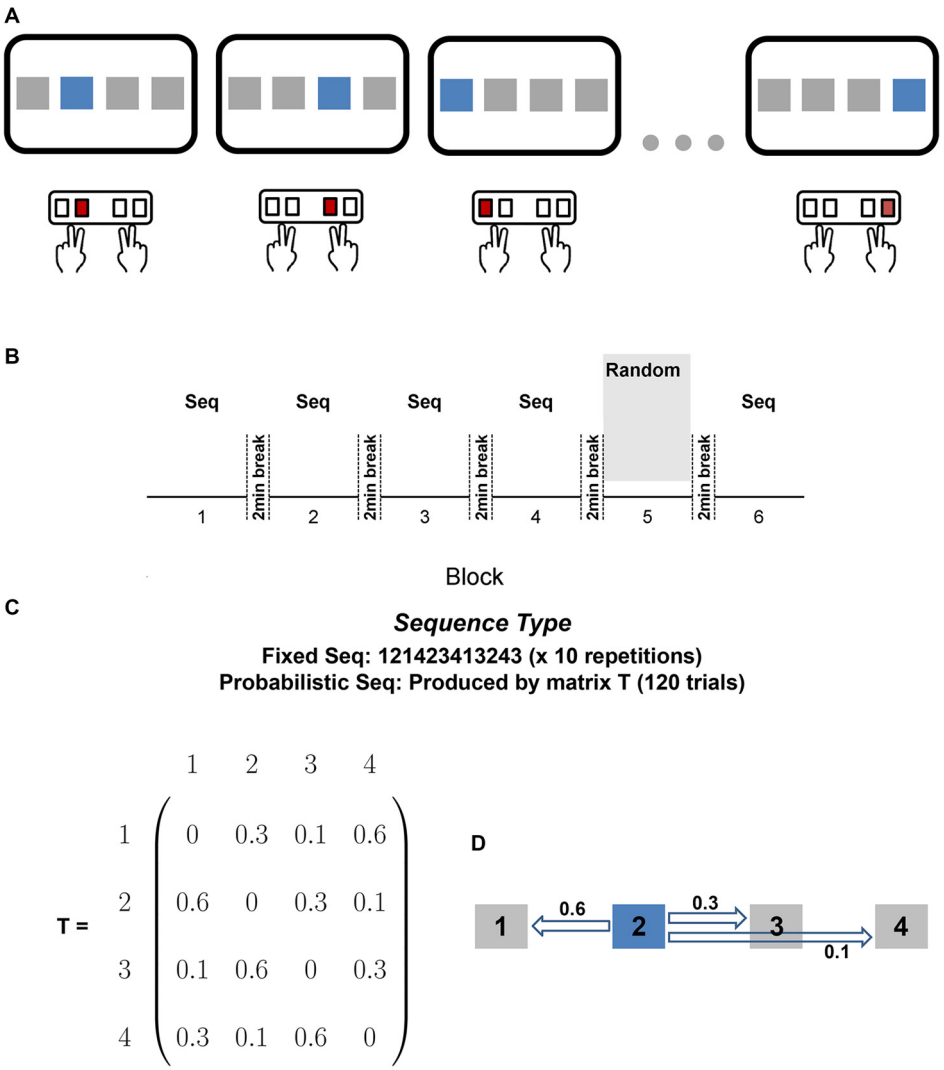

FIGURE 1 | (A) Experimental setup. At the beginning of each trial, a stimulus appeared in one of four squares on the screen and the participant pressed the key that corresponded to the location of the stimulus. Participants placed the middle finger of their left hand on the keyboard's "D" key, the index finger of their left hand on the "F" key, the index finger of their right hand on the "J" key, and the middle finger of their right hand on the "K" key. **(B)** Experimental paradigm. Participants performed the learning blocks (blocks 1–4) with either the fixed or probabilistic sequence, followed by randomly ordered stimuli in block 5, and ended with the same sequence in block 6. All blocks consisted of 120 trials. Participants were given a mandatory 2 min break between each block. **(C)** Sequence Types. Participants were randomly assigned to the fixed sequence group or the probabilistic sequence group. The probabilistic sequence was created using the probabilities defined in the transitional matrix, T. **(D)** Example of how the probabilistic sequence was created using matrix T. If the current stimulus is 2, there is a probability of 0.6 that the next stimulus will be 1, a 0.3 probability that the next stimulus will be 3, and a 0.1 probability that the next stimulus will be 4.

To measure the amount of declarative knowledge of the sequence, we calculated the recognition score as the number of correct chunks that participants chose in the recognition task. The recognition score was normalized by four as there were four correct chunks. To compare the recall score among participants, we calculated the number of three-element chunks that participants could recall. Given there were 12 three-element chunks in the fixed sequence, the number that a participant recalled was normalized by 12 to compute a percentage. To make the amount of declarative knowledge between probabilistic and fixed sequences comparable, the number of three-element chunks that participants could recall was also used in the two groups who performed the probabilistic sequence. Since participants only learned the stimulus transition with transitional probabilities of 0.3 and 0.6 (for details, see "Results" Section), there were 16 three-element chunks in the probabilistic sequence. Thus, the percent of recalled chunks was normalized by 16 in the two probabilistic sequence groups. Importantly, the chance level for guessing differed between the fixed and probabilistic sequence. Specifically, the chance level for a three-element chunk in the fixed sequence was 18.75% (i.e., given the first element, 75% chance for the second element and 25% chance for the

third element) while it was 25% for a three-element chunk in the probabilistic sequence (i.e., given the first element, 50% chance for the second and third elements), we corrected the percentage of recalled chunks by the chance level specific to each sequence group.

Statistical Analysis

A three-way (block × knowledge × sequence) repeated measures analysis of variance (ANOVA) was used to compare differences in RT between the blocks and groups. Separate pairwise comparisons were conducted on the priori contrasts of interest (block 1 vs. block 4 and block 4 vs. block 5) to determine any significant differences between the sequenced blocks and the random block. A three-way (block × knowledge × probability) ANOVA was used to compare differences in RT of stimuli with different probabilities in the two probabilistic groups. All repeated measures ANOVAs were performed in SAS with the MIXED procedure. Thus, the co-variance matrix structures were determined by the Akaike information criterion (AIC). A two-way (knowledge × sequence) ANOVA was employed to examine the effects of PK and sequence type on online, offline learning, and corrected offline learning. A two-way (knowledge × sequence) ANOVA was employed to examine the effects of PK and sequence type on the recall score. Given the violation of the normality assumption, the effects of PK and sequence type on the recognition score was examined by the Scheirer-Ray-Hare test. Tukey-Kramer *post hoc* tests were used to decompose any significant effects. Student's *t*-tests/Wilcoxon tests were used to examine whether recall/recognition scores were different from the corresponding chance level for each group. The statistical significance level was set as $\alpha = 0.05$.

RESULTS

Figure 2A shows the mean RT across the six blocks. The repeated measures ANOVA reveals a significant interaction between PK, sequence type, and block ($F_{(5,44)} = 2.79$, $p < 0.05$). *Post hoc* analyses with the Tukey-Kramer correction found that all four groups produced comparable RTs in all blocks (all $p > 0.2$). However, RT in two groups who performed the fixed sequences (i.e., PK_Fixed and NPK_Fixed) improved from blocks 1 to 4 and 6 (all $p < 0.0001$). In contrast, RT remained the same from block 1 to 4 in the other two probability sequence groups (i.e., PK_Prob and NPK_Prob, **Figure 2B**; all $p > 0.1$). Nevertheless, RT was faster in block 6 compared to block 1 in the NPK_Prob group ($p < 0.01$) and this improvement approached significance in the PK_Prob group ($p = 0.09$). In addition, when a random sequence was introduced in block 5, RT in the PK_Fixed and NPK_Fixed groups deteriorated (both $p < 0.0001$) while it remained the same between blocks 4 and 5 in the PK_Prob and NPK_Prob groups (both $p = 1$; **Figure 2C**).

The inferior learning in the probabilistic sequence (as expressed in no change in RT from block 1 to 4 and between blocks 4 and 5) is consistent with the hypothesis that probabilistic sequences are harder to learn compared to fixed sequences (Schvaneveldt and Gomez, 1998). However, given our hypothesis that participants typically acquire the sequence transitions of higher probabilities (Hunt and Aslin, 2001; Howard et al., 2004; Bornstein and Daw, 2012), the marginal learning effect on the probabilistic sequence likely resulted from the difference in RT among stimuli with different transitional probabilities (**Figure 1C**). Thus, we compared RTs between these stimuli (**Figure 2D**) in the probabilistic sequence. A three-way (block × knowledge × probability) repeated measures ANOVA found that PK does not significantly affect RT and there was a significant interaction between block and probability ($F_{(10,220)} = 17.07$, $p < 0.0001$). *Post hoc* analyses with the Tukey-Kramer correction revealed that RTs of stimuli with a transitional probability of 0.1 were comparable to that of stimuli with transitional probability of 0.3, while RTs of stimuli with transitional probability of 0.3 were slower than that of probability of 0.6 ($p < 0.01$). However, as learning progressed, RTs of stimuli with a transitional probability of 0.1 remained the same. In contrast, RTs improved from blocks 1 to 4 in stimuli with higher transitional probabilities 0.3 ($p < 0.01$) and 0.6 ($p < 0.0001$), suggesting learning of these higher transitional probabilities (**Figure 2E**). Additionally, introduction of a random sequence in block 5 did not impair RT of stimuli with transitional probabilities of 0.1 and 0.3, but RTs of stimuli with a transitional probability of 0.6 deteriorated in block 5 ($p < 0.0001$; **Figure 2F**). These results confirm that the participants learned stimulus transitions with higher probabilities, specifically 0.6 and perhaps 0.3.

Since participants only learned higher transitional probabilities when stimuli followed a probabilistic pattern, we re-compared the learning effects among groups by using RT for stimuli with transitional probabilities 0.3 and 0.6 in PK_Prob and NPK_Prob groups. A repeated measures ANOVA revealed a significant interaction among the effects of block, PK, and sequence ($F_{(5,44)} = 3.1$, $p < 0.05$). Tukey-Kramer-corrected *post hoc* analyses suggest that all groups had comparable mean RTs across all blocks (**Figure 3A**). In addition, all groups demonstrated improved mean RT from block 1 to 4 (all $p < 0.0001$) and deteriorated mean RT from block 4 to 5 (all $p < 0.005$). However, contrast analyses showed that the PK_Fixed group had the greatest change in RT from block 1 to 4 compared to the NPK_Fixed ($p < 0.05$), PK_Prob ($p < 0.0005$), and NPK_Prob groups ($p < 0.0005$; **Figure 3B**), while the latter three groups exhibited the same change in RT. Similarly, the RT change from block 4 to 5 was greater in the PK_Fixed group compared to the other three groups (all $p < 0.01$) who had the same RT change (**Figure 3C**). These results suggest that the PK_Fixed group learned better than the other three groups.

Although participants learned either fixed or probabilistic sequences with or without PK of the sequence, learning across trials exhibited different patterns (**Figure 4A**). Specifically, learning of a fixed sequence exhibits decreased RT within blocks while learning of a probabilistic sequence exhibits reduced RT after rest without practice. A two-way (knowledge × sequence) ANOVA found a significant effect

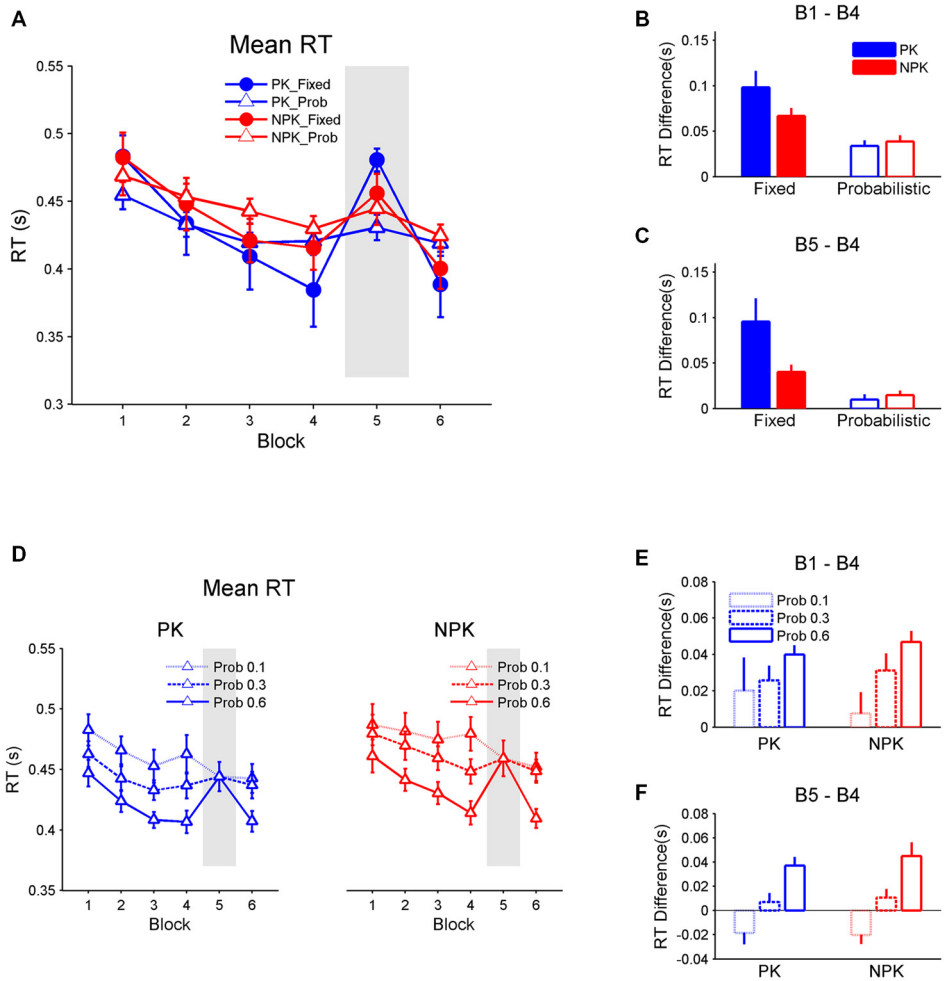

FIGURE 2 | (A) Mean RT and SE bars across the six blocks for all four groups. **(B)** Difference between the RT in block 1 and block 4 to assess whether sequence learning occurred. **(C)** Difference between block 5 and block 4 to assess whether RT increases in block 5 when a random sequence is presented. **(D)** Mean RT and SE bars across the six blocks for only the probabilistic sequence in which the three transitional probabilities (Pro 0.1, Pro 0.3, and Pro 0.6) have been extracted and plotted separately. **(E)** Difference between RT in block 1 and block 4. **(F)** In block 5 and block 4 separated for the three transitional probabilities in the probabilistic sequence. PK, preliminary knowledge; NPK, no preliminary knowledge; RT, reaction time; SE, standard error.

of sequence on offline learning ($F_{(1,44)} = 8.84$, $p < 0.005$). Particularly, the acquisition of the probabilistic sequence arises from greater offline learning compared to the acquisition of the fixed sequence (**Figure 4B**). Although sequence type was also found to significantly affect online learning ($F_{(1,44)} = 18.72$, $p < 0.0001$), it was shown that greater online learning was produced when a fixed sequence was learned (**Figure 4B**). Interestingly, when learning a probabilistic sequence, participants did not exhibit online learning. Instead, RT became slower within blocks. We further compared whether online or offline learning contributed more to the acquisition of a motor sequence. A two-way (knowledge × sequence) ANOVA on the RT difference between offline and online learning revealed a significant effect of sequence type ($F_{(1,44)} = 15.27$, $p < 0.0005$). Student's *t*-tests found equal online and offline learning when a fixed sequence is performed ($p = 0.59$), while greater offline compared to online learning was found when a probabilistic sequence was performed ($p < 0.0001$).

We also analyzed the corrected offline learning. The same results were found compared to the original offline learning data (**Figure 4C**). A two-way (knowledge × sequence) ANOVA found a significant effect of sequence ($F_{(1,44)} = 4.99$, $p < 0.05$). Specifically, there was greater corrected offline learning in PK_Prob and NPK_Prob groups compared to PK_Fixed and NPK_Fixed groups. These results suggest that offline learning rather than reactive inhibition/fatigue underlies the offline improvement in RT.

In the posttest, we found that the recognition score did not differ from chance (i.e., 50%) in all four groups and there were no effects of sequence type and PK on the scores. **Figure 5A** shows the percentage of recalled three-element chunks. It is clear that participants in the fixed sequence groups had higher than chance recall, while recall was at

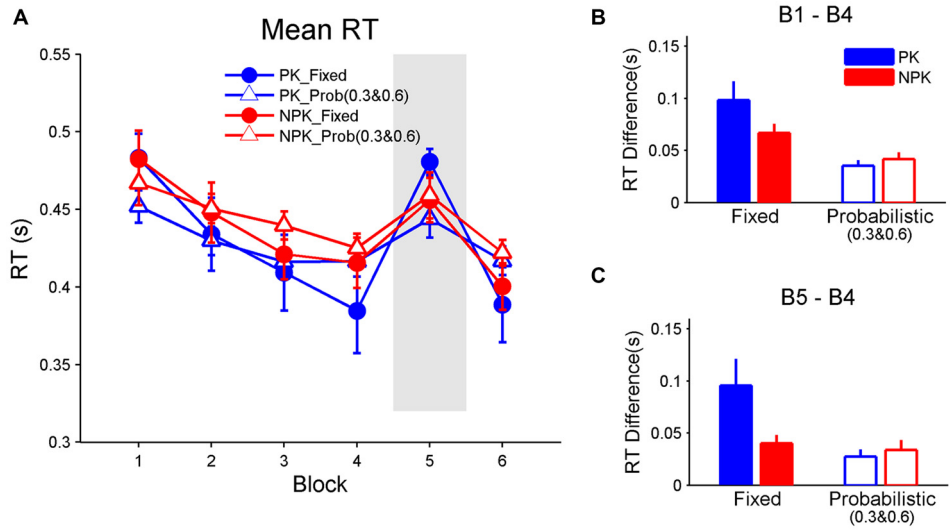

FIGURE 3 | Mean RT and SE bars to assess learning. Only the RT of stimuli with transitional probabilities of 0.3 and 0.6 were extracted and are shown for the probabilistic sequences. **(A)** Mean RT across the six blocks. **(B)** Difference between the RT in block 1 and block 4 to assess whether sequence learning occurred. **(C)** Difference between block 5 and block 4 to assess whether RT increases in block 5 when a random sequence is presented. PK, preliminary knowledge; NPK, no preliminary knowledge; RT, reaction time; SE, standard error.

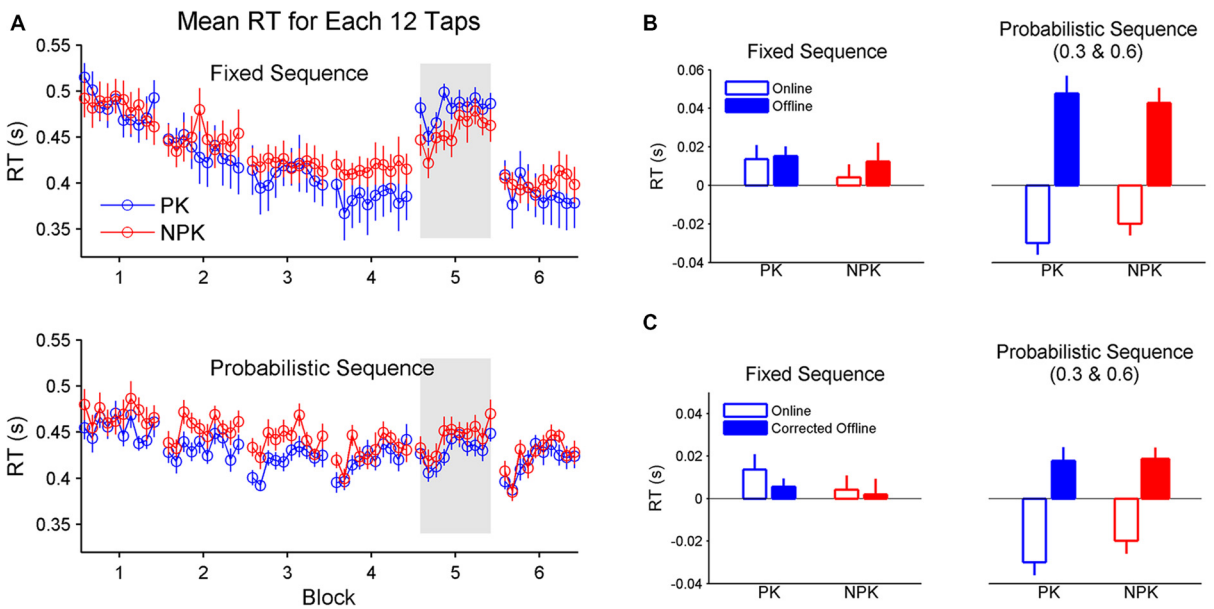

FIGURE 4 | (A) Mean RT of each 12 taps to reflect online and offline learning. **(B)** Comparison of online and offline learning between the groups. **(C)** Comparison of online and corrected offline learning between the groups. Error bars represent standard errors. PK, preliminary knowledge; NPK, no preliminary knowledge; RT, reaction time.

chance in the two probabilistic sequence groups. The corrected percentage according to the chance level was shown in **Figure 5B**. A two-way ANOVA found a significant effect of sequence type ($F_{(1,44)} = 6.75$, $p < 0.01$). Specifically, recall of the fixed sequence was superior compared to that of probabilistic sequence. In addition, using Student's *t*-tests with an adjusted *p* level of $\frac{\alpha}{4} = 0.0125$ to control the familywise error rate for the four simultaneous *t*-tests, the recall in the PK_Fixed was significantly higher than chance level ($p < 0.0001$) The recall in the NPK_Fixed did not differ from chance (but approached significance, $p = 0.0146$), In contrast, recall in the two groups that performed the probabilistic sequence (i.e., PK_Prob and

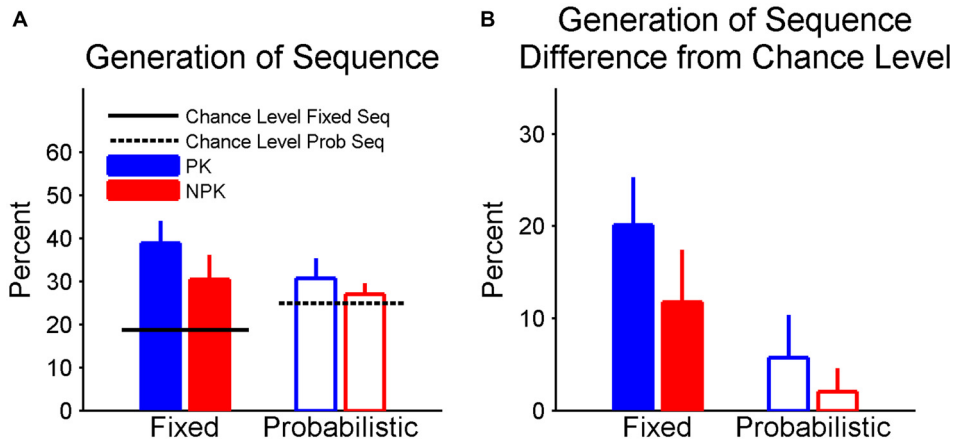

FIGURE 5 | (A) Percentage of three-element chunks recalled in the posttest. **(B)** Corrected percentage according to chance level. Error bars represent standard errors. PK, preliminary knowledge; NPK, no preliminary knowledge.

NPK_Prob) was not significantly different from the chance level (both $p > 0.2$).

DISCUSSION

In this study, we demonstrated that both fixed and probabilistic motor sequences can be learned quickly (i.e., in one training session). Further, this initial acquisition of a fixed sequence in the fast learning stage arises from both online and offline learning, while acquisition of a probabilistic sequence is driven predominantly by offline learning. Given that learning a probabilistic or fixed sequence requires greater procedural or declarative memory, respectively, our results suggest that a bias toward procedural or declarative memory modulates how a motor sequence is learned in the fast learning stage.

Offline learning, as a salient feature underlying motor sequence learning (Robertson et al., 2004a), can boost the memory of a newly acquired sequence 5–30 min after the initial acquisition (Albouy et al., 2006; Hotermans et al., 2006, 2008; Schmitz et al., 2009; Nettersheim et al., 2015) or consolidate the memory a few hours later without sleep (Robertson et al., 2004a; Brown and Robertson, 2007a,b) or after sleep (Walker et al., 2002; Robertson et al., 2004b; Censor et al., 2012; Nettersheim et al., 2015). Thus, offline learning has been widely considered to occur only after the initial acquisition of sequences that develops over the course of a single training session, referred to as fast learning (Honda et al., 1998; Karni et al., 1998; Walker et al., 2002; Dayan and Cohen, 2011; Censor et al., 2012). Unlike the widely-found offline learning that occurs following the fast learning stage, we observed offline learning that drives the fast acquisition of sequences within a first single training session. This result suggests that in addition to online learning (Cleeremans and McClelland, 1991; Bornstein and Daw, 2012), offline learning also contributes to rapid improvements in performance that allow sequences to be learned quickly in a single training session.

The concurrent effect of online and offline learning could be modulated by the involvement of declarative and procedural memory. It is widely accepted that both memory systems cooperate and compete during motor sequence learning (Meulemans et al., 1998; Brown and Robertson, 2007b). Remarkably, the presence of declarative memory inhibits offline learning of procedural memory and thus disruption of declarative memory induces offline improvement in procedural skills 4 h after the initial acquisition (Brown and Robertson, 2007a). In our study, similar effects of declarative and procedural memory were observed on offline learning in the fast learning stage. The recognition and recall tests were used to measure the engagement of declarative and procedural memory in the SRT task. Although the recognition test shows no differences in the amount of declarative knowledge acquired by participants regardless of the sequence type and PK (see details below), the recall scores reveal that, participants acquired less declarative knowledge of the probabilistic sequence. Notably, participants exhibited greater offline learning when performing probabilistic sequences, suggesting that the offline learning in the fast learning stage was strengthened when greater procedural memory and less declarative memory were required to learn the motor sequences. On the other hand, when greater declarative memory was involved in learning fixed sequences, as indicated by higher recall scores, reduced offline and greater online learning were observed. This inverse relationship between online and offline learning confirms the inhibition effect of declarative memory on offline learning. More importantly, our finding extends our understanding of the competition between multiple memory systems. That is, unlike previous studies that demonstrated this competition after skills are acquired (Poldrack et al., 2001; Foerde et al., 2006; Brown and Robertson, 2007b), we demonstrated that the competition begins as soon as learning starts and that declarative and procedural memory may be identified by their distinct behavioral expressions.

The offline learning observed within a single training session (i.e., the fast learning stage) is associated with procedural memory as is offline learning that takes place hours after the initial acquisition and is responsible for memory consolidation. However, it remains unclear whether this offline learning that allows fast initial acquisition of a motor sequence is related to offline learning that consolidates the memory of a newly acquired sequence. It is possible that offline learning that drives the fast acquisition is a precursor of the later occurring memory consolidation, or they may be the same process. To elucidate their relationship, further systematic investigations are needed.

A debate within the offline learning literature is whether offline improvement in performance after rest, referred to as reminiscence (Eysenck and Frith, 1977), results from fatigue or reactive inhibition (Rickard et al., 2008; Brawn et al., 2010) or an active learning mechanism (Eysenck and Frith, 1977; Robertson et al., 2004a). It has been suggested that offline learning and reactive inhibition/fatigue are usually combined to lead to reminiscence (Eysenck, 1965), thus making it difficult to determine if reactive inhibition/fatigue is a potential cause of reminiscence. However, observations from our data favor offline learning to reactive inhibition/fatigue as the primary mechanism underlying offline improvement in RT or reminiscence observed in the SRT task. Specifically, with the same amount of practice, only participants who performed the probabilistic sequence slowed down their RT, while such "fatigue" was not observed when participants performed a fixed sequence. In addition, if fatigue appeared as soon as participants in the probabilistic sequence groups started to perform the task, it would be unlikely that their learning would arise quickly (i.e., over four learning blocks) and to a comparable level as the participants in the fixed sequence groups who did not exhibit fatigue. Moreover, according to Eysenck and Frith (1977), reminiscence is task-specific. For example, reminiscence that results from reactive inhibition or fatigue usually occurs in a task that does not involve learning, where performance on the task is already perfect when an individual starts to perform the task. In contrast, reminiscence that arises from offline learning usually takes place in a learning task. Obviously, the SRT task involves sequence learning and our data demonstrated that participants learned the sequence. Further evidence supporting offline learning rather than reactive inhibition or fatigue comes from the observation on corrected offline learning. In the probabilistic sequence groups, performance after the short break is superior to the best performance level before the break. Therefore, without fully excluding the effect of reactive inhibition/fatigue, our results favor the statement that the offline improvement in RT is driven by offline learning rather than reactive inhibition or fatigue. Meanwhile, we suggest that it is necessary to systematically examine the reactive inhibition or fatigue effects in future sequence learning studies.

Although it appears that offline learning rather than reactive inhibition or fatigue is the primary mechanism underlying the offline improvement in RT, the cause of increased RT when learning a probabilistic sequence is unclear. One likely reason is the interference of stimuli transitions with a probability of 0.1. It has been found that adults learned a sequence by iteratively updating the internal model of the motor sequence (Cleeremans and McClelland, 1991; Bornstein and Daw, 2012, 2013; Verstynen et al., 2012) and our data provide consistent evidence that participants acquired the stimulus transitions with probabilities of 0.3 and 0.6. However, the introduction of stimulus transition governed by a probability of 0.1 may mislead the updating of the internal model (i.e., transitional probability matrix) and thus impair RT when the probabilistic sequence was performed.

In addition to the primary findings on online and offline learning, our results provide insights into the learning of probabilistic sequences. Sequence structure plays a critical role in motor sequence learning (Curran and Keele, 1993; Jiménez et al., 1996; Bennett et al., 2007; Song et al., 2007). To date, a variety of probabilistic sequences have been used in the SRT task, but only a few studies have employed probabilistic sequences that represent the stochastically related events of daily life, such as sequences produced by a finite state grammar (Jiménez et al., 1996) or a Markov chain. We found that participants acquired stimulus transitions with higher probabilities of 0.3 and 0.6 and the learning of these higher stimulus transitions was comparable to that of the fixed sequence. Moreover, the facilitating effect of PK of a sequence depends on the sequence structure, which is consistent with previous studies (Jiménez et al., 1996; Stefaniak et al., 2008). Specifically, PK only facilitates the learning of a simple sequence, such a fixed sequence (Curran and Keele, 1993; Frensch and Miner, 1994; Curran, 1997; Destrebecqz, 2004; Stefaniak et al., 2008) and not a sequence with a complex structure.

Finally, one caveat worthy of further study is the measurement of the amount of declarative knowledge. Both recognition and recall tests are most widely used to examine procedural learning in the SRT task (Shanks and Johnstone, 1999; Wilkinson and Shanks, 2004; Destrebecqz and Peigneux, 2005). In particular, these tests examine whether participants can explicitly recollect the acquired sequence knowledge. However, results from the recognition tests are equivocal in the literature (Perruchet and Amorim, 1992; Willingham et al., 1993; Reed and Johnson, 1994; Shanks and Johnstone, 1999). Similarly in our study, unlike the recall tests demonstrating the common finding that probabilistic sequence learning favors more procedural memory (Jiménez et al., 1996; Song et al., 2007), the recognition tests reveals no difference in the amount of acquired declarative knowledge despite the sequence type and PK. In addition, the recognition scores in all four groups were not greater than chance. Given that in the recognition test, participants were presented with sequence segments and were asked to determine whether these segments are from the sequence they learned or a new sequence they did not see in the SRT task, it is hard to know whether the chance-level score was due to the participant's inability to explicitly recollect sequence knowledge or that the participant did not learn some segments of the sequence. These two possibilities that may simultaneously account for the chance-level recognition must be addressed by other tests in future studies. Moreover, in our study, only four correct sequence segments were given to participants,

while there were more than 10 segments within the learned sequence, the chance-level recognition score was caused possibly because some participants may learn segments other than the four displayed in the recognition test.

In summary, we found that concurrent online and offline learning allows motor sequences to be acquired quickly in the fast learning stage and can be identified by their manifestations in the progressive changes in RT. Remarkably, online and offline learning can be mediated by the declarative and procedural memory that are required to learn motor sequences. In addition, the modulation of online and offline learning may reflect the competition between both memory systems during motor sequence learning that begins in the fast learning stage. How the offline learning that drives the initial acquisition of sequences is related to the offline learning that is responsible for memory consolidation occurring hours after the initial acquisition remains to be investigated.

AUTHOR CONTRIBUTIONS

YD, SP, IS, and JEC designed the experiment. IS performed the experiment. YD and SP analyzed the data and prepared all tables and figures. YD, SP, and JEC wrote the manuscript. All authors reviewed the manuscript.

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Cognitive Fatigue Facilitates Procedural Sequence Learning

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Enhanced procedural learning has been evidenced in conditions where cognitive control is diminished, including hypnosis, disruption of prefrontal activity and non-optimal time of the day. Another condition depleting the availability of controlled resources is cognitive fatigue (CF). We tested the hypothesis that CF, eventually leading to diminished cognitive control, facilitates procedural sequence learning. In a two-day experiment, 23 young healthy adults were administered a serial reaction time task (SRTT) following the induction of high or low levels of CF, in a counterbalanced order. CF was induced using the Time load Dual-back (TloadDback) paradigm, a dual working memory task that allows tailoring cognitive load levels to the individual's optimal performance capacity. In line with our hypothesis, reaction times (RT) in the SRTT were faster in the high- than in the low-level fatigue condition, and performance improvement was higher for the sequential than the motor components. Altogether, our results suggest a paradoxical, facilitating impact of CF on procedural motor sequence learning. We propose that facilitated learning in the high-level fatigue condition stems from a reduction in the cognitive resources devoted to cognitive control processes that normally oppose automatic procedural acquisition mechanisms.

Keywords: cognitive fatigue, motor sequence learning, memory competition, serial reaction time (SRT) task, skill learning, procedural learning

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INTRODUCTION

Animal studies (e.g., White and McDonald, 2002) and clinical evidence in humans (e.g., ; Heindel et al., 1989; Tranel et al., 1994) show that memory is not a unitary phenomenon. Rather, it is best understood as the result of a combination of different systems or brain processes that either operate in parallel or enter in competition. On one hand, memory systems can cooperate in a compensatory way (e.g., during route recognition in Huntington disease, hippocampus activity can compensate for the gradual dysfunction of the caudate nuclei; Voermans et al., 2004). On the other hand, brain systems can interact in a competitive relationship (Hartley and Burgess, 2005) to access and integrate information in such a way that disabling one system gives free rein to another to mediate the learning process (for a review see Krupa, 2009). For instance, there is a competitive relationship between the striatal and medial temporal lobe (MTL) regions, in such a way that implicit memory performance is better when striatal activity is high and MTL activity is low; and conversely explicit memory performance is better when MTL activity increases and striatal activity diminishes (Poldrack et al., 2001). Likewise, there is a negative coupling between the activity of the anterior cingulate/medial prefrontal cortex and the striatum during explicit but not implicit memory

retrieval (Destrebecqz et al., 2005). Since implicit memory was exclusively associated with striatal activity in this latter study, it is suggested that the influence of implicit processes can be successfully controlled by conscious knowledge during explicit memory retrieval.

The competition between the prefrontal cortex and basal ganglia systems has also been invoked for the control of behavior (Daw et al., 2005). In this framework, experimental manipulations that reduce the efficiency of executive control and attentional systems, e.g., the disruption of dorsolateral prefrontal cortex activity by transcranial magnetic stimulation (TMS; Galea et al., 2010; Smittenaar et al., 2013), hypnosis (Nemeth et al., 2013) or increased working memory demands (Filoteo et al., 2010) have been shown to enhance consolidation and the acquisition of procedural skills. Altogether, these studies suggest that learning in a particular memory system is facilitated under circumstances in which the expression of other competing memory systems is hampered.

Another condition depleting the availability of controlled resources is mental or cognitive fatigue (CF), defined as the decrease in cognitive resources developing over time on sustained cognitive demands independently of sleepiness (Trejo et al., 2005). CF is associated with impaired cognitive control (Lorist et al., 2005), high-level information processing (Tanaka et al., 2012) and sustained attention (Langner et al., 2010). Exposure to High Cognitive Load (HCL) levels, conditions where the time to process ongoing cognitive demands is restricted, also leads to increased CF (Borragán et al., submitted). Magnetoencephalographic data suggest that impaired activity in the anterior cingulate and dorsolateral prefrontal cortical regions triggers the subjective feeling of CF and the decision to rest (Ishii et al., 2014). Accordingly, arterial spin labeling perfusion fMRI has evidenced deactivation in the fronto-parietal network during rest after sustained mental workload (Lim et al., 2010). In this framework, CF might directly diminish available cognitive reserves and facilitate the disengagement of resources consuming controlled top-down memory systems. Hence, reduced goal-directed attention with CF would eventually lead to stimulus-driven performance (Boksem et al., 2005).

In the present study, we tested the hypothesis that CF would facilitate performance in automatic, procedural forms of learning that do not require, or are potentially hampered by, controlled cognitive resources. To do so, we investigated whether triggering high levels of CF may enhance acquisition performance in a motor procedural serial reaction time (SRT) task. At the neuroanatomical level, we assumed that mostly basal ganglia activity would subtend learning in the high CF condition, considering that high CF deplete the fronto-parietal resources underlying attentional and executive functions (Lorist et al., 2005; Lim et al., 2010; Ishii et al., 2014). More specifically, we reasoned that triggering high levels of CF before learning would hamper the prefrontal executive resources competing with subcortical activity and support the controlled declarative memory component of the task. Indeed, striatal activity supports habit formation (Yin and Knowlton, 2006) and implicit sequence learning (Destrebecqz et al., 2005), and increasing the working memory load biases neural competition in favor of habit memory mechanisms (Foerde et al., 2006). As a result, implicit motor procedural learning that mainly relies on striatal activity should develop better.

MATERIALS AND METHODS

Participants

Twenty-three French-speaking participants (17 women, 4 left-handed; mean age ± SD 23.04 ± 4.14 years) without any history of psychiatric or neurological disease gave their written, informed consent to participate in the present study conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Faculty of Psychological Sciences (Université Libre de Bruxelles, ULB). All participants had normal to acceptable sleep quality in the past month (Pittsburgh Sleep Quality Index score <7; Buysse et al., 1989). Participants also exhibited moderate to neutral chronotype (31 > morningness–eveningness questionnaire score < 70; Horne and Ostberg, 1976).

Material and Tasks

Cognitive Fatigue Induction: Time load Dual-back (TloadDback Task)

The Time load Dual-back (TloadDback; Borragán et al., submitted) is a task in which different levels of cognitive load can be induced and individually adjusted by modifying the time available to process and manipulate the ongoing task demands. Basically, the TloadDback task is a dual task featuring a classical N-back working-memory updating task (Kirchner, 1958) and a parity number decision task. Digits and letters are displayed in alternation on the screen, and participants are instructed to press the space bar with their left hand every time the displayed letter is the same as the penultimate letter, or to indicate whether the displayed digit is odd or even by pressing "1" or "2" on the numeric keypad. Combining two tasks featuring different requirements for information processing ensures a large recruitment of working memory resources, an involvement that can be adjusted with the pace at which the information is processed. During a pre-test session, the maximal load level (i.e., the fastest stimulus time duration (STD) allowing accuracy performance >85%) is determined separately for each participant. This maximal load level corresponds to the HCL condition. In the Low Cognitive Load (LCL) condition, stimulus presentation rate is made 1/3 slower [i.e., STD (LCL) = STD (HCL) + 1/2 STD (HCL)]. Hence, both LCL and HCL conditions have the same level of complexity, but the available processing time is proportionally different and tailored to each participant's processing capacity. The duration of the task is 16 min. The evolution of CF is assessed: (a) at the subjective level using a Visual Analogue Scale for fatigue (VASf; Lee et al., 1991) before and after the TloadDback task; and (b) objectively by computing the evolution of performance within the TloadDback task (Lorist et al., 2000; van der Linden et al., 2003; Campagne et al., 2004; Faber et al., 2012). Performance levels are computed over four successive time periods (t1, t2, t3, t4) including each ± 20% of the total number trials.

Procedural Learning: Serial Reaction Time Task

We used a tactile variant of the classical Serial Reaction Time Task (SRTT; Nissen and Bullemer, 1987). In this version (Borragán et al., 2015), stimuli (i.e., the drawing of a car) were presented using E-Prime Software (Psychology Software Tools) at one out of the four corners on a computer screen (16 inches; refresh rate of 60 Hz) adapted for tactile responses (Magic Touch Add-On Touch Screen, KeyTec-Inc.). Participants were instructed to press the location of the stimulus with their right hand as quickly and as accurately as possible. The stimulus remained on the screen until subject's response, with the next stimulus being displayed immediately after the response (response stimulus interval [RSI] = 0 ms). The learning session consisted of eight blocks (B1 to B8; 96 stimuli/block) for an approximate duration of 6–7 min. Unbeknownst to participants, a fixed 12-element sequence of positions (**A**: 1, 4, 2, 1, 3, 2, 4, 1, 3, 4, 2, 3 or **B**: 3, 2, 4, 1, 3, 4, 1, 2, 4, 3, 1, 4) was repeated during six blocks (B2 to B6 and B8). In blocks B1 and B7, the succession of positions was pseudo-random. Trills (e.g., 1, 2, 1), runs (e.g., 1, 2, 3, 4) and repetitions (e.g., 1, 1) are excluded both in regular sequences A and B and in pseudo-random sequences (Goedert and Willingham, 2002). Sequence A (respectively, B) was used for SRT learning on day 1, and sequence B (respectively, A) for SRT learning on day 2, in a counterbalanced order.

Sequence Generation Task

At the end of the experimental session on day 2, participants were informed about the presence of a repeated sequence of stimuli in the majority of the SRTT blocks, and that their knowledge about the regularities of the sequence practiced on day 2 will be assessed in a generation task (Destrebecqz and Cleeremans, 2001). The generation task is an adaptation to sequence learning of the process dissociation procedure (PDP; Jacoby, 1991). It aims at providing a measure of how implicit and explicit memory components contribute to performance in a single task. In the Inclusion condition, participants have to reproduce the learned sequence of stimuli by pointing to the successive positions on the tactile screen for 96 trials (i.e., 1 block). If they claim having no explicit memory of the sequence, they are encouraged to follow their best feeling. Hence, generation performance can be due both to explicit and implicit knowledge in this Inclusion condition. Contrarily, in the Exclusion condition, participants are asked to generate a sequence of positions that is different from the learned sequence, also for 96 trials. In this case, continued generation of learned elements in spite of the exclusion instructions indicate a lack of conscious knowledge, as participants are unable to prevent producing familiar elements. Inclusion and Exclusion condition order was counterbalanced between subjects.

Generation performance in the Exclusion and Inclusion conditions is computed as the percentage of generated triplets (or chunks) belonging to the learned sequence (i.e., maximal 100% score is obtained with 94 correctly generated triplets out of 94, as the total number of stimuli is 96). Chance level is 33%. In addition, an index of explicit knowledge is calculated by computing the difference between inclusion and exclusion scores (I-E). A higher index signifies a higher level of explicit knowledge and conscious control over the learned sequence (for details, see Destrebecqz et al., 2005).

Procedure

Our experimental design is illustrated **Figure 1**. To ensure similar levels of vigilance over the 3 days of the experiment, a 5-min version of the psychomotor vigilance task (PVT; Dinges and Powell, 1985) was administered at the beginning of each session. On day 0, a pretest session determined the maximal cognitive load capacity for each participant through the TloadDback task.

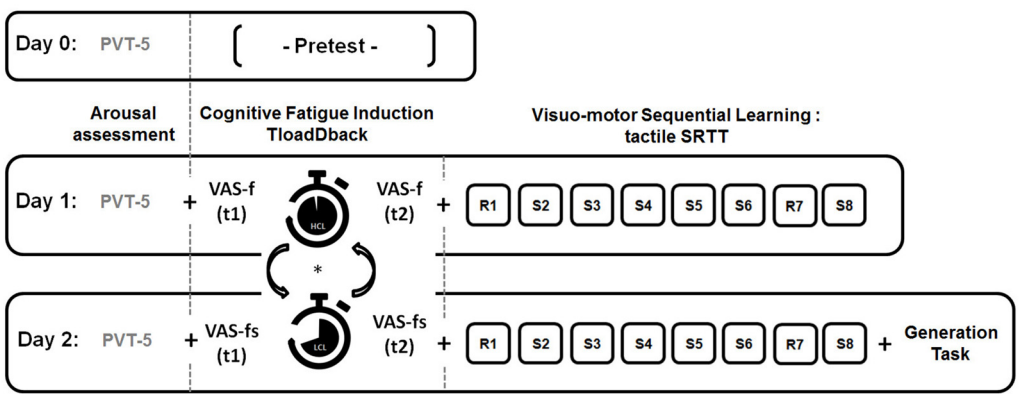

FIGURE 1 | Experimental design. On Day 0, participants are administered a pre-test to determine their maximal cognitive load capacity on the TloadDback task (i.e., fastest pace allowing accuracy performance >85%) . On Day 1 and Day 2, they perform the TloadDback task either in a High Cognitive Load (HCL) or in a Low Cognitive Load (LCL) condition, in counterbalanced order. Immediately before and after completion of the TloadDback task, participants complete the Visual Analogue Scale of fatigue (VAS-f). They are then administered the Serial Reaction Time Task (SRTT) using either repeated sequence A or B, in counterbalanced order. Additionally, at the end of the Day 2 session, they are asked to accomplish a generation task to test their knowledge about the sequential patterns in the last learned sequence. Vigilance levels prior to the beginning of the experiment are measured every day using a psychomotor vigilance task (PVT-5). Each session lasted approximately 30 min.

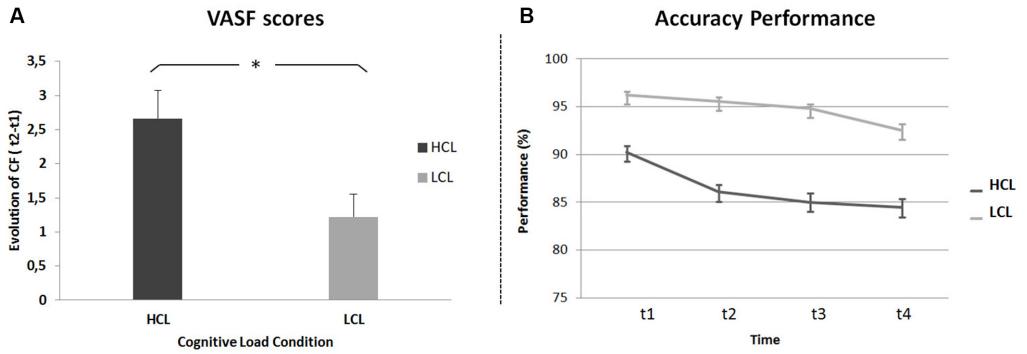

FIGURE 2 | Cognitive Fatigue (CF). (A) Task-related CF (difference between VAS-f scores before and after the TloadDback task) in high (HCL) and low (LCL) cognitive load conditions. **(B)** Evolution of performance (accuracy scores) across four quartiles (± 3 min each) during the TloadDback in the HCL and the LCL conditions. Error bars represent standard errors.

On day 1, the TloadDback task was administered either in the HCL or the LCL condition (the order was counterbalanced between participants). Subjective evolution of CF was calculated by subtracting the VASf scores before the TloadDback from the VASf scores after. Immediately after the TloadDback task, participants were administered the SRTT learning session using either sequence A or B (counterbalanced). The same procedure was repeated on day 2 using the other SRTT sequence (B or A). Finally, the generation task was administered at the end of day 2.

RESULTS

Sleep Quality and Baseline Vigilance Levels Within the Experiment

Sleep duration and sleep quality for the nights preceding the testing sessions did not differ significantly from each other (*p*s > 0.7). Mean ± standard deviation for sleep duration and sleep quality were Night 0 = 7.63 ± 1.45 h and 4.5 ± 0.9; Night 1 = 7.62 ± 0.94 h and 4.32 ± 0.8; Night 2 = 7.93 ± 1.17 h and 4.45 ± 0.8 (as derived from the St-Mary Hospital Questionnaire, Ellis et al., 1981).

A repeated-measures ANOVA conducted on Reciprocal Reaction Times on the PVT (i.e., mean 1/RT; Basner and Dinges, 2011) with Day (D0, D1 and D2) as the within-subject factor was not significant ($F_{(1,22)}$ = 0.14, p > 0.86; $M \pm SD$ Day 0 = 0.3 ± 0.02, Day 1 = 0.3 ± 0.03 and Day 2 = 0.31 ± 0.03), which did not support the assumption of differences in vigilance levels between the experimental sessions.

Induction of Cognitive Fatigue

For subjective measures, a repeated-measures ANOVA was run on CF scores (i.e., the difference between VAS-fatigue (VAS-f) scores before (t1) and after (t4) the TloadDback task) with Cognitive Load (HCL and LCL) as the within-subject factor and condition administration Order (HCL then LCL vs. LCL then HCL) as the between-subjects factor. Results disclosed a main effect of Cognitive Load ($F_{(1,21)}$ = 8, p < 0.05, MSE = 2.90; η^2 = 0.27), with higher CF in the HCL (VAS-f score 2.66 ± 2) than the LCL (1.22 ± 1.59) condition (**Figure 2A**). The analysis did not show any other significant effect or interaction (all p values > 0.45).

To investigate the evolution of CF during the TloadDback task, a repeated-measures ANOVA was computed on weighted accuracy scores with Cognitive Load condition (HCL vs. LCL) and Time on Task (t1 vs. t2 vs. t3 vs. t4) as within-subject factors and administration Order (HCL-LCL vs. LCL-HCL) as the between-subjects factor. The analysis disclosed a main effect of Cognitive Load ($F_{(1,20)}$ = 24.3, p < 0.001; MSE = 1; η^2 = 0.55) with higher performance levels during the LCL (94.8 ± 1.6%) than the HCL (86.4 ± 2.62%) condition, although performance was above the required accuracy level (i.e., 85%) in both conditions. The Time on Task effect was also significant ($F_{(3,60)}$ = 9.14, p < 0.001; MSE = 0.16; η^2 = 0.31). *Post hoc* tests revealed higher performance at the beginning than at the end of practice (93.2 > 90.8 > 89.9 > 88.5%; t1 > (t3 = t4) and t2 > t4; *p*s < 0.01; **Figure 2B**). Finally, the Cognitive Load by Time on Task interaction was significant ($F_{(3,60)}$ = 3.6, p < 0.05; MSE = 0.10; η^2 = 0.12), indicating a different evolution of performance in the HCL and LCL conditions. As illustrated in **Figure 2B**, performance decreased in both the HCL and LCL conditions from the beginning to the end of the task (t1 > t4; Tukey's *post hoc ps* < 0.05), but decreased faster in the HCL (t1 > (t2 > (t3 = t4))) than in the LCL condition ((t1 = t2 = t3) > t4). These results suggest that, as expected, cognitive demands and resulting CF were higher in the HCL condition. All other effects were non-significant (all p values > 0.4).

Serial Reaction Time Task (SRTT)

Reaction times (RTs) for only correct responses were averaged per block (Borragán et al., 2015). RTs >3 standard deviations from the mean were excluded, and responses given outside of the stimulus target (the 5 × 6 cm^2 at each corner of the screen) were considered as errors. Analyses conducted on accuracy scores only disclosed slightly more errors in

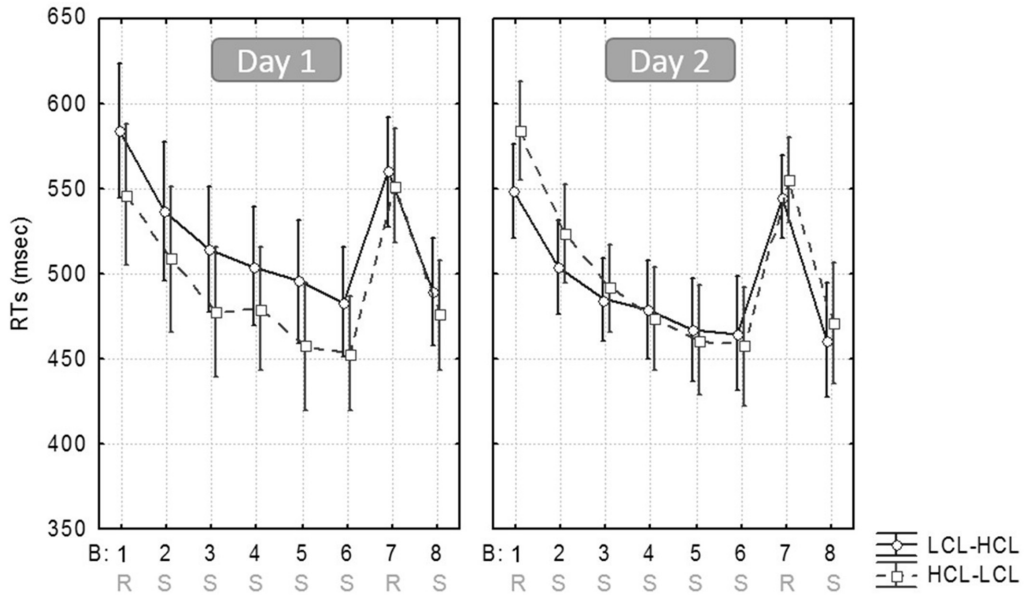

FIGURE 3 | SRTT performance. Mean RT/block in the high (HCL) and low (LCL) cognitive load conditions (HCL vs. LCL), for participants learning on Day 1 in the LCL then on Day 2 in the HCL condition (LCL–HCL), and participants learning first in the HCL then in the LCL condition (HCL–LCL).

the Random than in the Sequential blocks (3.13 ± 1.4% vs. 3.74 ± 2.38%; ($F_{(1,19)}$ = 22.7, *MSE* = 0.76; $p < 0.001$; $\eta^2 = 0.54$), with no interaction involving any other factors (all *p*s > 0.16). Therefore, and given the low proportion of errors, subsequent analyses were only computed for RTs. **Figure 3** illustrates the evolution of speed performance in the two experimental conditions (HCL and LCL) during the two successive days.

As a reminder, our analyses aimed at investigating whether sequence learning is enhanced after a high level of CF is induced (HCL condition), as compared to after a low level of CF (LCL condition). A repeated-measures ANOVA was conducted on response speed (mean RT/block) with Cognitive Load (HCL vs. LCL), Block Type (Sequential vs. Random) and Task Practice (i.e., Beginning [Blocks 1–2] vs. End [Blocks 7-average (6/8)] of the learning session) as within-subject factors, and Sequence (A vs. B) and Condition Order (HCL-LCL vs. LCL-HCL) as between-subjects factors. Note that Sequential blocks 6 and 8 were averaged to obtain a more accurate measure of performance at the end of the learning session in comparison with the intermediate Random block 7 (Borragán et al., 2015).

In looking at learning effects, results revealed a main effect of Block Type ($F_{(1,19)}$ = 149.61, *MSE* = 1234; $p < 0.001$; $\eta^2 = 0.89$), with slower RTs for Random (557 ± 44.83 ms) than for Sequential (492 ± 46 ms) blocks, indicating an advantage of the repeated sequence (i.e., a learning effect). There was also a main effect of Task Practice ($F_{(1,19)}$ = 42.06, *MSE* = 1063; $p < 0.001$; $\eta^2 = 0.69$), with faster RTs at the end (508 ± 41 ms) than at the beginning (540 ± 48 ms) of the SRTT session. The interaction between Block Type and Task Practice was significant ($F_{(1,19)}$ = 33.06, *MSE* = 380; $p < 0.001$; $\eta^2 = 0.63$): RT differences between Sequential and Random blocks were higher at the end (83 ± 33 ms) than at the beginning (47 ± 21 ms) of the SRTT session ($p < 0.001$), indicating a progressive learning of the sequential regularities (see **Figure 4A**).

Regarding the effect of CF, the main effect of Cognitive Load was non-significant ($p > 0.48$) but there was a significant interaction between Cognitive Load and Block Type ($F_{(1,19)}$ = 4.46, *MSE* = 252; $p < 0.05$; $\eta^2 = 0.19$). *Post hoc* tests disclosed significantly faster RTs in the HCL than the LCL condition for Sequential (487 ± 46 ms vs. 497 ± 53 ms; $p < 0.05$) but not for Random blocks (556 ± 41 ms vs. 557 ± 54 ms; $p > 0.76$; **Figure 4B**), suggesting that CF mostly had a positive impact on performance for the sequential component of procedural learning in the SRTT. Also, the interaction between Cognitive Load and Condition Order factors was significant ($F_{(1,19)}$ = 6.59, *MSE* = 2324; $p < 0.02$; $\eta^2 = 0.26$). *Post hoc* tests revealed faster RTs that were marginally significant (irrespective of Sequential or Random blocks) with regard to only high CF for participants who received the LCL condition first (LCL vs. HCL = 536 ± 71 ms vs. 512 ± 57 ms; $p = 0.06$). This was not the case for participants who completed the HCL condition first (HCL vs. LCL = 532 ± 60 ms vs. 518 ± 76 ms, $p > 0.54$).

Generation Task

Exclusion generation scores (% of chunks belonging to the sequence learned on day 2) were above chance level in the HCL [single-sample *t*-test against 33% value, $t_{(12)}$ = 3.13,

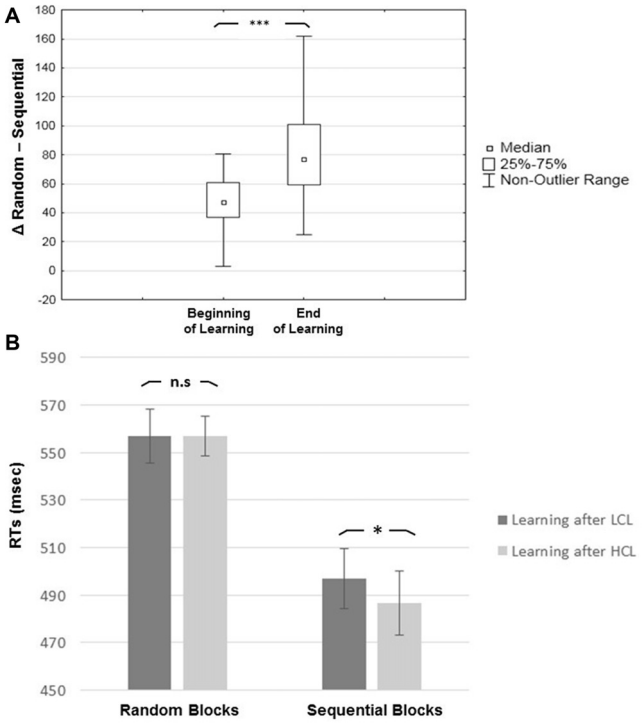

FIGURE 4 | Learning effects. (A) Learning effect (RTs for random minus sequential blocks). **(B)** Type of Block by Condition interaction, showing that the positive effect of CF is present for sequential but not random blocks. Error bar represent one standard deviation from the mean; Asterisks indicate *p*-value significance after Tukey *post hoc* correction: $*p < 0.05$; $**p < 0.01$ and $***p < 0.001$. n.s: non-significant.

$p < 0.02$] but not in the LCL ($p > 0.1$) condition. This suggests that participants in the LCL condition had more control and explicit knowledge about the learned sequence than participants in the HCL condition. However, a one-way ANOVA computed for generation scores with Instruction (Inclusion vs. Exclusion) as the within-subject factor and Cognitive Load (HCL vs. LCL) as between-subjects factor failed to reveal main or interaction effects (all $ps > 0.3$). Additionally, the index of explicit knowledge (inclusion minus exclusion scores) did not significantly differ from zero in either the HCL ($p > 0.35$) or LCL ($p > 0.5$) conditions, suggesting a lack of conscious knowledge about the regularities embedded in the sequential material.

DISCUSSION

The present study aimed at exploring a paradoxical, facilitating effect of CF due to prior exposure to HCL on procedural sequence learning in a SRTT. CF was successfully elicited in our experiment. Indeed, in comparing the HCL with the LCL condition, subjective fatigue (VASf) scores showed more of an increase in the HCL condition, with accuracy performance during the TloadDback task being lower and decreasing more rapidly. As expected, there was sequence learning in both CF conditions, with faster RTs for repeated than for random sequences of stimuli. Importantly, the improved performance for repeated and not random sequences from increased CF levels indicates that the facilitating effect of CF is restricted to the sequential component of motor sequence learning. Finally, performance in the generation task indicates that learning in the SRTT remained essentially implicit in both LCL and HCL conditions, although analysis of exclusion scores suggests less top-down control about sequential knowledge at high CF levels. These results corroborate the proposal that facilitative learning effects on one memory system may stem from the disengagement of another competing memory system (Foerde et al., 2006; Brown and Robertson, 2007a,b).

Prior studies have already reported enhanced procedural learning in conditions where cognitive control is reduced (Foerde et al., 2006; Filoteo et al., 2010; Galea et al., 2010; Nemeth et al., 2013; Delpouve et al., 2014). To the best of our knowledge, the present study is the first to report a facilitation of procedural learning after increased CF due to prior exposure to HCL levels. In this framework, CF might be a factor that directly diminishes available cognitive reserves, and eventually facilitates the disengagement of the controlled top-down memory systems that are demanding in terms of cognitive resources. This proposal is in agreement with the view that mental or CF as a reduction in goal-directed attention eventually leading to performing in a stimulus-driven fashion (Boksem et al., 2005). In the present study, we hypothesize that it is essentially activity in the basal ganglia that supported the learning process in the high CF condition, assuming that high CF levels had actually depleted the fronto-parietal resources that underlie attentional and executive functions (Lorist et al., 2005; Lim et al., 2010; Ishii et al., 2014). Indeed, striatal activity, which is associated with habit formation (Yin and Knowlton, 2006) and automatic detection of complex regularities (Peigneux et al., 2000), supports the implicit processing of sequential patterns (Destrebecqz et al., 2005). Furthermore, increasing the working memory load actually biases the competition in favor of habit memory mechanisms (Foerde et al., 2006). Accordingly, we used the TloadDback task to saturate working memory resources for a period of time in order to induce CF (Borragán et al., submitted). Notably however, we demonstrated the aftereffects of sustained cognitive load in terms of persistent CF here, which reflects a temporary inability to regain the sufficient cognitive resources to drive top-down controlled processes during the learning episode. Notwithstanding, we recognize that a limitation of the present study is the lack of brain activity recordings to support the functional hypotheses. Future neuroimaging studies should address this issue of an imbalance between the neural substrates of competing memory systems in different CF conditions. Additionally, our participants were healthy young adults, and it is unclear how cognitive performance is modulated by fatigue as a function of age. Although the topic is still barely explored, and was beyond the scope of the present study, we argue that individual adjustment to each participant's maximal cognitive load in the TloadDback task normalizes for a possible effect of age. Indeed, a different, adjusted cognitive

load would be defined for older or younger participants as a function of their capacity, thus equating cognitive demands. Notwithstanding, future studies should test whether CF and its effects evolve with age even in controlled cognitive load conditions.

Our results show that CF is specifically beneficial for the acquisition of the sequential components in the SRTT, but not the motor learning components (i.e., performance in random blocks). Additionally, the analysis of exclusion scores in the generation task suggests that participants performed slightly better in repeating learned sequential patterns in the LCL than in the HCL condition. This suggests less control over the learned sequence in the HCL condition. Together with the finding of faster RTs for sequential blocks in the HCL condition, these results are in agreement with the proposal that learning was more automatic in this resource-depleting condition. Also in line with this proposal, other studies have shown that testing participants at their non-optimal time of the day (i.e., when they feel the least ability to perform cognitively demanding tasks) is actually associated with an increased performance in implicit learning and procedural memory (May et al., 2005; Delpouve et al., 2014), whereas performance deteriorates in an explicit memory task (May et al., 2005). We show that, independently of time-of-day, which was a random factor in this study, previous cognitive demands and the ensuing CF influence the relative involvement of controlled and automatic memory systems on performance in a SRT task. Notably, our results cannot be explained by sleep disturbances known to trigger CF (Akerstedt et al., 2004), and vigilance levels were similar during pre-testing and both HCL and LCL conditions in this within-subject design.

Cooperative and competitive interactions among different memory systems is a currently developing topic of interest in the cognitive neurosciences. Whereas some memory systems exhibit dependency relationships, others might act more independently under certain circumstances (Klein et al., 2002; Voermans et al., 2004; Hartley and Burgess, 2005), which might represent an adaptive and evolutionary competitive mechanism (Klein et al., 2002) that remains to be fully understood. Presently, it has come to be recognized that competitive relationships in memory systems are dynamic in nature and are modulated by various factors, such as the presence or absence of sleep during the consolidation period (Orban et al., 2006; Brown and Robertson, 2007b; Albouy et al., 2008, 2013; Rauchs et al., 2008) and available resource levels (Foerde et al., 2006; Filoteo et al., 2010), that can themselves be associated with CF.

To conclude, our results challenge the idea that CF results only in negative consequences on cognition. Aside from representing a useful signal that cognitive resources are saturated and that there is a need for rest and/or change of activity, CF may also modify the balance between memory systems in such a way that it facilitates the automatic acquisition of novel skills. Finally, our results stress the need to consider CF as a moderating factor in learning and memory performance and that the impact of CF on the different cognitive components involved in a given task should be assessed separately.

AUTHOR CONTRIBUTIONS

GB: tested conceptualization of the hypothesis. He conducted the experimental testing and the statistical analysis. Finally, he wrote the article. HS: tested conceptualization of the hypothesis. AD: methodological advice. PP: tested conceptualization of the hypothesis and statistical analysis.

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Mirror Visual Feedback-Induced Performance Improvement and the Influence of Hand Dominance

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Mirror visual feedback (MVF) is a promising technique in clinical settings that can be used to augment performance of an untrained limb. Several studies with healthy volunteers and patients using transcranial magnetic stimulation (TMS) or functional magnetic resonance imaging (fMRI) indicate that functional alterations within primary motor cortex (M1) might be one candidate mechanism that could explain MVF-induced changes in behavior. Until now, most studies have used MVF to improve performance of the non-dominant hand (NDH). The question remains if the behavioral effect of MVF differs according to hand dominance. Here, we conducted a study with two groups of young, healthy right-handed volunteers who performed a complex ball-rotation task while receiving MVF of the dominant (n = 16, group 1, MVF_{DH}) or NDH (n = 16, group 2, MVF_{NDH}). We found no significant differences in baseline performance of the untrained hand between groups before MVF was applied. Furthermore, there was no significant difference in the amount of performance improvement between MVF_{DH} and MVF_{NDH} indicating that the outcome of MVF seems not to be influenced by hand dominance. Thus our findings might have important implications in neurorehabilitation suggesting that patients suffering from unilateral motor impairments might benefit from MVF regardless of the dominance of the affected limb.

Keywords: mirror visual feedback (MVF), hand dominance, motor learning, motor skill learning, handedness

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INTRODUCTION

Mirror visual feedback (MVF) is a promising technique in the context of neurorehabilitation to induce performance improvements without training. For MVF, a mirror is placed in the subject's midsagittal plane with one limb behind the mirror. Then the subject performs a motor task with one limb in front of the mirror while watching its reflection giving the illusion of the other limb moving. Importantly, the opposite limb behind the mirror should be at rest throughout the MVF task. MVF was originally used by Ramachandran et al. (1995) to reduce phantom limb pain in amputees. Since then, MVF has been successfully applied to improve motor deficits in stroke patients (Altschuler et al., 1999; Yavuzer et al., 2008; Dohle et al., 2009). Recently, several studies have indicated that MVF is also capable of improving performance of an untrained limb in both young and old volunteers without neurological deficits (Hoff et al., 2015; von Rein et al., 2015). Moreover, it has been shown that MVF leads to functional alterations

in the human motor system as assessed by transcranial magnetic stimulation (TMS; Nojima et al., 2012). In support of this, recent studies using continuous theta burst stimulation (cTBS) or transcranial direct current stimulation (tDCS) provide further evidence that functional alterations in motor cortex (M1) contralateral to MVF might play a crucial role in mediating performance improvements of the untrained hand (Nojima et al., 2012; Hoff et al., 2015; von Rein et al., 2015). For example, upregulating excitability within M1 contralateral to MVF by means of anodal tDCS has been shown to induce superior performance improvements in both healthy younger and older adults relative to sham stimulation (Hoff et al., 2015; von Rein et al., 2015). However, the exact underlying mechanisms of MVF-induced performance improvements still remain controversial, since other TMS studies indicated different MVF-induced effects within and between M1s (Garry et al., 2005; Fukumura et al., 2007; Lappchen et al., 2012; Avanzino et al., 2014). Functional magnetic resonance imaging (fMRI) studies of MVF provide further evidence that functional alterations are not limited to M1 but also affect other motor-related brain areas such as dorsal premotor cortex (dPMC), ventral premotor cortex (vPMC) and supplementary motor area (SMA; Hamzei et al., 2012).

Interestingly, most of the above mentioned studies investigated the effect of MVF on the non-dominant hand (NDH) by performing the MVF-task with the DH. It still remains elusive if similar behavioral effects of MVF can be observed for the DH by training with the NDH. This in turn might be important in the context of neurorehabilitation of sensorimotor function after stroke. Previous studies have shown that the level of impairment is stronger in patients with motor deficits of the NDH as compared to the DH (Harris and Eng, 2006). Hence, the question whether the factor hand dominance influences the effectiveness of MVF is of high clinical interest, but not yet investigated. To address this question, we conducted a study with two groups of young, healthy right-handed volunteers who performed a complex ball-rotation task where one group performed the task in front of the mirror with the DH and hereby received MVF from the DH (MVF_{DH}) while the other group received MVF from the NDH (MVF_{NDH}). The aim of the present study was to investigate: (a) potential baseline differences of the untrained hand (primary outcome measure, either DH or NDH) and (b) differential effects of MVF on the untrained hand (either DH or NDH). Our assumption was that baseline performance of the NDH is worse than that of the DH in a complex fine-motor task (Todor and Kyprie, 1980; Triggs et al., 1997; Brouwer et al., 2001; Garry et al., 2004; Goble and Brown, 2008). Furthermore, we hypothesized that the beneficial effect of MVF will be more pronounced for the NDH as compared to the DH due to a quicker saturation of performance in the DH (Ridding and Flavel, 2006).

MATERIALS AND METHODS

Subjects

A total number of 32 right-handed healthy young volunteers (mean age: 26.78 ± 0.78 years; range 20–38 years; 19 females) participated in the present study. All volunteers gave their written informed consent before starting the experiment. The study was performed in accordance with the Declaration of Helsinki, and was approved by the local ethics committee of the University of Leipzig. None of the volunteers had a history of neurological illness, and during the time of the experiment none of the volunteers was taking any central-acting drugs. All volunteers were task naïve and right-handed, as assessed with the Edinburgh Handedness Questionnaire (mean handedness score of 88.19 ± 2.95; Oldfield, 1971). Highly skilled musicians or sportsmen were excluded from the study, even though some of the volunteers were currently doing sports on a regular basis or were experienced in playing a musical instrument. Total hours of sports per week and hours of fine-motor training per week (e.g., playing a musical instrument, knitting, doing handcrafts, playing videogames with keypad or joystick) were assessed with a questionnaire. Sixteen volunteers were enrolled in the first study group, who performed the MVF-task with the DH (MVF_{DH}), 16 volunteers were enrolled in the second study group (MVF_{NDH}), who performed the MVF-task with the NDH (for details, see **Table 1** for group demographics). Before and after the experiment, all volunteers rated their levels of attention, fatigue and discomfort on a visual analog scale (VAS).

Experimental Procedure

We used a modified version of the complex fine-motor ball-rotation task introduced by Nojima et al. (2012). All volunteers performed the ball-rotation task with two cork balls (diameter 30 mm; weight 10 g) with their DH and NDH in a specific order and direction as described below. During the task, volunteers were seated in a comfortable chair with their elbows flexed

TABLE 1 | Group demographics.

Group	Age (years)	Gender (female/male)	LQ	Sports/week (hours)	Fine-motor training/week (hours)
MVF_{DH} $n = 16$	26.56 ± 0.91	9/7	87.75 ± 4.17	2.84 ± 0.45	0.22 ± 0.19
MVF_{NDH} $n = 16$	27 ± 1.30	10/6	88.63 ± 4.32	3.09 ± 0.48	0.06 ± 0.06

LQ, Laterality Quotient as assessed with the Edinburgh Handedness Scale [range: −100 (full left-handed) to + 100 (full right-handed)]. Hours of sports per week and hours of fine-motor training per week (e.g., playing a musical instrument, knitting, doing handcrafts, playing videogames with keypad or joystick) were assessed with a questionnaire. All values are depicted as mean ± standard error (SE) of the mean. Statistical analysis revealed no differences in age, gender, LQ, sports/week, fine-motor training/week between groups.

at 90° and with their pronated hands resting on a desk in front of them. In both groups, volunteers rotated the balls with the NDH always in a counterclockwise direction and with the DH always in a clockwise direction. The number of ball-rotations/min was counted and used to assess motor dexterity. Motor performance was videotaped throughout the experiment and analyzed (number of ball-rotations/min) offline by an experimenter who was blinded to the study procedures.

To assess baseline performance, volunteers in MVF_{DH} were asked to rotate two cork balls with their NDH as fast as possible in a counterclockwise direction for 1 min (untrained hand pre). Subsequently, the training period with MVF was conducted: volunteers in MVF_{DH} were instructed to rotate the balls with the DH (trained hand) in a clockwise direction as quickly as possible while observing the movement in a mirror placed between their arms. The MVF-task was performed for 10 trials (trial length 1 min each), separated by 30 s break to prevent muscle fatigue, adding up to a total of 15 min of MVF-training (**Figure 1A**). Volunteers in MVF_{NDH} conducted the same task, but switched hands respectively (**Figure 1B**): baseline performance was assessed by 1 min training with the DH in a clockwise direction (untrained hand pre). Then they were asked to complete the set of 10 trials of training (1 min each with 30 s between the trials) with the NDH (trained hand) in a counterclockwise orientation while MVF was provided. During MVF-training, direct view of the training hand was prevented by a wooden barrier and volunteers were instructed to concentrate on the movement in the mirror and to relax the untrained hand behind the mirror in both study groups (**Figure 1C**). To facilitate the mirror illusion, it was taken care that the mirror image of the training hand got superimposed on the untrained hand behind the mirror and volunteers were instructed to take off any jewelry of their hands prior to the experiment. After this training phase, performance of the untrained hand was retested for 1 min (untrained hand post): volunteers in MVF_{DH} rotated the balls with their NDH in a counterclockwise direction, volunteers in MVF_{NDH} performed the task with the DH in a clockwise direction. Importantly, during performance of the untrained hand (pre-MVF and post-MVF) volunteers in both groups were instructed to watch their moving hand.

Statistical Analyses

Statistical analyses were conducted using the Statistical Software Package for Social Sciences (IBM SPSS Version 22). For all analyses, motor performance was assessed as the number of ball-rotations/min both for the untrained hand before (pre-) MVF and after (post-) MVF and for the trained hand during the training period. According to our research aims we first tested for differences in baseline performance of the untrained hand. Hence, the number of ball-rotations/min with the untrained hand pre-MVF was compared between groups (MVF_{DH} vs. MVF_{NDH}) using an independent samples *t*-test. Second, in order to assess potential influences of hand dominance on the effect of MVF on performance improvements of the untrained

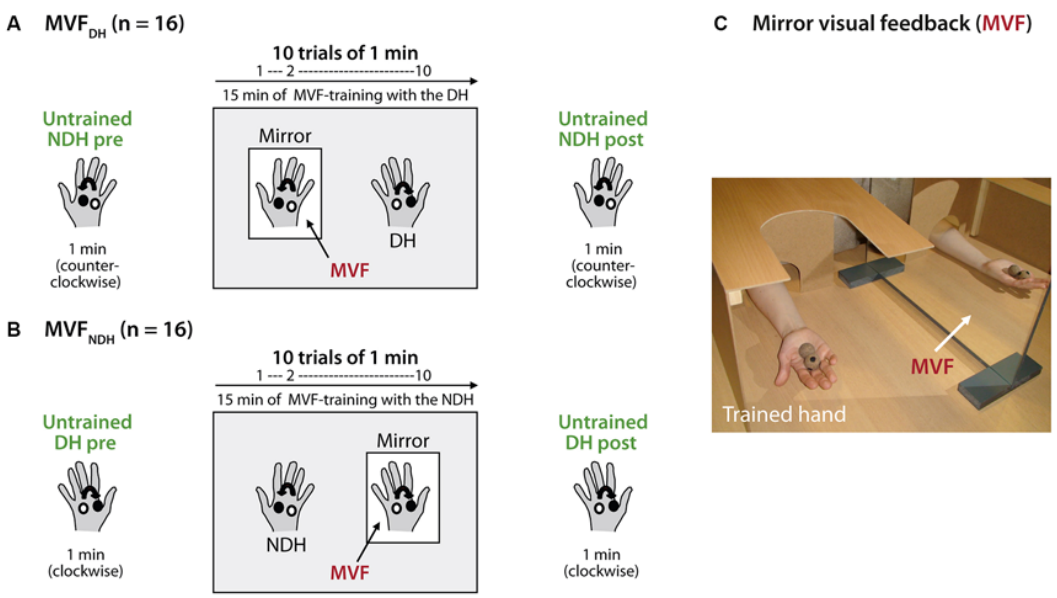

FIGURE 1 | Experimental setup and design. Volunteers performed a complex ball-rotation task with two cork balls. **(A)** Volunteers in MVF_{DH} rotated the balls with their non-dominant hand (NDH) in a counterclockwise direction for 1 min (untrained hand pre), followed by a 15 min training period with the dominant hand (DH) in a clockwise direction while mirror visual feedback (MVF) was provided (10 trials of 1 min each with 30 s break in between). After the training phase, the performance of the NDH was retested (untrained hand post). **(B)** Volunteers in MVF_{NDH} received the same instructions and conducted the same task just *vice versa*: MVF-training was performed with the NDH while the DH was assessed before and after MVF (untrained hand pre and post). In both groups, volunteers rotated the balls with the NDH always in a counterclockwise direction and with the DH always in a clockwise direction. **(C)** During the task, volunteers in both groups were seated in a comfortable chair with their elbows flexed at 90° and with their pronated hands rested on a desk in front of them. While MVF was provided, a mirror was placed in the subject's midsagittalplane and the performing hand was covered by a wooden barrier to prevent direct view. See text for details.

hand, a repeated-measures ANOVA (ANOVA-RM) with factor TRIAL (untrained hand pre vs. untrained hand post) and GROUP (MVF_{DH} vs. MVF_{NDH}) was performed. This analysis was supported by a second analysis, where absolute performance improvement (untrained hand post—untrained hand pre) of the untrained hand was compared across groups (MVF_{DH} vs. MVF_{NDH}) with an independent samples *t*-test. Furthermore, we performed a control analysis in order to ensure that volunteers improved with their trained hand during MVF. Hence, the number of ball-rotations/min of the trained hand in the first trial of training (T1) was compared between groups (MVF_{DH} vs. MVF_{NDH}) using an independent samples *t*-test. Trained hand performance over the whole training period was evaluated using another ANOVA-RM with factor TRIAL (T1–10) and GROUP (MVF_{DH} vs. MVF_{NDH}). If necessary, Greenhouse-Geisser correction was applied, and *P*-values were corrected for multiple comparisons using Bonferroni correction. A *P*-value of <0.05 was considered to be significant. As a measure of the effect size, the Eta-squared (η^2) is reported for each ANOVA. As proposed by Miles and Shevlin (2001), a η^2 of ≥ 0.02 is considered to be a small, a η^2 of ≥ 0.13 a medium and a η^2 of ≥ 0.26 a large effect. Behavioral data are presented as mean ± standard error (SE).

RESULTS

Volunteers in both groups did not differ with regard to age [$t_{(30)} = -0.276$, $P = 0.784$] gender [$t_{(30)} = -0.349$, $P = 0.729$], laterality quotient [LQ; $t_{(30)} = -0.146$, $P = 0.885$], their weekly hours of sports [$t_{(30)} = -0.381$; $P = 0.706$] or fine-motor training [$t_{(30)} = 0.789$; $P = 0.437$; see also **Table 1**]. Both groups did not differ regarding their level of fatigue [$t_{(30)} = -1.125$, $P = 0.270$] or discomfort [$t_{(30)} = -0.338$, $P = 0.737$] prior to the experiment. We found, however, a statistically significant difference on the VAS in attention at baseline between groups [$t_{(30)} = -2.590$, $P = 0.015$] as well as a significant increase in attention in both groups: by 1.13 ± 0.26 on the VAS in MVF_{DH} [$t_{(15)} = -4.392$; $P = 0.001$] and by 0.38 ± 0.02 on the VAS in MVF_{NDH} [$t_{(15)} = -2.423$; $P = 0.029$]. To exclude a correlation between the attention prior to the experiment and the performance improvement of the untrained hand, we performed a bivariate correlation and did not find a significant interaction [$r = 0.10$; $P = 0.589$]. We furthermore did not find a significant correlation between the change in attention and the performance improvement of the untrained hand [$r = 0.12$; $P = 0.502$] in a second bivariate correlation. See **Table 2** for a complete breakdown of attention, fatigue and discomfort levels.

Performance of the Untrained Hand

There was no difference in baseline performance of the untrained hand between groups [MVF_{DH} pre-MVF: 31.94 ± 3.19; MVF_{NDH} pre-MVF: 33.50 ± 2.75 ball-rotations/min, $t_{(30)} = -0.371$; $P = 0.713$; **Figure 2A**]. Performance of the untrained hand improved in both groups significantly by 9.44 ± 1.40 ball-rotations/min in MVF_{DH} [$t_{(15)} = -6.730$; $P < 0.001$] and by 6.19 ± 2.01 ball-rotations/min in MVF_{NDH} [$t_{(15)} = -3.080$; $P = 0.008$; **Figure 2A**]. There were no significant differences in behavioral improvements of the untrained hand after MVF between groups [ANOVA-RM with factor TRIAL (untrained

TABLE 2 | Visual analog scale (VAS).

Group	Before	After
MVF_{DH}		
Attention (1–10)	7.00 ± 0.27	8.13 ± 0.27
Fatigue (1–10)	6.88 ± 0.48	7.88 ± 0.41
Discomfort (1–10)	1.13 ± 0.13	1.00 ± 0.14
MVF_{NDH}		
Attention (1–10)	8.19 ± 0.37	8.59 ± 0.34
Fatigue (1–10)	7.56 ± 0.38	7.75 ± 0.39
Discomfort (1–10)	1.19 ± 0.0	1.06 ± 0.06

Before and after the MVF-task, attention, fatigue and discomfort were assessed with the VAS questionnaire. Attention scale, 1–10: 1, no attention; 10, highest level of attention. Fatigue scale, 1–10: 1, high level of fatigue; 10, no fatigue. Discomfort scale, 1–10: 1, no discomfort; 10, highest level of discomfort. All values are presented as mean ± standard error (SE) of the mean.

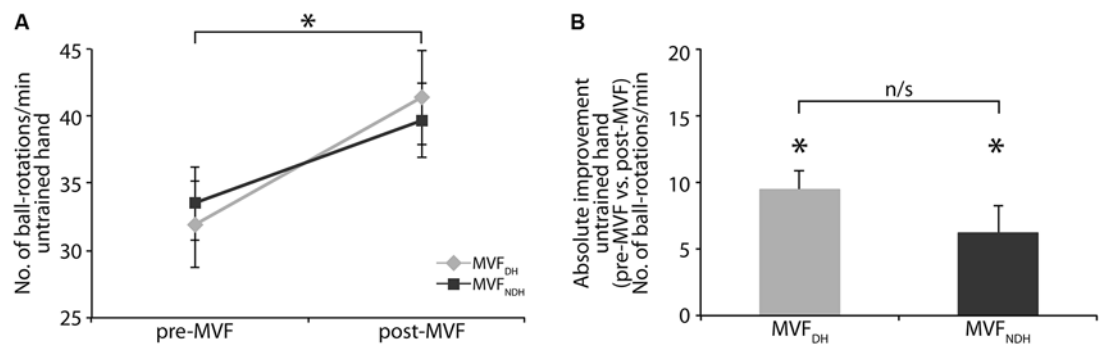

FIGURE 2 | Effect of MVF on motor performance of the untrained hand. (A) Number of ball-rotations/min of the untrained hand before and after MVF. Note that baseline performance of the untrained hand did not differ between groups. **(B)** Absolute performance improvement of the untrained hand. Both groups improved their performance with the untrained hand significantly. There was no significant difference in absolute performance improvement of the untrained hand between groups. The plot shows mean values, and whiskers represent standard error (SE) values. *$P < 0.05$; n/s, not significant.

hand pre vs. untrained hand post) × GROUP (MVF_{DH} vs. MVF_{NDH}): $F_{(1,30)} = 1.760$; $P = 0.195$; $\eta^2 = 0.055$]. A comparison of the absolute amount of performance improvement showed no significant difference between groups [$t_{(30)} = 1.327$; $P = 0.195$; **Figure 2B**]. There was no correlation between the absolute amount of performance improvement of the untrained hand (post-MVF − pre-MVF) and the absolute amount of performance improvement of the trained hand (T10−T1) in neither of the groups [MVF_{DH}: $r = 0.20$; $P = 0.461$; MVF_{NDH}: $r = -0.28$; $P = 0.301$].

Performance of the Trained Hand

In the first trial of MVF-training (T1), there was no significant difference in performance of the trained hand between groups [MVF_{DH}: 23.13 ± 4.75; MVF_{NDH}: 17.63 ± 3.41 ball-rotations/min, $t_{(27.208)} = 0.942$; $P = 0.355$]. Performing the ball-rotation task with MVF during the training phase (trials 1–10) resulted in significant performance gains of the trained hand in both groups. On average participants improved by 23.19 ± 3.15 ball-rotations/min in MVF_{DH} and by 17.06 ± 2.00 ball-rotations/min in MVF_{NDH} [ANOVA-RM with factor TRIAL (T1–10): $F_{(4.330,129.885)} = 41.073$; $P < 0.001$; $\eta^2 = 0.578$] while there was no significant difference in the learning rate between groups [ANOVA-RM with factor TRIAL (T1–10) × GROUP (MVF_{DH} vs. MVF_{NDH}): $F_{(4.330,129.885)} = 1.070$; $P = 0.376$; $\eta^2 = 0.034$]. See **Table 3** for details of group data.

DISCUSSION

The aim of the present study was to investigate whether MVF from the DH and NDH during motor skill learning differentially affects performance of the untrained hand.

Contrary to our hypothesis, there was no significant difference in baseline performance of the untrained hand between groups before MVF. Both groups improved the dexterity of the untrained hand significantly and there was no significant difference in the amount of performance improvement. Moreover, there was no significant difference in the learning rate of the trained hand during the training phase with MVF.

Our results seem to be in contrast with other studies showing worse performance of the NDH in motor-tasks like finger tapping or the pegboard task (Triggs et al., 1997; Brouwer et al., 2001; Garry et al., 2004). One potential explanation for these divergent results might be related to the fact that the motor-task in the present study (ball-rotation task) is a more complex and attentionally demanding motor skill that was completely novel to participants. In line with this, several other studies have argued that the dominant and non-dominant arm have complementary roles during complex motor skill tasks, with the dominant arm specializing in specification and control of arm/joint trajectory and the non-dominant arm preferentially encoding sensory-mediated error correction (Sainburg and Kalakanis, 2000; Sainburg and Wang, 2002; Bagesteiro and Sainburg, 2003).

We showed that the untrained hand improved significantly, irrespective if volunteers used their DH or NDH hand for the MVF-task. Hence, MVF-induced performance improvements do

TABLE 3 | Group data of the untrained hand pre-MVF and post-MVF and of the trained hand during learning phase (T1–T10) in the ball-rotation task.

	pre-MVF	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	post-MVF
MVF_{DH}	31.94 ± 3.19	23.13 ± 4.75	29.38 ± 4.52	34.88 ± 4.20	37.44 ± 3.48	37.88 ± 3.48	39.88 ± 3.36	41.69 ± 3.41	43.63 ± 3.48	44.63 ± 3.24	46.31 ± 3.33	41.38 ± 3.49
MVF_{NDH}	33.50 ± 2.75	17.63 ± 3.41	21.19 ± 3.89	25.00 ± 3.65	26.88 ± 4.02	25.56 ± 3.51	27.56 ± 3.14	31.13 ± 3.50	32.88 ± 3.17	33.88 ± 3.62	34.69 ± 2.87	39.69 ± 2.75

Behavioral data for the trained and untrained hand. Performance of the untrained hand before and after MVF-training (trials 1–10). Performing the ball-rotation task with MVF during learning phase (trials 1–10) resulted in significant performance gains of the trained hand in both groups while there was no difference in the learning rate between groups. For details, see text. Data are presented as mean ± standard error (SE) of the mean.

not seem to be affected by hand dominance, at least in right-handed volunteers.

The question remains whether MVF-induced performance improvements of the untrained hand are due to intermanual transfer or other unknown factors. Intermanual transfer, a phenomenon where unilateral skill training improves not only the trained, but also the untrained hand (Obayashi, 2004; Perez et al., 2007; Camus et al., 2009) is well described for different motor paradigms. However, studies reported conflicting results concerning the directionality of transfer between the DH and the NDH. For example, studies showed a symmetric transfer to both directions (Imamizu and Shimojo, 1995; van Mier and Petersen, 2006) as well as a greater transfer from the NDH to the DH (Taylor and Heilman, 1980; Parlow and Kinsbourne, 1990; Lavrysen et al., 2003) while other studies showed the reverse phenomenon (Parlow and Dewey, 1991; Halsband, 1992; Thut et al., 1996; Criscimagna-Hemminger et al., 2003; Redding and Wallace, 2008). Moreover, other studies have shown that some aspects of the same visuo-motor task transferred only in one direction while others in the other direction (Sainburg and Wang, 2002). The diversity in the literature seems to reflect the complexity of the phenomenon of intermanual transfer and suggests that there is a dependency between a- and/or symmetry and the task and paradigm used. Concerning intermanual transfer, one could argue that MVF is not the driving mechanism behind the observed performance improvements in the untrained hand. We believe, however, that pure intermanual transfer cannot explain the observed MVF-induced behavioral improvement. In favor of this, Nojima et al. (2012) performed a control experiment with the same complex ball-rotation task and showed no performance improvements of the untrained left hand when motor-training with the right hand was performed without MVF. Interestingly, a recent study by Reissig et al. (2015) showed divergent findings. Here, the authors found no difference between a MVF group and a group that received no MVF during training. Obvious reasons behind these opposing findings need to be addressed in future studies. However, as pointed out by Reissig et al. (2015) one obvious explanation might be related to the fact that the kinesthetic illusion for MVF, which seems to be important for the observed behavioral effects, might have been different between studies.

Apart from that, the underlying neural mechanisms of MVF and intermanual transfer seem to be divergent: for example, Perez et al. (2007) and Camus et al. (2009) showed that alterations in intracortical and interhemispheric inhibition (IHI) between homologous M1s predominantly contribute to intermanual transfer. On the other hand, Nojima et al. (2012) could not find any alterations in IHI after MFV was applied. Furthermore, they showed that even in callosotomized patients, MVF-induced performance improvements were still observable (Nojima et al., 2013). Hence, MVF as compared to intermanual transfer may rely on different underlying mechanisms. This, however, needs to be further investigated in future studies, since at least one study indicated potential alterations in IHI when MVF was provided for a simple paced finger tapping movement (Avanzino et al., 2014).

Future Implications and Summary

We here provide novel evidence that MVF from the DH as well as NDH is capable of improving the dexterity of the untrained hand in a complex fine-motor task. In the present study, we investigated whether the behavioral effect of MVF differs according to hand dominance. Future studies should explore potential differences or similarities between the directionality of MVF on a neurophysiological level. Furthermore, future research should investigate the observed behavioral effects of MVF in left-handed volunteers, as well, to see if hand dominance affects their dexterity, differently. Since our volunteers only conducted the task once, we cannot exclude the possibility that hand dominance may induce differences in the amount of MVF-induced performance improvement after several sessions of MVF.

Since MVF is successfully used in the context of neurorehabilitation as an adjuvant strategy to augment performance in the paretic arm after focal brain lesion (Altschuler et al., 1999; Yavuzer et al., 2008; Dohle et al., 2009) and to reduce pain in patients with complex regional pain syndrome (Moseley, 2004), our findings might have important implications from a clinical perspective and support the application of MVF in patients regardless of the dominance of the affected limb.

AUTHOR CONTRIBUTIONS

VR and PR and AV designed the study. VR performed the experiment. PR, VR, CJS analyzed the data. VR, EK, MH, BS, CJS, PR interpreted results of the experiment. PR and VR drafted the manuscript. PR, VR, EK, MH, CJS and BS edited and revised the manuscript. All authors approved the final version of the manuscript.

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A Day Awake Attenuates Motor Learning-Induced Increases in Corticomotor Excitability

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The "synaptic homeostasis hypothesis" proposes that the brain's capacity to exhibit synaptic plasticity is reduced during the day but restores when sleeping. While this prediction has been confirmed for declarative memories, it is currently unknown whether it is also the case for motor memories. We quantified practice-induced changes in corticomotor excitability in response to repetitive motor sequence training as an indirect marker of synaptic plasticity in the primary motor cortex (M1). Subjects either practiced a motor sequence in the morning and a new motor sequence in the evening, i.e., after a 12 h period of wakefulness (*wake group*); or they practiced a sequence in the evening and a new sequence in the morning, i.e., after a 12 h period including sleep (*sleep group*). In both wake and sleep groups motor training improved movement performance irrespective of the time of day. Learning a new sequence in the morning triggered a clear increase in corticomotor excitability suggesting that motor training triggered synaptic adaptation in the M1 that was absent when a new sequence was learned in the evening. Thus, the magnitude of the practice-induced increase in corticomotor excitability was significantly influenced by time of day while the magnitude of motor performance improvements were not. These results suggest that the motor cortex's potential to efficiently adapt to the environment by quickly adjusting synaptic strength in an activity-dependent manner is higher in the morning than in the evening.

Keywords: synaptic homeostasis hypothesis, transcranial magnetic stimulation, finger sequence tapping, motor learning, sleep

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INTRODUCTION

The synaptic homeostasis hypothesis (Tononi and Cirelli, 2003, 2006) assumes that a net increase in synaptic strength occurs when awake due to long-term potentiation (LTP) triggered by learning (Muellbacher et al., 2002; Silva, 2003; Rosenkranz et al., 2007a) or due to synaptic plasticity reflecting statistical regularities of the environment experienced during wakefulness (Cirelli and Tononi, 2000; Tononi and Cirelli, 2001, 2003; Huber et al., 2013). This increase in synaptic strength is believed to reduce neuronal selectivity, i.e., firing in response to a specific stimulus, but also limits the capacity to undergo further synaptic plasticity (saturation of learning capabilities; Tononi and Cirelli, 2003, 2006, 2014). The synaptic homeostasis hypothesis predicts that sleep "downscales" or renormalizes the overall synaptic strength hereby improving signal-to-noise

ratio and restoring the brain's energy balance and cellular homeostasis (Tononi and Cirelli, 2014). Using a plausible computational model of sleep-dependent renormalization, it has been predicted that the human brain's ability to form new memories is hereby renormalized in the morning following sleep (Olcese et al., 2010). In accordance with this latter prediction, behavioral studies testing the formation of declarative memories showed that sleep was beneficial for memory consolidation (Born et al., 2006; Gais et al., 2006) and that learning capacity was higher in the morning (i.e., after 12 h including sleep) than in the evening (i.e., after 12 h without sleep; Kvint et al., 2011). Moreover, sleep deprivation caused a substantial impairment in learning capacity (McDermott et al., 2003; Yoo et al., 2007; Mander et al., 2011). By contrast, for motor learning, and most notably for sequence learning, it has been shown that while sleep is beneficial for consolidation and retention performance, particularly when performance saturation was reached during prior training (Kvint et al., 2011), behavioral measurements of sequence learning capacity did not differ in the morning compared to the evening (Fischer et al., 2002; Walker et al., 2002; Brawn et al., 2010; Kvint et al., 2011; Sale et al., 2013).

Here, we test the prediction that motor learning-induced synaptic plasticity is attenuated after a period of wakefulness. Transcranial magnetic stimulation (TMS) was used to estimate a person's capacity to undergo synaptic plasticity in the primary motor cortex (M1) either after 12 h of wakefulness or after the same period including sleep. Synaptic plasticity was probed in response to repetitive training of a five-element motor sequence which has been shown to modify the functional organization of the motor system, a phenomenon known as use-dependent plasticity (Classen et al., 1998; Muellbacher et al., 2002; Ziemann et al., 2004; Rosenkranz and Rothwell, 2006; Stefan et al., 2006; Perez et al., 2007; Rosenkranz et al., 2007a,b; Huang et al., 2011; Zhang et al., 2011; Bisio et al., 2015).

In humans, use-dependent plasticity within M1 is indicated by larger motor-evoked potentials (MEP) after training than at baseline (Classen et al., 1998; Perez et al., 2007; Rosenkranz et al., 2007a,b). This increase in corticomotor excitability most likely results from training-induced synaptic plasticity leading to strengthening of intracortical neuronal ensembles (Rioult-Pedotti et al., 1998, 2000; Bütefisch et al., 2000), it is *N*-Methyl-D-aspartate (NMDA) receptor-dependent and it is strongly reduced by γ-aminobutyric acid type A ($GABA_A$) receptor mediated inhibition (Bütefisch et al., 2000). Moreover, use-dependent plasticity occluded subsequent induction of LTP via paired-associative stimulation (PAS) protocols in accordance to principles of homeostatic metaplasticity as predicted by the Bienenstock-Cooper-Munro theory (Kirkwood et al., 1996; Stefan et al., 2006; Rosenkranz et al., 2007a), thus suggesting that this type of learning saturates synaptic plasticity. Together, these findings strongly suggest that use-dependent plasticity activates LTP-like mechanisms in humans (Bütefisch et al., 2000; Stefan et al., 2006; Rosenkranz et al., 2007a) which are reflected by changes in corticomotor excitability.

The synaptic homeostasis hypothesis predicts that the brain's capacity to undergo synaptic plasticity is reduced after a prolonged period awake, while this ability is restored after a night of sleep. In line with this theory, we hypothesize that inducing use-dependent plasticity in the morning by practicing one motor sequence will result in larger increases in corticomotor excitability than practicing a new motor sequence in the evening because overall synaptic strengthening during the waking day will diminish the potential to further increase synaptic efficiency.

MATERIALS AND METHODS

Subjects

Nineteen naïve (no musicians, no prior experience with the task), healthy, right-handed (Oldfield, 1971) subjects (1 female, mean ± SD age; 21.9 ± 1.1 years) participated in this experiment. All subjects signed a written informed consent prior to participation and were screened for adverse reactions to TMS when they complied with the inclusion criteria. The experimental procedure was approved by the local Ethics Committee for Biomedical Research at the Catholic University of Leuven in accordance to The Code of Ethics of the World Medical Association (Helsinki, 1964).

General Setup

Participants were seated in a comfortable chair with their right forearm resting in a neutral position and performed the behavioral task on a laptop positioned in front of them (for details see below). Subjects wore a tight fitting swimming cap which allowed to outline the TMS coil position and helped placing the TMS coil appropriately in each session.

Electromyographic Recordings (EMG) and TMS

EMG recordings and TMS acquisition were performed in accordance to a standard protocol described in Alaerts et al. (2011). Focal TMS was applied with a 70 mm figure-of-eight coil connected to a Magstim 200 stimulator (Magstim, Whitland, Dyfed, UK). The coil was positioned over M1 of the left hemisphere, tangential to the scalp with the handle pointing backwards and laterally at 45° away from the mid-sagittal line (Pascual-Leone et al., 2002). The optimal scalp position ("hotspot") for stimulating the right first dorsal interosseous (FDI) and its rest motor threshold (rMT; lowest stimulus intensity evoking MEPs with an amplitude of at least 50 μV in 5 out of 10 consecutive stimuli) were determined (Rossini et al., 1994; **Table 1**).

Disposable Ag-AgCl surface electrodes (Blue sensor SP Surface) were used to record EMG from the FDI. The first electrode was placed on the belly, the second on the tendon of the muscle and a third on a bony prominence (reference electrode). The signals were sampled at 5000 Hz (CED Power 1401, Cambridge Electronic Design, UK), amplified, band-pass filtered (5–1000 Hz), and stored on a PC for offline analysis. Pre-stimulus EMG recordings were used to assess the presence of unwanted background EMG activity in the 110–10 ms time interval preceding the magnetic pulse.

TABLE 1 | Subject data.

			rMT (%)		Hotspot			
	Age (yrs)	Oldfield (%)	Ses 1	Ses2	Ses 1	Ses 2	Sleep (h)	Sleep quality (0–10)
Wake (n = 9)	21.8 ± 1.2	81.7 ± 17.0	36.1 ± 3.9	35.8 ± 3.1	x: 5.3 ± 1.0 y: 0.8 ± 0.4	x: 5.2 ± 0.8 y: 0.4 ± 0.5	7.2 ± 1.0	7.5 ± 1.4
Sleep (n = 10)	21.9 ± 1.1	90.5 ± 12.6	37.1 ± 5.4	37.3 ± 5.5	x: 4.8 ± 0.9 y: 0.7 ± 0.8	x: 4.8 ± 0.9 y: 0.8 ± 0.9	8.1 ± 1.9 7.1 ± 0.8	6.7 ± 1.9 6.6 ± 1.5

Groups were matched regarding age and gender (n = 19; 1 female). The rest motor threshold (rMT) indicates the lowest stimulus intensity evoking MEPs with amplitudes of at least 50 μV in 5 out of 10 consecutive stimuli. Hotspot location is reported as the distance in cm relative to the vertex. The table shows x, y coordinates with x being the lateral-medial distance (positive values are located left to the nasion-inion line) and y the anterior-posterior distance (positive values are located anterior to the vertex). Subjects were asked about their hours of sleep and sleep quality (i.e., score between 0–10) before the practice sessions. The wake group was asked about the night before the morning session and the sleep group about both the night before the evening as the next morning session. No differences were observed considering rMT, hotspot location or hours of sleep between groups ($t \leq 0.73$; $p \geq 0.48$) and between sessions ($t < 0.76$; $p > 0.47$). Data are represented as mean ± SD.

Corticomotor excitability was quantified by measuring input-output curves (IO curve) using 90, 115, 140, 165 and 190% of rMT. One IO curve consisted of 20 MEPs per intensity. They were acquired in two blocks of 50 MEPs so that per block 10 stimulations were acquired for each of the five intensities. In between blocks a rest period of approximately 2 min was provided. Within one block, the interstimulation interval ranged from 5–9 s resulting in a total block time of 6 min 30 s.

Behavioral Task

Subjects performed a computerized sequence tapping task (presented with E-Prime; Psychology Software Tools, Inc. Sharpsburg, PA, USA) adapted from Karni et al. (1998). The sequence to be executed was depicted on top of the laptop screen using a numbering system, with 1, 2, 3, and 4 corresponding to the index, middle, ring and little fingers of the right hand respectively. Throughout the experiment three different yet equally difficult sequences were used (A: 4–1–3–2–4; B: 2–3–1–4–2; C: 3–4–2–1–3). While tapping the sequence a black dot appeared on the screen below the current number every time the subject pressed a key indicating that a response was recorded without giving any accuracy feedback (**Figure 1A**). When a sequence was completed, the screen was refreshed so that the same sequence appeared on top without any black dots present. One experimental trial consisted of typing the given sequence for 30 s as many times as possible followed by a rest period of 30 s to prevent fatigue.

Experimental Protocol

Subjects participated in a familiarization session, first practice session and second practice session. Each session required the acquisition of a new motor sequence (i.e., A, B or C with the order randomized across participants) which was repeatedly practiced within that session. During the familiarization session TMS was used to determine the FDI hotspot and rMT. Afterwards three experimental trials were performed (i.e., 30 s tapping of e.g., sequence A, followed by 30 s rest) which lasted 3 min in total. Subjects were then randomly assigned to one of two experimental groups.

The first experimental group, the *wake group*, started their first session at 8 a.m. (**Figure 1B**). The FDI hotspot and rMT were determined and corticomotor excitability was measured in the form of an IO curve. Subjects then performed motor training, i.e., they practiced a new motor sequence (e.g., sequence B) for 12 experimental trials (i.e., 30 s tapping followed by 30 s rest) which lasted 12 min in total. Subjects then left the lab and followed their typical daily routine and returned for their second session at 8 p.m., which followed the identical procedure but, importantly, a new motor sequence was acquired (e.g., C).

A similar procedure was followed in the second experimental group, the *sleep group*, but the first practice session took place in the evening at 8 p.m. After this first session subjects went home for a night of sleep and returned to the lab at 8 a.m. the following morning for the second session. The presentation of sequences A, B, and C was randomly assigned to familiarization, session 1 and session 2, and differed across subjects.

Before and during the testing day(s), subjects did not perform strenuous exercise, had no more than two cups of coffee a day and followed their normal sleep rhythm without taking additional naps during the day (as instructed and verified via self-report; **Table 1**).

Data Analysis and Statistics

Key presses were recorded and accuracy (%) was calculated as the number of correct sequences divided by all completed sequences during each 30 s trial. Performance speed was measured as the time (s) between key presses, i.e., the inter-tap interval (ITI). A performance score was calculated for each subject and trial by dividing the accuracy percentage by the ITI, with higher scores indicating better performance (also see de Beukelaar et al., 2014). A repeated measures analysis of variance (ANOVA) was performed on performance scores with the between-subject factor *group* (wake, sleep) and the within-subject factors *session* (1st, 2nd) and *training block* (trial 1–12).

Corticomotor excitability was quantified by MEP peak-to-peak amplitude. MEP amplitude is known to be modulated by EMG background activation since slight voluntary

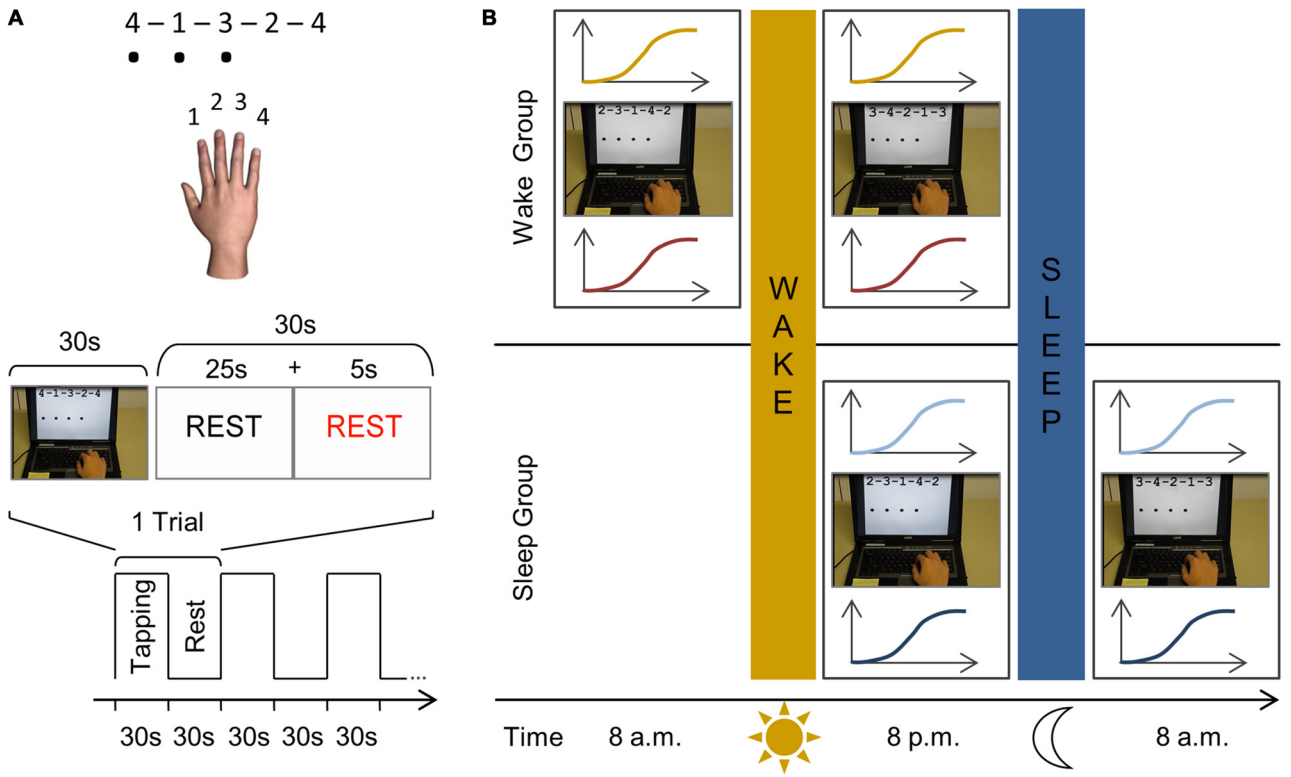

FIGURE 1 | Schematic representation of the finger sequencing task and experimental protocol. (A) The motor task is performed with the right dominant hand on a laptop keyboard. Three different five-element sequences are used throughout the experiment, each consisting of four numeric keys (A: 4–1–3–2–4; B: 2–3–1–4–2; C: 3–4–2–1–3). Each number represented a finger; with "1" being the index finger, "2" the middle finger, etc. The sequence to be executed (e.g., sequence A) was shown on the computer screen using the same numbering system to reduce the likelihood of the task including a working memory component. While performing the task, a black dot appeared on the screen indicating that a key had been pressed. Key presses were recorded and no feedback was provided regarding task accuracy. An experimental trial consisted of 30 s of sequence tapping followed by a rest period of 30 s to prevent fatigue. During the practice sessions, this experimental trial is repeated 12 times so that subjects are mass trained for 12 min in total. Participants were instructed to type the sequences as quickly and as accurately as possible and were motivated continuously throughout the experiment. **(B)** In both groups, we first determined the rest motor threshold (rMT) and hotspot for each subject. In the wake group, subjects are first tested at 8 a.m. and IO curves are measured before (yellow) and after training (red) the finger sequencing task for 12 min (e.g., sequence B). At 8 p.m., following a normal day, subjects are tested in an identical manner as during the morning session, however they are trained on a different sequence (e.g., sequence C). In the sleep group the first session takes place at 8 p.m. when IO curves are measured before (light blue) and after (dark blue) they practiced the finger sequencing task (e.g., sequence B). After a night's sleep, they are tested again using the same procedure at 8 a.m. yet practicing a novel sequence (e.g., sequence C).

contractions of the target muscle might increase MEP amplitude (Barker et al., 1986, 1987; Hess et al., 1987; Rothwell et al., 1987; Devanne et al., 1997; Nollet et al., 2003). Therefore pre-stimulus EMG recordings were used to assess the presence of unwanted background EMG activity in the 110–10 ms preceding the magnetic pulse and were quantified via root mean square scores (RMS) across this interval. For each subject and over all trials we calculated the mean and standard deviations of the background EMG so that values over + 2.5 standard deviation were removed from the analysis. Furthermore we considered MEP peak-to-peak amplitudes which exceeded Q3 + 1.5 × (Q3 & Q1) as outliers (3.1%) that were removed from further analysis, with Q1 denoting the first quartile and Q3 the third quartile computed over the whole set of trials for each subject. MEP amplitudes were averaged for each stimulation intensity of each IO curve that was recorded and these averages where then subjected to group statistics.

We first tested whether motor practice changed corticomotor excitability as quantified by the IO curve and whether these changes would differ between the first and second session. This analysis was performed separately for each experimental group using a repeated measures ANOVA (rmANOVA) with the within-subject factors *session* (1st, 2nd), *pre-post* (pre, post) and *intensity* (90, 115, 140, 165 and 190%). Next we tested whether baseline corticomotor excitability (i.e., measured prior to motor practice) changed from the first to the second session and calculated for each group a rmANOVA for the IO curve measured at pre, using the factors *session* (1st, 2nd) and *intensity* (90, 115, 140, 165 and 190%).

Finally we directly compared whether changes in corticomotor excitability induced by motor practice differed

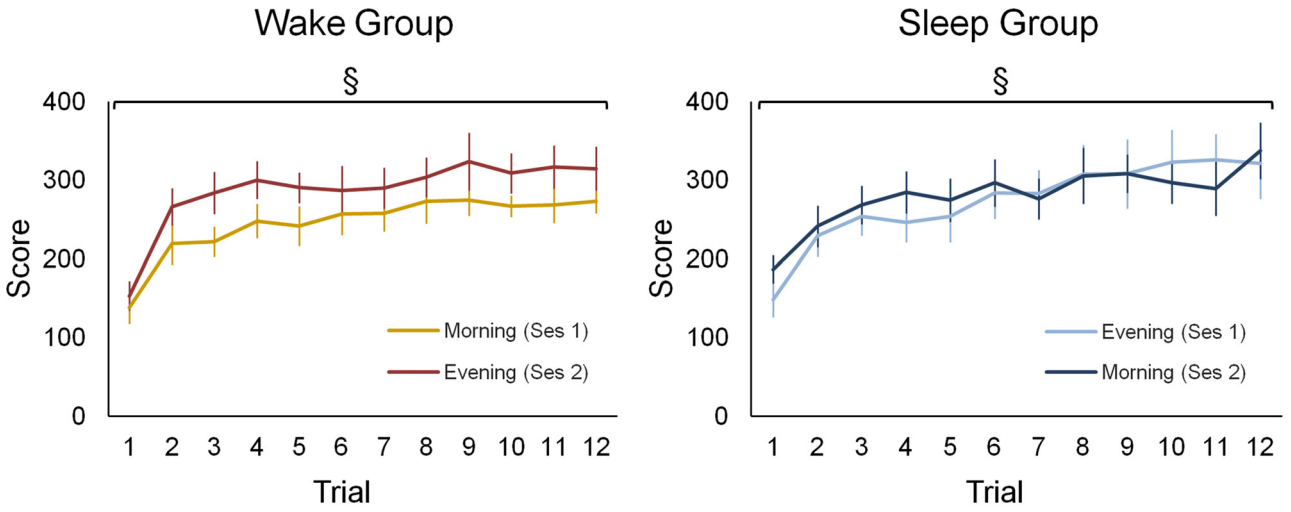

FIGURE 2 | Behavioral data of the wake and sleep group during both practice sessions. For both groups and in each session a separate learning curve is shown for the sequence tapping task. There was a significant main effect for training block ($F_{(11,187)} = 34.61$; $p < 0.001$), yet no main effect for group or a higher interaction containing this group factor was found ($F < 2.56$; $p > 0.12$). A main effect for session ($F_{(1,17)} = 5.01$; $p < 0.05$) indicates that during the second session a higher performance is achieved in both groups. §Represents a main training block effect ($p < 0.001$). Vertical bars indicate standard errors (SEs).

between sessions and groups. Therefore, we calculated the integral underneath the IO curve measured before and after motor practice (Carson et al., 2013), and calculated a facilitation index (FacInd) by:

$$\text{FacInd} = \int_{\text{Intensity1-5}} \text{MEP}_{\text{post}} \Big/ \int_{\text{Intensity1-5}} \text{MEP}_{\text{pre}}$$

FacInd > 1 indicates that an increase in corticomotor excitability is observed from pre to post training, while a FacInd < 1 represents a decrease. The FacInds were calculated for the two sessions and the two groups and were entered into a repeated measures ANOVA with the between-subject factor *group* (wake, sleep) and within-subject factor *session* (1st, 2nd).

The alpha level for all statistical tests was set to 0.05 and significant interactions were further analyzed by the use of a Fisher's LSD *post hoc* analysis. All statistical analyses were performed with Statistica 8 (StatSoft, OK, USA).

RESULTS

Behavioral Results

Both groups improved motor sequence performance during each of the practice sessions to a similar extent (**Figure 2**). Accordingly, statistics revealed a main effect for *training block* ($F_{(11,187)} = 34.61$; $p < 0.001$) but no main effect for *group* or a higher interaction containing the factor *group* ($F < 2.56$; $p > 0.12$). Additionally, performance was generally better in session 2 (310.52 ± 91.74) than in session 1 (297.97 ± 100.79) as indicated by a significant *session* main effect ($F_{(1,17)} = 5.01$; $p < 0.05$). However, there was no statistical evidence to suggest that learning gains were differential influenced be waking or sleeping since the *session* × *trial* interaction failed to reach significance ($F_{(11,187)} = 0.78$; $p = 0.66$).

Neural Results

Wake Group

In the wake group, corticomotor excitability increased due to practice in the morning session while no such increase is seen in the evening session (**Figure 3A**). This is supported by a significant *session* × *prepost* × intensity interaction ($F_{(4,32)} = 3.31$; $p < 0.05$) and by follow up analyses revealing a significant prepost × intensity interaction ($F_{(4,32)} = 4.23$; $p < 0.01$) for the morning session, while significance was not reached in the evening session ($F_{(4,32)} = 2.54$; $p = 0.06$). This indicates that motor practice changed corticomotor excitability more strongly in the morning than in the evening.

When comparing the pre-training IO curves between the two experimental sessions, baseline excitability increased over a 12 h-day awake as indicated by a significant session × intensity interaction for the pre curves of both sessions ($F_{(4,32)} = 5.96$; $p < 0.01$; **Figure 3B**).

Sleep Group

In the sleep group, motor practice did not cause a significant increase of corticomotor excitability in the evening session (prepost × intensity interaction: $F_{(4,36)} = 0.45$; $p = 0.77$) while a significant increase was observed during the following morning session ($F_{(4,36)} = 2.68$; $p < 0.05$), i.e., after a night of sleep (**Figure 3A**). However, the session × prepost × intensity interaction did not reach significance most likely due to large inter-individual variability ($F_{(4,36)} = 1.94$; $p = 0.13$).

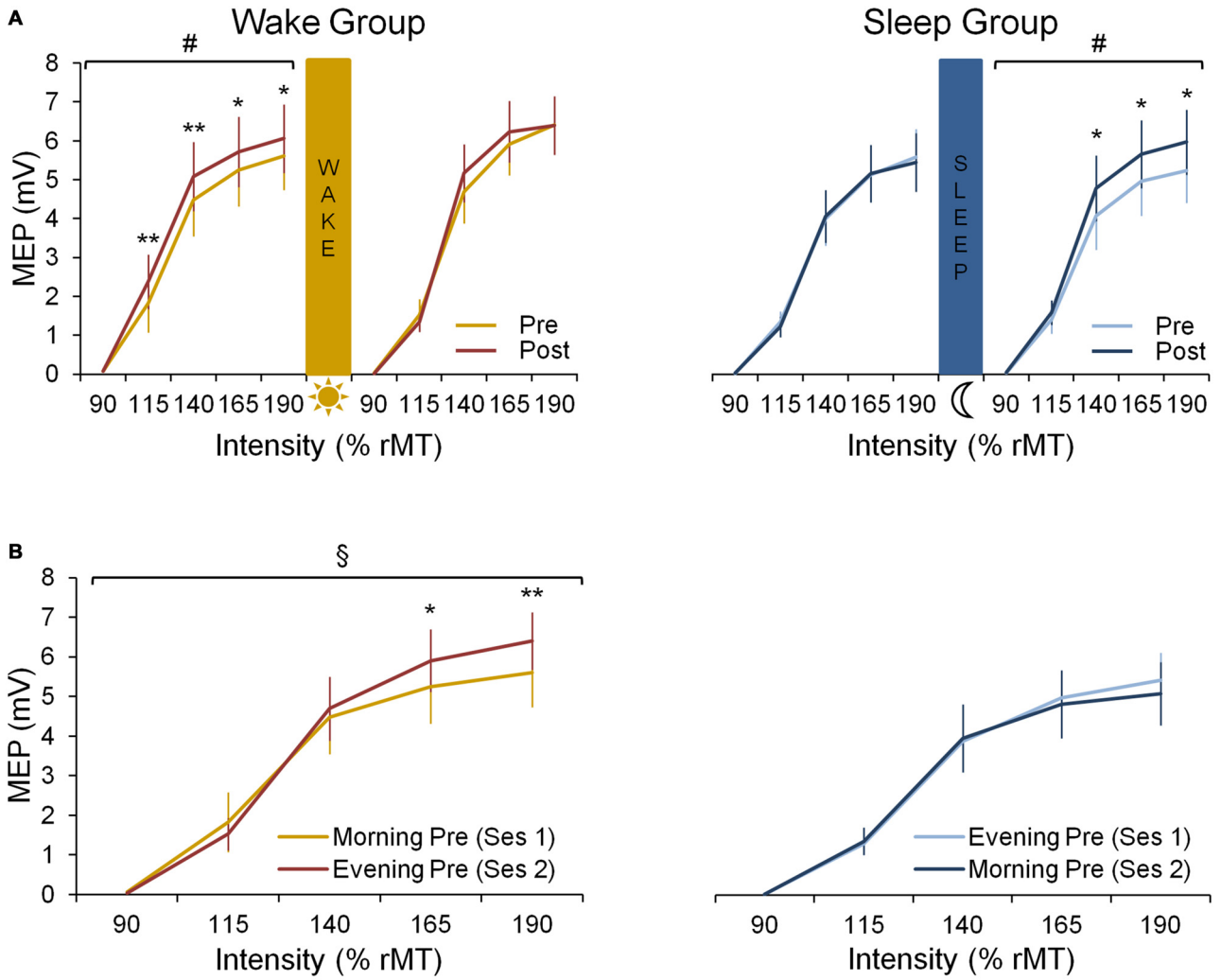

FIGURE 3 | Neural data. (A) Input-0utput (IO) curves pre- and post-training in the first and the second practice session for both wake and sleep group. In the wake group (left panel) we show an increase in corticomotor excitability from pre-training (yellow curve) to post-training (red curve) in the morning session at 8 a.m. ($F_{(4,32)} = 4.23$; $p < 0.01$) but not in the evening session at 8 p.m. A Fisher LSD *post hoc* analysis shows that this effect in the morning is found for suprathreshold stimulation with intensities ≥ 115% rMT. In the sleep group (right panel) we show no increase in corticomotor excitability from pre-training (light blue curve) to post-training (dark blue curve) in their first session being the evening session at 8 p.m. During the second session on the consecutive morning at 8 a.m., a significant increase in excitability is seen from pre- to post-training ($F_{(4,36)} = 2.68$; $p < 0.05$), especially for supratreshold stimulation with intensities ≥ 140% rMT. **(B)** IO curves obtained pre-training in both sessions for both wake and sleep group. For the wake group an increase during the day is observed since the pre-training curve obtained in the evening session is increased compared to the pre curve in the morning ($F_{(4,32)} = 5.96$; $p < 0.01$), especially for supratreshold intensities ≥ 165% rMT. For the sleep group no difference between both pre curves is observed ($p = 0.27$). #Indicates a prepost × intensity interaction effect ($p < 0.05$); §Indicates a session × intensity interaction effect ($p < 0.01$); significant Fisher LSD *post hoc* analyses are represented by *$p < 0.001$ and **$p < 0.0001$. Vertical bars indicate SEs.

When investigating the evolution of the pre-training IO curves, we found no session × intensity interaction in the sleep group indicating that there was no significant change in baseline excitability overnight ($F_{(4,36)} = 1.34$; $p = 0.27$; **Figure 3B**).

FacInd

The FacInd was calculated to directly test whether the potential to undergo changes in corticomotor excitability differed when practice sessions were either separated by 12 h awake (wake group) or 12 h including sleep (sleep group). **Figure 4** shows that the FacInd of the wake group was higher in the morning (indicating that excitability changed by approximately 14.4 ± 18.6% in response to motor practice) than in the evening (excitability changes were only 4.4 ± 13.5%). The sleep group, by contrast, exhibited the opposite pattern with a lower FacInd in the evening (−1.3 ± 8.1%) than the next morning (22.7 ± 31.8%), i.e., after a night of sleep. Importantly, statistics revealed a significant group × session interaction ($F_{(1,8)} = 5.36$; $p < 0.05$) suggesting that wakefulness decreases the ability to change corticomotor excitability in response to motor practice whereas the ability to exhibit use-dependent neural

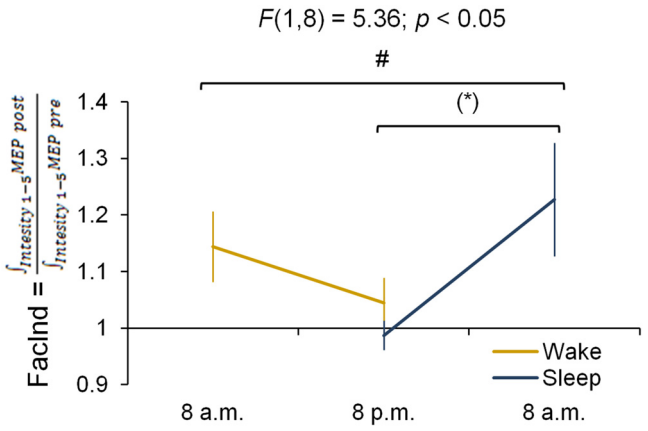

FIGURE 4 | Facilitation Index (FacInd). The FacInd is calculated as a general measurement quantifying the potential to undergo learning-induced changes of corticomotor excitability. We divide the integrated motor-evoked potentials (MEP) amplitudes collected for each intensity after practice (i.e., area under the IO curve post-training) by the integrated MEP amplitudes of those collected before (i.e., area under the IO curve pre-training). This calculation shows that the potential to exhibit training-induced neural changes is dependent on time of day and is higher in the morning compared to the evening in both experimental groups $^{*}p = 0.052$. $^{\#}$Indicates a group × session interaction effect ($p < 0.05$); marginally significant Fisher LSD *post hoc* analysis is represented by $^{*}p = 0.052$. Vertical bars indicate SEs.

changes was re-established at the next morning after a night of sleep.

DISCUSSION

In the present study, we tested the prediction that motor learning-induced synaptic plasticity is attenuated after a period of wakefulness. The capacity to undergo synaptic plasticity was probed by measuring changes in corticomotor excitability in response to acquiring a finger sequence tapping task, a learning paradigm that is well-known to induce use-dependent plasticity in M1. Our main finding is that the capacity to increase corticomotor excitability in response to motor practice (a marker of training-induced synaptic strengthening) is reduced after 12 h of wakefulness during the day.

Behavioral Data

In this study, we used a sequence tapping task to induce use-dependent plasticity. The advantage of this task is that a similar learning process can be induced twice, a requirement of our experimental design. In order to account for potential differences in complexity, we randomized the presentation of sequences in a balanced order. Furthermore, a familiarization session was performed, so that subjects knew the general paradigm in order to minimize novelty effects.

Unlike the neural measurements, the behavioral data did not show differential performance gains in the morning compared to the evening sessions. It is important to note that task performance is not a "pure" measurement of memory formation because it is strongly influenced by fatigue, attention, alertness and motivation (Karni et al., 1998; Robertson et al., 2004a,b). For the sequence tapping task used here it is well-known that time of day does not result in differential motor learning gains (Fischer et al., 2002; Walker et al., 2003; Brawn et al., 2010), and that the beneficial effect of sleep has only been demonstrated for retention performance (i.e., an indirect marker of consolidation) but not for restoring motor learning capacity. Thus, the absence of behavioral differences between morning and evening sessions when estimating sequence learning is highly consistent with previous findings.

Furthermore, the lack of differential behavioral findings between morning and evening sessions could be explained by the overall simplicity of the sequence tapping task in combination with the relative short acquisition phase in relation to the total training time. More specifically, Walker et al. (2002) reported an overall performance increase for sequence tapping of 59.3% over 12 × 30 s training trials with the largest increase occurring during the first 3 training trials (38.8%). Therefore, shortening the training period (e.g., less and/or shorter training trials) to prevent subjects to reach a performance plateau by the end of training could potentially be a more sensitive procedure to reflect more subtle time of day effects on behavior. Note also that the behavioral performance measurements are likely to reflect different learning processes: initially, skill acquisition ensures that the sequence is correctly represented at the neural level and that it is fluently performed which might cause large gains early in learning. By contrast, in a later phase, repetitive practice of the sequence is likely to activate mechanisms related to use-dependent plasticity; i.e., neural changes that are induced by extensively repeating movements within a specified time window (Classen et al., 1998; Bütefisch et al., 2000; Stefan et al., 2006; Rosenkranz et al., 2007a). This might explain why memory specific neurophysiological processes are not always accurately reflected by behavioral changes (Urbain et al., 2013).

Influence of Waking vs. Sleep on the Potential for Increasing Corticomotor Excitability in Response to Motor Training

We found that the potential for increasing corticomotor excitability, measured as the difference between IO curves recorded before and after motor practice (similar to Lotze et al., 2003; Perez et al., 2004; Jensen et al., 2005; Stefan et al., 2006; Rosenkranz et al., 2007a,b; Zhang et al., 2011), was higher in the morning than the evening. It is important to keep in mind that we infer synaptic plasticity based on changes of corticomotor excitability as measured by single pulse TMS. This assumption is based on a large body of evidence reporting a robust increase of corticomotor excitability in response to extensive motor practice inducing use-dependent plasticity (Classen et al., 1998; Perez et al., 2007; Rosenkranz et al., 2007a,b), or other plasticity inducing protocols using transcranial direct current stimulation (tDCS; Romero Lauro et al., 2014), PAS (Stefan et al., 2000; Ridding and Uy, 2003) or theta-burst stimulation (Jacobs et al., 2012). Importantly, this rise in corticomotor excitability after extensive motor training has been shown to be of cortical origin (rather than reflecting changes at e.g., the spinal

level; Rioult-Pedotti et al., 1998, 2000; Bütefisch et al., 2000), is specific for motor learning rather than for motor performance (Rosenkranz et al., 2007a), and it is abolished when synaptic plasticity is reduced either by blocking NMDA receptors or by increasing GABAergic inhibition with pharmacological agents (Bütefisch et al., 2000). Although there is compelling evidence that learning a finger sequence tapping task typically results in increased corticomotor excitability early after training, this might not be the case for all motor tasks (e.g., Tunovic et al., 2014) reported a delayed increase in corticomotor excitability). An alternative approach to probe neuroplasticity of human M1 is to experimentally induced LTP (typically by a PAS_{LTP} protocol) after motor training has been performed. According to models of homeostatic metaplasticity, the effect of LTP-inducing PAS (i.e., PAS_{LTP}) is either reduced or even reversed to long-term depression (LTD) depending on the extent to which synaptic plasticity has been induced by prior motor practice. Combining motor training and PAS protocols is an elegant approach to test synaptic plasticity, however, the efficiency of PAS_{LTP} has been shown to be dependent on corticosteroid levels which are typically lowest early in the morning. Accordingly, PAS_{LTP} effects have been shown to be significantly smaller in the morning than in the evening (Sale et al., 2008). In the context of our paradigm this represents a potential confound and could therefore not be applied. Note however, that only the response to PAS_{LTP} was influenced by corticosteroid levels whereas MEP amplitudes were comparable over the day. Moreover, we controlled other confounding factors like the background EMG across the pre and the post session excluding the possibility that excitability changes were caused by pre-contraction. Therefore, we argue that the increase in corticomotor excitability in response to a standardized practice protocol as quantified by the FacInd is a surrogate marker of a person's ability to undergo neuroplastic changes at the synaptic level (see also Rosenkranz et al., 2007b). Under this assumption, our data suggest that a day awake decreases the potential to show neural changes due to motor learning.

Our data indicate that there is no causal link between practice-induced changes in corticomotor excitability and practice-induced changes of motor behavior (Bestmann and Krakauer, 2015). It has been suggested that there is no straightforward relationship between MEP size (i.e., IO curve) and behavioral output following learning (Muellbacher et al., 2000, 2001; McDonnell and Ridding, 2006; Bagce et al., 2013). From our data, it is apparent that behavior can improve significantly even though corticomotor excitability remains virtually unchanged (as observed after a day of wakefulness). These short-term changes in corticomotor excitability as obtained in the morning sessions appear to indicate that M1 underwent adaptive changes resulting in increased efficiency of the activated neural network (Bestmann and Krakauer, 2015). In other words, a change in corticomotor excitability is not essential to learn a novel motor task, however, in order to efficiently learn the task the neural system needs to adapt. However, we showed that this capacity to learn and to efficiently adapt to the changing world around us is attenuated after a day of wakefulness. This interpretation is in line with the predictions of the synaptic homeostasis hypothesis proposing that one central function of sleep is to downscale overall synaptic strength, thus maintaining the brain's efficiency by ensuring that neurons fire sparsely but selectively for important inputs. In this manner energy consumption is maintained at a sustainable level, and most importantly for our study the ability to learn is restored (Yoo et al., 2007).

Potentiation of Synaptic Strength During Wakefulness

Our findings also support the notion that synaptic strength is potentiated during the day (Tononi and Cirelli, 2003, 2006, 2014) since corticomotor excitability measured prior to motor training increased from the morning to the evening in the wake group consistent with previous findings in humans (Huber et al., 2013) and animal models (Vyazovskiy et al., 2008).

Contrary to the wake group, in the sleep group we found only a slight non-significant decrease in baseline corticomotor excitability after a night of sleep. One has to note, though, that the sleep group did not participate in extra motor training during the day and that the synapses of the muscular representation probed with TMS might not have been strongly potentiated prior to the evening motor training. This is a key difference to the wake group since these subjects were exposed to intensive motor training in the morning. Furthermore, TMS stimulates pyramidal neurons in layer 5 transsynaptically, i.e., via interneurons located in layer 2/3 (Di Lazzaro and Ziemann, 2013). Higher MEPs might not only result from synaptic strengthening occurring within M1 but also from potentiated inputs to these M1 interneurons in layer 2/3 deriving from other areas (Bestmann and Krakauer, 2015). One primary candidate area that might have been activated by the tapping task is the striatum which has been shown to be involved in sequence learning and has dense reciprocal connections with M1 (Doyon et al., 2003; Doyon and Benali, 2005). Other likely input areas to M1 that also undergo changes in response to motor practice are parieto-premotor networks (Doyon and Benali, 2005). Even though these areas outside of M1 contribute to all phases of sequence learning it has been suggested that the time course is slightly different: thus while M1 probably undergoes most prominent synaptic changes during and immediately after practice, the striatum is believed to become increasingly more important during memory consolidation, i.e., during the first minutes and hours after the training has finished (Shadmehr and Holcomb, 1997; Doyon and Ungerleider, 2002; Frankland and Bontempi, 2005; Censor et al., 2010). Consequently we propose that short-term changes of corticomotor excitability as observed when comparing pre to post-training measurements might be predominantly driven by fast neuroplastic changes (which certainly involve M1), while long-term changes in corticomotor excitability as observed when comparing baseline excitability between the morning and the evening test might additionally be influenced by slow neuroplastic changes that occurred during consolidation and potentially, also in areas outside of M1.

Interpretational Issues

The present study was designed in light of the synaptic homeostasis hypothesis, i.e., whether motor learning capacity is

reduced after a day awake but restored in the morning after a night of sleep. Our results are in line with this prediction; however, the present study design does not allow us to dissociate the influence of sleep from the influence of circadian rhythms. Indeed it has been shown that performance of certain motor tasks show time of day effects (Miller et al., 1992; Wyse et al., 1994; Atkinson and Reilly, 1996; Edwards et al., 2007; Keisler et al., 2007) and it is possible that the ability to undergo changes in corticomotor excitability in response to repetitive motor training is also influenced by circadian rhythms. However, previous studies using plasticity inducing brain stimulation protocols would predict the opposite pattern of results than obtained in our present study (Sale et al., 2010). Future research is needed that objectively measures sleep quality by the use of electroencephalography (EEG) and experimentally modulates slow wave sleep which seems to be most related to synaptic downscaling and investigates whether, for example, slow wave sleep perturbation impacts on the renormalization of motor learning capacity. It is also important to note that we, tested two different groups of subjects. Even though, our groups were well matched regarding age, gender, over day activity and sleeping hours it might be advantageous to use a cross-over design in future studies.

CONCLUSION

In this study, we show that the learning-induced synaptic plasticity caused by acquiring a finger sequence tapping task decreases after a day awake. Our findings are in line with the synaptic homeostasis hypothesis which states that synaptic strength is potentiated during the day and sleep restores learning capacity by maintaining synaptic potentiation within an optimal range. Hence, sleep ensures that M1 circuits can undergo reorganization to perform the practiced movements with high efficiency; a mechanism which is attenuated with time spent awake. Although our findings are in accordance with this hypothesis, future studies should objectively measure sleep quality and vary sleep independently of time of day to provide more direct evidence regarding the restorative role of sleep in synaptic homeostasis.

AUTHOR CONTRIBUTIONS

TTdeB designed the study; collected, analyzed and interpreted the data; drafted and revised the manuscript; gave final approval. JVS collected, analyzed and interpreted the data; drafted and revised the manuscript; gave final approval. RH interpreted the data; revised the manuscript; gave final approval. NW designed the study; interpreted the data; revised the manuscript; gave final approval.

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A Developmental Perspective in Learning the Mirror-Drawing Task

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Is there late maturation of skill learning? This notion has been raised to explain an adult advantage in learning a variety of tasks, such as auditory temporal-interval discrimination, locomotion adaptation, and drawing visually-distorted spatial patterns (mirror-drawing, MD). Here, we test this assertion by following the practice of the MD task in two 5 min daily sessions separated by a 10 min break, over the course of 2 days, in 5–6-year-old kindergarten children, 7–8-year-old second-graders, and young adults. In the MD task, participants were required to trace a square while looking at their hand only as a reflection in a mirror. Kindergarteners did not show learning of the visual-motor mapping, and on average, did not produce even one full side of a square correctly. Second-graders showed increased online movement control with longer strokes, and robust learning of the visual-motor mapping, resulting in a between-day increase in the number of correctly drawn sides with no loss in accuracy. Overall, kindergarteners and second-graders producing at least one correct polygon-side on Day 1 were more likely to improve their performance between days. Adults showed better performance with improvements in the number of correctly drawn sides between- and within-days, and in accuracy between days. It has been suggested that 5-year-olds cannot learn the task due to their inability to detect and encapsulate previously produced accurate movements. Our findings suggest, instead, that these children lacked initial, accurate performance that could be enhanced through training. Recently, it has been shown that in a simple grapho-motor task the three age-groups improved their speed of performance within a session and between-days, while maintaining accuracy scores. Taken together, these data suggest that children's motor skill learning depends on the task's characteristics and their adopting an efficient and mature performance strategy enabling initial success that can be improved through training.

Keywords: mirror-tracing, motor skill learning, motor-control, consolidation, long-term memory

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INTRODUCTION

Children are often thought to have superior skill learning abilities compared with adults. This notion has been invoked in relation to "critical" early life periods in several domains (e.g., language, Johnson and Newport, 1989; visual stereopsis, Blake and Hirsch, 1975; Packwood and Gordon, 1975). Some studies support this notion (e.g., performance of older children vs. adults on the probabilistic sequence learning task, Fischer et al., 2007; Janacsek et al., 2012; Nemeth et al., 2013). However, most laboratory studies fail to support this notion, and report an age advantage

in learning of skills such as auditory temporal-interval discrimination, locomotion adaptation, applying a linguistic rule, deterministic sequence learning, and drawing visually-distorted spatial patterns (mirror-drawing (MD); e.g., Ferrel-Chapus et al., 2002; Thomas et al., 2004; Ferman and Karni, 2010; Vasudevan et al., 2011; Lejeune et al., 2013; Hodel et al., 2014). An age advantage was most frequently reported for learning within a session, but also between consecutive practice days (Huyck and Wright, 2011). One of the tasks young children failed to learn was the MD task (Ferrel-Chapus et al., 2002).

The MD task has been used in the study of skill learning since 1910 (e.g., Starch, 1910; Clinton, 1930; Ballard et al., 1993; Voderholzer et al., 2011). In this task, participants are required to trace a shape (commonly a polygon, e.g., a star, diamond, square or a triangle) and stay within the boundaries of a double line, while only seeing an inverted reflection of their hand through a mirror. Mirror learning reflects the formation of new associations between vision—rotated by 180°—and arm movement (Edelstein et al., 2004; Miall and Cole, 2007).

In motor skill-learning tasks, initial performance presumably reflects controlled processes, such as trial and error and adaptation of performance solutions, which mature with age. Later performance reflects selection of a given task solution mode and its optimization as a function of repetition (Anderson, 1982; Logan, 1988; Chein and Schneider, 2005; Adi-Japha et al., 2008; Roebers and Kauer, 2009). It has been suggested that adults adapt within a few trials to the mirror inversion because of their explicit bidirectional visuo-motor awareness of space (enabling efficient coding of visual information into movement in opposite directions) vs. unidirectional awareness in younger children, and because of their better online control of movement. The shift in visuo-motor awareness and movement control occurs at about 8 years of age, and is established at about age 11 (Ferrel et al., 2001; Ferrel-Chapus et al., 2002). On a diamond-shape MD task, in which visual feedback was rotated by 180° and appeared on a computer screen, the performance of 5-year-old children was characterized by direction changes within polygon-sides, even after many repetitions (Ferrel-Chapus et al., 2002). It has been suggested that 5-year-olds, unlike 7-year-old children, cannot learn the task because they "cannot detect accurate movements and reproduce the same programming for the next movements" (Ferrel-Chapus et al., 2002, p. 515). These findings stand in sharp contrast to 5-year-olds' successful learning of a recently introduced simple grapho-motor task, the Invented Letter Task, in which direct visual feedback is afforded (Julius and Adi-Japha, 2015).

Five-year-olds were not the only age-group to show difficulties in learning the MD task. Ferrel-Chapus et al. (2002) compared visuo-manual coordination of children aged 5-, 7-, 9-, and 11-years and adults in mirror tracing a diamond. Only the 11-year-olds reached a performance level similar to that of adults within nine repeats, a finding recently replicated by Finn et al. (2016). Like the 5-year-olds, the 7-year-olds performed fast, ballistic movements, increasing their velocity from trial to trial, while a large number of pauses accompanied their movement. However, 7-year-olds showed less directional changes than 5-year-olds, while producing a similar number of polygon-sides. This reflected performance of some polygon-sides without directional changes. Lejeune et al. (2013) also studied age related differences in an MD task in children aged 7 and 10 years, and in adults, but used a triangle. In this task, four blocks of three trials were administered to the participants. Their findings largely replicated the findings of Ferrel-Chapus et al. (2002), indicating significant age-related differences in speed of performance, and in the number of errors produced. Lejeune et al. (2013) reported that all three age groups learned the MD task.

Studies testing the formation of visual-manual associations while adapting to other experimental conditions, also report age-advantages by which younger children adapt at a slower rate and with greater performance variability (Konczak et al., 2003; Contreras-Vidal et al., 2005; Bo et al., 2006; Kagerer and Clark, 2014). For example, when visual feedback for straight lines (in a center-out task) was rotated at 45°, 4-year-olds produced movements with the highest variability, adapting less well than 6- and 8-year-olds (Contreras-Vidal et al., 2005).

To the best of our knowledge, no study has tested between-day performance developmentally, using the MD task. Post-training performance has been tested, but only in a specific age group: children aged 10–13 years. Specifically, Prehn-Kristensen et al. (2009) tested 12 h post-training performance and Vicari et al. (2005) tested 24 h post-training performance. Both studies showed improvements in performance following the training session. For example, Vicari et al. (2005) tested the MD task in four 10-min sessions, the last session taking place the day after the initial testing. Typically developing children showed between-session improvements in both speed and accuracy during the initial training day, and a larger increase 24 h post-training. Between-session improvements were assessed while comparing performance following session completion. The current study employed a similar design.

Trial-to-trial assessment of the MD task shows performance loss between the last trial of a previous session and the first trial of the next session in adults (Snoddy, 1926) and in children (Prehn-Kristensen et al., 2009). It has been suggested that the consolidation of task-related memories amalgamates the fine-tuning motor process needed for task initiation with the memory trace, resulting in a decrease in this performance loss with practice (Buitrago et al., 2004). However, the effect of sleep dependent consolidation processes seems to differ by task performance level, being maximal at intermediate levels (Stickgold, 2009). Consolidation effects may therefore depend on task repetitions and age (Wilhelm et al., 2012). These data suggest that next-session performance is affected by many factors, and that the rate of increase in performance following a next-session, tested within a day and between days on the MD task, may differ between children in different age-groups, and between children and adults.

The Current Study

In the current study, 5–6-year-old kindergarten children, 7–8-year-old second-graders, and young adults practiced the MD task for two sessions on two consecutive days. We aimed to

investigate why it is that young children do not learn the MD task. It is not clear whether: (A) They cannot produce any correct polygon-sides, and therefore do not have an initial correct model to repeat and presumably optimize; or (B) Initial successful production is not repeated, as suggested by Ferrel-Chapus et al. (2002). We further tested several kinematic measures to characterize differences in online control of movement between age groups. Repetitions of MD production were tested over two consecutive training days because within a training day, learning could occur but not lead to performance gains. It has been shown that learning following repetition may be evident only when demonstrated in between-day improvement (Huyck and Wright, 2011; Soderstrom and Bjork, 2015).

Following the difficulties experienced by 5-year-olds in producing strokes without directional change, as described by Ferrel-Chapus et al. (2002), and following the success of 7-year-olds on the MD task described by Lejeune et al. (2013), we hypothesized that mirror learning would mature with age into adulthood.

Furthermore, Ferrel-Chapus et al. (2002) made a distinction between the 7-year-olds who were able to correctly produce one or more segments of the MD shape without directional changes, and the 5-year-olds who were not able to do so even after many repetitions. We hypothesized that improvement on the MD task in terms of correct polygon-sides requires some initial correct performance experience (as in the case of the 7-year-olds). To test this notion, we compared improvement in performance between those children who produced correct sides during initial training, and those who did not.

Specifically, we hypothesized that:

1. Kindergarten children aged 5–6- years would not be able to learn the MD task because they would hardly produce any correct polygon-sides.
2. Production kinematics would differ between the two younger age groups, and 7–8-year-olds would show better online control of movement.
3. Second-grade students aged 7–8 years would be able to perform the MD task, and with training, would improve more than 5–6 year-olds kindergarteners because they would learn the MD visuo-motor association with repeats.
4. Adults would learn the MD task better than second-grade students, in terms of speed and accuracy.
5. Only children who could produce correct polygon-sides on initial training would improve their performance following subsequent training.

MATERIALS AND METHODS

Participants

Full data were acquired for 58 of 60 participants recruited to participate in the study. These included 19 kindergarten children (9 boys, 10 girls), aged 5 years 7 months, to 6 years 8 months (M = 6.17 years, SD = 0.33); 19 second-grade children (10 boys, 9 girls), ranging in age from 7 years 6 months, to 8 years 11 months (M = 8 years, SD = 0.43); and 20 young adults (10 men, 10 women), aged 19 years 1 month to 29 years 5 months (M = 23.92 years, SD = 3.01). One kindergartener did not want to take part in the second session of the first day. One second-grader did not want to take part in the second day of the study. Participants were recruited from centrally located regions in Israel, with medium-high socio-economic status. Israeli Ministry of Education approval of the study was received (approval number: 10.32/235/2010, 10.32/514/2011), and parents of children signed Ministry of Education consent forms. Adults signed a university-standard consent form. According to parental reports, the children recruited for the study did not have any known neurological conditions or sleep disorders. Furthermore, kindergarten teachers, as well as school teachers, identify children at risk for developmental delay in the first 3 months of the school year (Ministry of Education, 2007). These children were not included. All participants were right-handed, based on the Hand Dominance Questionnaire (Oldfield, 1971). The parents of the younger participants answered 10 age-matched questions on the questionnaire for their children (RH: range 7–10, M = 8.75, SD = 1.16; range 7–10, M = 8.75, SD = 0.97, kindergarten and second-grade, respectively). Adults answered all 14 questions (range 10–13, M = 12.05, SD = 0.89).

The participants of the current study were part of a larger study focusing on the association between motor skill learning (assessed using the Invented Letter Task) and academic achievements (Julius, 2015). The participants (except for one adult) were assessed on additional measures, including tests of visuo-motor skills known as predictors of handwriting in children (Feder and Majnemer, 2007). These tests are reported below.

Procedure

All sessions were conducted in a quiet room, over two consecutive days. Token rewards such as school supplies, were distributed to the children at the end of each day. In the instance of the children, handedness and visuo-motor skills were tested in sessions separate from the MD task. In the adults, these were assessed following the last MD session.

The Mirror Drawing Task

For the MD task we employed the shape of a square that does not require diagonal lines, because diagonal lines are considered more complex to produce (Gullaud-Toussaint and Vinter, 2003; Feder and Majnemer, 2007). The MD task apparatus consisted of a box that was constructed to hold a mirror allowing the participants to see the target page, but not to see their hand. Based on our preliminary trials, and bearing in mind the age-dependent abilities of the younger participants in this study, we adopted a square shape as our MD task. Following Vicari et al. (2005), we used timed sessions, but shortened the length of the session compared to Vicari et al. (2005) from 10-min sessions to 5 min per session. The task was performed over two consecutive days; two 5-min sessions, 10 min apart, were held on two consecutive days. Participants traced the outline of a square between two lines while seeing their hand only through a mirror. They were not able to see the square directly, as the MD apparatus blocked their vision.

The length of each side of the square was 11.5 cm, and the distance between the inner and outer contour was 0.9 cm. Participants were instructed to complete as many squares as possible during the allotted time while staying within the double line—the inner and outer parameters of the square. Upon completion of a square, another sheet of paper was placed in the MD apparatus by the experimenter. On several occasions, the younger children requested a new sheet before completing a square.

Coding

Following the observation by Ferrel-Chapus et al. (2002) regarding the differences between 5- and 7-year-olds' correct movement-segments, only correct movement-segments of at least one polygon-side length (i.e., one side of a square) were analyzed. Thus, similar to Vicari et al. (2005), we measured speed according to the number of correctly produced polygon-sides in each session (rather than the overall number of polygon-sides). Correct polygon-sides were defined as sides of the square produced with no lines that crossed over either the inner or the outer parameter of the square, and no pen-lifts, with at least one correctly performed corner turn. The ratio of incorrect polygon-sides to the total number of polygon-sides served as the main error measures of analyses. Interrater reliability (ICC measure), calculated for 12 participants (4 in each age group, overall 20% of the data), was 0.95 ($p < 0.001$).

In order to characterize the MD solution strategy used by the two children's groups, additional kinematic measures were coded. These included the total number of sides of a square completed (correct and incorrect polygon-sides), and all pathway line crossings (either escaping from between the double line, or entering the double line) committed per shape. Following Vicari et al. (2005), an error ratio measure (overall number line crossings divided by the number of polygon-sides produced, per session) was calculated. Reversals (direction change) and pen-lifts per shape were also coded (Ferrel-Chapus et al., 2002; Gullaud-Toussaint and Vinter, 2003), and their ratio to the total number of polygon-sides produced was calculated.

Visuo-Motor Skills

The Beery Buktenica developmental test of visuo-motor integration (Beery-VMI) is frequently administered to evaluate the quality of abilities that may underlie problematic handwriting. The main idea is that the acquisition and preservation of readable handwriting requires one to be able to recognize shapes; to use vision to control arm, hand, and finger movements; and to coordinate the movements of these effectors accurately. Three subtests of the Beery-VMI were developed to test these abilities in children between 2- and 17-years. The test is the norm referenced for American children from 2- to 18-years.

The visual perception subtest (VP) measures whether children can discriminate geometric figures. The visuo-motor integration subtest (VMI) is used to assess children's ability to copy similar geometric figures, while the motor coordination subtest (MC) requires children to draw figures in between lines (Beery et al., 1997). All three tests use 27 geometric figures, starting with simple figures and ending with more complex ones. All children participating in the study had a standardized VMI score $\geq$85, apart from one second-grader who had a standard score of 76. Adults, evaluated using 18-year-olds standards, scored 86 or above. Performance below the 5th percentile (standardized score $<$75) is appropriate for the definition of a motor impairment which impacts children's daily life (Lingam et al., 2009).

The MC-subtest was used in the current study as a covariate of motor performance. The MC-subtest was preferred because it has the same visual features as the MD task, but it enables normal visual feedback. In the MC-subtest, the participant draws a line within each of the 24 figures. The line is drawn in a gap between an inner and an outer borderline (as in the MD task). Two dots define the beginning and the end of the line ("draw a line from the black dot to the gray dot. Try to stay inside the track"). Completion time is within a maximum of 5 min. Participants were not allowed to use an eraser. All correctly drawn figures (i.e., between the lines) were scored. Standardized MC scores were: M = 92.70, SD = 17.23; M = 84.11, SD = 9.54; M = 92.42, SD = 7.54, for kindergarteners, second-graders and adults, respectively, range = 76–115; raw scores were: M = 13.15, SD = 3.51; M = 14.70, SD = 2.41; M = 25.37, SD = 1.86, respectively. Analysis of Variance (ANOVA) indicated a significant group differences in raw scores $F_{(2,55)} = 117.65$, $p < 0.001$, that emerged because adults had much higher scores than the two younger groups (Bonferroni, p's < 0.001) that did not differ significantly ($p = 0.22$). Differences in standardized between these groups were insignificant as well $F_{(1,36)} = 1.82$, $p = 0.08$.

Analytic Plan

The aim of the current study was to understand why 5-year-olds (and possibly some 7-year-olds) do not learn the MD task. To this end, we compared the performance of three age-groups on the number of correctly performed polygon-sides, and the ratio of incorrect polygon sides. Differences between the three age-groups on the measures of the number of correct polygon-sides produced and the ratio of incorrect polygon-sides were studied using a 2 (Day) $\times$ 2 (Session) $\times$ 3 (Group) Analysis of Variance for repeated measures (rmANOVA). The main effects were reported, but where these were followed by interactions, only the interactions were explained. Based on the hypotheses that 5–6-year-old kindergarten children would not show learning, and that 7–8-year-olds second-graders would learn, but less so than adults, significant Group main effects were followed by comparing the 5–6- to the 7–8-year-olds (first contrast), and by comparing the 7–8-year-olds to adults (second contrast). Similarly, interactions were followed by using interaction contrast analysis. In case of violations of equality of variances appropriate testing procedures were used, correcting for degrees of freedom.

Because learning may be affected by motor ability, the rmANOVAs were repeated with the raw scores of the Beery MC-subtest as a covariate. To differentiate between the effects of age-group and motor ability on learning, one condition

is that the age-group variable and MC-subtest scores must be independent before entering the analyses. As indicted in "Visuo-Motor Skills" Section above, this held true only for the raw (and standardized) scores of the two younger age-groups. The second condition is that the age-group × MC-subtest interaction with respect to the dependent variable be insignificant (Miller and Chapman, 2001). We therefore restricted the analysis with the MC-subtest used as a covariate to the two younger age-groups, and reported the analyses only after verifying a non-significant interaction term in a preliminary analysis (i.e., a non-significant group by MC-subtest interactions when the MC-subtest is incorporated into the analysis). Analyses done with raw scores used as a covariate were then repeated with standardized scores used as a covariate.

In order to characterize developmental changes in performance strategy (from ballistic to online movement control), we analyzed the total number of polygon-sides, and the ratios per polygon-side of pen-lifts, pathway line-crossing errors, and reversals. These analyses pertained only to the two younger age groups, and were carried out between- as well as within-groups.

Parametric as well as non-parametric tests were used to test the hypothesis that of the 38 children, only children who could produce correct polygon-sides on initial training would improve their performance following subsequent training. The definition of what constituted initial training and subsequent training was based upon the emergence of learning gains in second-graders vs. kindergarteners. We tested improvement differences on subsequent training between those children who did produce correct polygon-sides on the initial portion of the training and those who did not. The non-parametric analysis was performed using Sign tests and a *z*-ratio test. The Sign test compared subsequent-training improvement among those who did/did not produce correct sides in initial training. The *z*-ratio test compared the proportion of subsequent-training improvement between these two groups. Our hypothesis would be supported if the *z*-ratio test were to find that among those children producing correct sides during initial training, there would be a significant larger proportion of children who improved vs. those who did not produce correct polygon-sides. The parametric analyses compared the magnitude of improvements between these two groups.

RESULTS

Examples of MD production by age group are provided in **Figure 1**. **Figure 2** presents the number of correct polygon-sides, and the ratio of incorrect polygon-sides to the total number of polygon-sides for each age group: 5–6-year-old kindergarten children, 7–8-year-old second-graders, and young adults.

Number of Correct Polygon-Sides

The 2 (Day) × 2 (Session) × 3 (Group) rmANOVA pertaining to the number of correct polygon-sides indicated a main effect of Group $F_{(2,55)} = 117.27$, $p < 0.001$, $\eta^2 = 0.81$, and a main effect of Day $F_{(1,55)} = 32.71$, $p < 0.001$, $\eta_p^2 = 0.37$, modulated by a Group × Day interaction $F_{(2,55)} = 17.03$, $p < 0.001$, $\eta_p^2 = 0.38$. The group contrast comparisons revealed that the between-day improvement (the difference in improvement during Day 2 vs. during Day 1) was greater for the second-graders than for the kindergarteners $F_{(1,19.74)} = 12.09$, $p < 0.01$, and greater for the adults than for the second-graders $F_{(1,22.10)} = 13.35$, $p < 0.01$. Only the second-grade students and adults improved between days ($F_{(1,18)} = 15.31$, $p < 0.01$; $F_{(1,19)} = 24.29$, $p < 0.001$, respectively).

The rmANOVA further indicated a main effect of Session $F_{(1,55)} = 61.62$, $p < 0.001$, $\eta_p^2 = 0.53$, that was modulated by a Group × Session interaction $F_{(2,55)} = 46.23$, $p < 0.001$, $\eta_p^2 = 0.63$. The group contrast comparisons indicated that the improvement from Session 1 to Session 2 was greater in the adults than in the second-graders ($F_{(1,26.71)} = 42.46$, $p < 0.001$) because only the adults improved between sessions, $F_{(1,19)} = 65.19$, $p < 0.001$. No other interactions emerged.

These data suggest a different rate of improvement between groups. Kindergarten children did not improve their performance as a result of training. Second-graders gained between days more than kindergarteners. Adults gained more than second-graders between sessions and between days.

The Ratio of Incorrect Polygon-Sides

The rmANOVA pertaining to the ratio of incorrect polygon-sides (number of incorrect polygon-sides/total number of polygon-sides) indicated a main effect of Group $F_{(2,55)} = 941.76$, $p < 0.001$, $\eta^2 = 0.97$, and a main effect of Day $F_{(1,55)} = 12.07$, $p < 0.001$, $\eta_p^2 = 0.18$, modulated by a Group × Day interaction $F_{(2,55)} = 6.41$, $p < 0.01$, $\eta_p^2 = 0.19$. The group contrast comparisons indicated that adults improved between days significantly more than second-graders ($F_{(1,35.58)} = 4.54$, $p < 0.05$), because only the adults improved between days, $F_{(1,19)} = 23.43$, $p < 0.001$.

The rmANOVA further indicated a main effect of Session $F_{(1,55)} = 8.61$, $p < 0.01$, $\eta_p^2 = 0.14$, whereby the performance during the second session was more accurate than during the first. No other interactions emerged.

Developmental Differences Between Kindergarteners and Second-Graders Beyond Motor-Coordination Ability

It may be suggested that group differences in learning partially reflect differences in motor ability, rather than in learning, *per se*. To test this possibility, the above analyses were repeated with the raw (and standardized) scores of the MC-subtest of the Beery-VMI as a covariate in analyses that pertained to the two groups of children. In the MC-subtest, participants draw figures between a double-line (see "Materials and Methods" Section), while afforded with visual feedback. The results of the MC-subset did not differ significantly between kindergarteners and second-graders. A preliminary analysis indicated that group × MC-subtest was insignificant in the analyses of the number of correct polygon-sides and the ratio of incorrect polygon-sides.

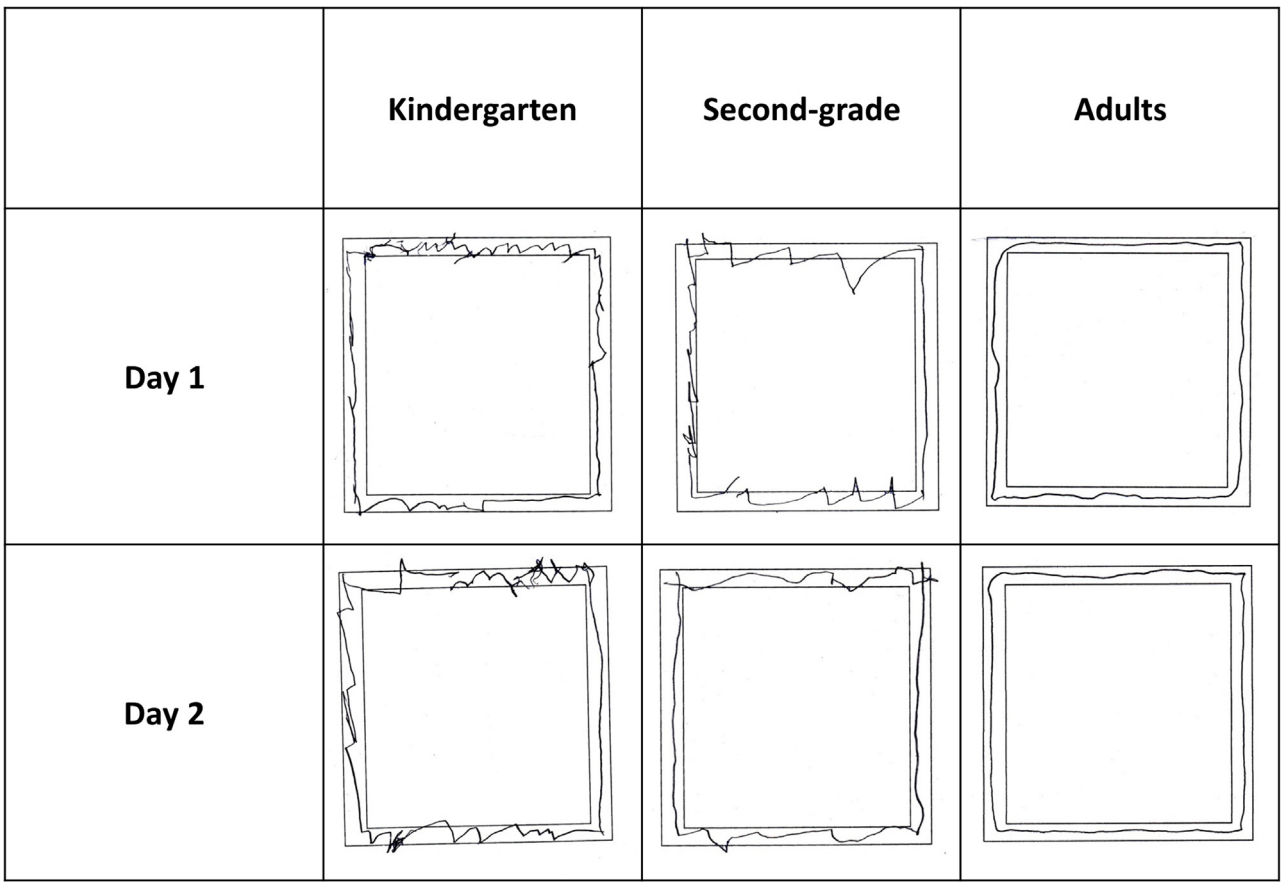

FIGURE 1 | Examples of mirror drawing (MD) production by age group: 5–6-year-old kindergarteners, 7–8-year-old second-graders, and young adults.

The rmANOVAs pertaining to the number of correct polygon-sides, in the presence of the raw MC-subtest as a covariate, indicated a significant effect of Group ($F_{(1,35)} = 13.87$, $p < 0.01$, $\eta_p^2 = 0.28$). The Group main effect was modulated by a Group × Day interaction ($F_{(2,53)} = 12.14$, $p < 0.01$, $\eta_p^2 = 0.26$), because only second-graders improved between days. The analyses pertaining to the ratio of incorrect polygon-sides indicated only a main effect of group ($F_{(1,35)} = 7.81$, $p < 0.01$, $\eta_p^2 = 0.18$) due to the better performance of the second-graders. The same pattern of results was received when the standardized MC-subtests were used as a covariate. These analyses indicate that differences between kindergarteners and second-graders could not be fully accounted for by differences in MC.

In Depth Analysis of the Differences Between Kindergarteners and Second-Graders

In order to test the source of the difference in the learning profile between kindergarteners and second-graders, a detailed kinematic analysis was carried out between age-groups and within age-groups. Four additional measures are presented: the total number of polygon-sides produced, the ratios of pen-lifts, pathway line-crossing errors, and reversals per polygon-side (**Figure 3**). It should be noted that for all analyses the Group × Day interactions, whenever appearing, were retained even when the Beery MC-subtest (either raw or standardized) was used as a covariate. For simplicity, we report here the analysis without the covariate.

Total Polygon-Sides

The between group analysis of the total number of polygon-sides indicated a main effect of Group, $F_{(1,36)} = 13.76$, $p < 0.001$, $\eta^2 = 0.28$. Furthermore, the analysis indicated a main effect of Day $F_{(1,36)} = 56.88$, $p < 0.001$, $\eta_p^2 = 0.61$, modulated by a Group × Day interaction $F_{(1,36)} = 42.07$, $p < 0.001$, $\eta_p^2 = 0.54$. There was also a main effect of Session, $F_{(1,36)} = 19.71$, $p < 0.01$, $\eta_p^2 = 0.36$, modulated by a Group × Session interaction, $F_{(1,36)} = 16.77$, $p < 0.001$, $\eta_p^2 = 0.31$. The interactions emerged because only second-grade students increased the total number of polygon-sides produced between days and between sessions ($F_{(1,18)} = 80.30$, 33.46, respectively, ps < 0.001). No other interactions emerged. Significant interactions were retained even when the MC-subtest was added as a covariate.

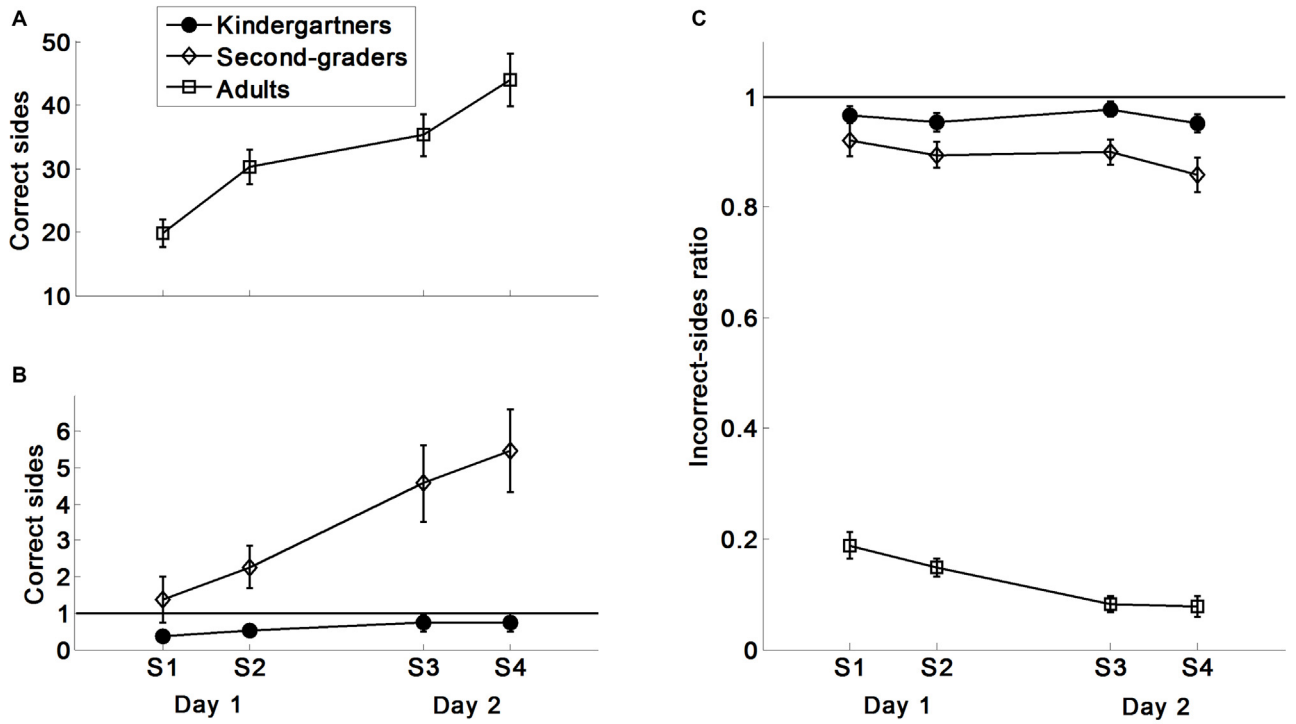

FIGURE 2 | Main outcome measures: the number of correct sides and the ratio of incorrect sides/total sides for children and adults. (A) Correct sides in adults. **(B)** Correct sides in children. **(C)** Incorrect-sides ratio.

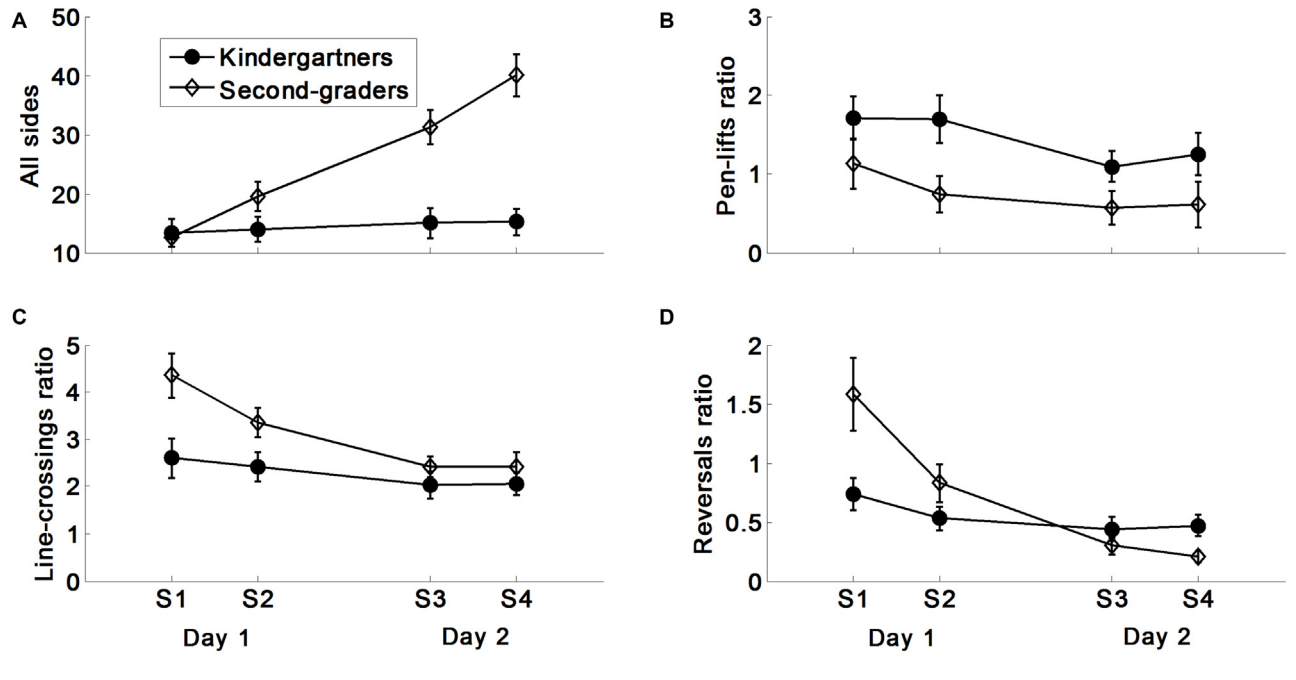

FIGURE 3 | Children's production characteristics. (A) Overall number of sides. **(B)** Pen-lifts ratio. **(C)** Line-crossings ratio. **(D)** Reversals ratio. The ratios were computed as number per side.

Pen Lifts

The analysis of the rate of pen-lifts per polygon-sides indicated an overall higher rate of pen-lifts in kindergarteners, $F_{(1,36)} = 4.33$, $p < 0.05$, $\eta^2 = 0.11$. The analysis further indicated that the number of pen-lifts was lower on the second day (i.e., when performance was summed over the two sessions) $F_{(1,36)} = 10.59$, $p < 0.01$, $\eta_p^2 = 0.23$, for both groups. No other main effects or interactions emerged. The Day main effect was not significant after the MC-subtest was added as a covariate.

Line-Crossing Errors

The analysis of the rate of line-crossing errors indicated a main effect of Group, $F_{(1,36)} = 5.17$, $p < 0.03$, $\eta^2 = 0.13$. Furthermore, the analysis indicated a main effect of Day $F_{(1,36)} = 22.47$, $p < 0.001$, $\eta_p^2 = 0.38$, modulated by a Group × Day interaction $F_{(1,36)} = 6.00$, $p < 0.02$, $\eta_p^2 = 0.14$. The interaction emerged because only second-grade students decreased their line-crossing error-rate between days ($F_{(1,18)} = 25.28$, $p < 0.001$). The rmANOVA further indicated a main effect of Session, $F_{(1,36)} = 4.57$, $p < 0.05$, $\eta_p^2 = 0.11$, modulated by a Day × Session interaction, because line-crossing error-rate significantly decreased only on Day 1, $t_{(38)} = 2.93$, $p < 0.01$, and to a much lower extent on Day 2. No other interactions emerged. Only the Group × Day interaction remained after the MC-subtest was added as a covariate.

Reversals

Unlike out-of-line errors and pen-lifts, reversals were not defined as errors. They primarily indicate to what extent children learned the visual-motor map. The analysis of reversals indicated no overall group differences. In contrast, all other main effects and interactions emerged as significant, including the triple interaction of Day × Session × Group, $F_{(1,36)} = 4.86$, $p < 0.04$, $\eta_p^2 = 0.12$ (for all other mean effects and interactions $F_{(1,36)} = 6.10$, ps < 0.02). The triple interaction emerged because on Day 1 Session 1, second-graders began with a higher rate of reversals than kindergarteners, $t_{(36)} = 2.67$, $p < 0.02$. However, the rate of second-graders' reversals decreased across sessions ($F_{(1,18)} = 18.17$, $p < 0.001$), while that of kindergarteners was maintained. Finally, in the last session (Day 2 Session 2), second-graders had a lower rate of reversals than kindergarteners, $t_{(36)} = 2.57$, $p < 0.02$. These data suggest that only second-grade students learned the visual-motor map. It should be noted that when the Beery MC-subtest was used as covariate the triple interaction decreased, and was at the $p = 0.066$ level only.

Within-Group Analyses

Tables 1, **2** include a detailed analysis of the learning within each of the age-groups (these tables match **Figures 2**, **3**). **Table 1** indicates that across days, kindergarten children did not improve their performance significantly, in terms of either correct polygon-sides, or the rate of incorrect polygon-sides, while second-grade students significantly improved in the former, with no decrease in the latter.

Table 2 shows that the second-graders improved on the additional four kinematic measures within the first day, and between days. This explains, at least partially, their between-day improvement in the number of correct polygon-sides produced (**Figure 2**). Kindergarten children showed improvement only in the ratio of pen-lifts to polygon-sides produced between days (reduction of pen-lifts ratio while maintaining their overall number of polygon-sides produced), suggesting that similar to second-graders, these children produced longer strokes on Day 2 than on Day 1.

Learning of the MD Task in Children Who did/did not Produce Correct Polygon-Sides on Day 1

On average, kindergarten children did not produce even one correct polygon-side, and had above 95% inaccurate polygon-sides throughout the experiment (**Figure 2**). Second graders had on average more than three polygon-sides correctly produced by the end of Day 1. The achievements of second-graders on Day 2 suggest that having at least one polygon-side correctly performed on Day 1 was a sufficient experience for a between-day improvement. Among the 14 second-graders who produced one or more polygon-sides on Day 1, 11 improved (by at least by one polygon-side), two maintained their performance level, and one child preformed less well on Day 2 than on Day 1 (Sign-test, $p < 0.001$).

Of the 38 children, 23 produced correct polygon-sides on Day 1. Of these 23 children, 15 improved between days, five maintained their performance, and three children performed less well on Day 2 than on Day 1 (Sign-test, $p < 0.01$). Of the 15 children who did not produce any correct polygon-sides on Day 1, only five improved between days. These data suggest that children who had at least one polygon-side correctly performed on Day 1, were more likely to improve between days than their peers (15/23, vs. 5/15, $z = 1.9241$, $p = 0.0548$). Furthermore, among the 20 children who improved their performance between days (6 kindergarteners, 14 s graders), 15 (4 kindergarteners, 11 second-graders) had at least one polygon-side correctly produced on Day 1 (Binomial, $p < 0.05$), suggesting that between-day improvers were more likely to already have some experience in correctly producing polygon-sides.

The 23 children who had correct sides on Day 1 significantly improved their performance between days, $t_{(22)} = 3.47$, $p < 0.01$, and their improvement was larger than that of their peers, $F_{(1,36)} = 4.33$, $p < 0.05$. The 15 children who did not produce any correct sides on Day 1 did not show a significant between day improvement, $t_{(14)} = 1.83$, $p > 0.09$. Both groups did not change their accuracy scores between days. These data corroborate the notion that only children who had some experience in solving the MD task on Day 1, were able to succeed more on Day 2 because of practice. The children who did not have this experience (and kindergarten on average) did not improve between days.

In order to test the difference in the learning profile *within* Day 1 between those who produced and those who did not produce correct polygon-sides, the performance of these two groups was compared on the additional four kinematic measures. The analysis indicated that both groups similarly increased their overall number of sides produced

TABLE 1 | Within-group comparison of correct sides and incorrect sides ratio, across sessions and days.

	Between days: $(D_1S_1 + D_1S_2) - (D_2S_1 + D_2S_2)$	Between sessions: $(D_1S_1 + D_2S_1) - (D_1S_2 + D_2S_2)$	Day × Session	Within day 1: $D_1S_2 - D_1S_1$	$D_2S_1 - D_1S_2$	Within day 2: $D_2S_2 - D_2S_1$
	F day	*F* session	*F* interaction	*t*	*t*	*t*
Correct sides						
Kindergarten	2.57	0.32	0.81	1.37	1.00	0.00
Second-grade	15.31***	2.88	0.01	1.44	2.55*	1.04
Young adults	24.29***	65.19***	0.72	7.84***	2.20*	4.73***
Error rate: incorrect sides/total sides						
Kindergarten	0.68	2.65	1.15	0.91	1.24	2.42*
Second-grade	1.65	3.46	0.12	0.88	0.28	1.65
Young adults	23.43***	2.47	1.18	1.90	3.44**	0.08

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Kindergarten, n = 19; Second-grade, n = 19; Young adults, n = 20. D = Day. S = Session.*

TABLE 2 | Within-group comparison of kinematics measures across sessions and days.

	Between days: $(D_1S_1 + D_1S_2) - (D_2S_1 + D_2S_2)$	Between sessions: $(D_1S_1 + D_2S_1) - (D_1S_2 + D_2S_2)$	Day × Session	Within day 1: $D_1S_2 - D_1S_1$	$D_2S_1 - D_1S_2$	Within day 2: $D_2S_2 - D_2S_1$
	F day	*F* session	*F* interaction	*t*	*t*	*t*
Overall number of sides						
Kindergarten	1.04	1.43	0.10	0.45	0.58	0.11
Second-grade	80.30***	33.46***	0.77	4.95***	5.82***	4.34***
Pen-lifts ratio						
Kindergarten	5.87*	0.35	0.58	0.18	2.89**	0.91
Second-grade	4.87*	1.53	3.82	2.25*	2.29*	0.24
Line-crossings ratio						
Kindergarten	2.68	0.32	0.39	0.85	1.47	0.13
Second-grade	25.28***	4.26*	7.59*	3.11**	3.67**	0.65
Reversals ratio						
Kindergarten	2.09	0.89	3.96	1.83	0.69	0.26
Second-grade	21.78***	18.17***	18.03***	4.68**	4.25**	2.25*

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Kindergarten, n = 19; Second-grade, n = 19. D = Day. S = Session.*

$F_{(1,36)} = 7.61$, $p < 0.01$, reduced their ratio of line-crossing, $F_{(1,36)} = 10.53$, $p < 0.01$, and of reversals, $F_{(1,36)} = 21.44$, $p < 0.001$ with no group interactions. However the children who produced correct polygon-sides on Day 1 had an overall higher number of sides produced, $F_{(1,36)} = 7.61$, $p < 0.001$, and lower ratio of pen-lifts $F_{(1,36)} = 41.75$, $p < 0.001$. These data suggest that both groups improved on some of the kinematic measures; however, the children who produced correct polygon-sides on Day1 had an overall better performance than their peers did. These children (like second-graders vs. kindergarteners on average) were able to produce longer strokes.

DISCUSSION

The current study tested developmental differences in within-day and between-day learning on the MD task. Three age groups were tested: 5–6-year-old kindergarten children, 7–8-year-old second-graders, and young adults. Kindergarten children produced on average less than one correct polygon-side per session, had less than 5% accurate polygon-sides produced overall, and did not improve their performance significantly throughout the experiment. The use of many oriented short segments characterized their performance. Second-grade students showed better online control of movement, enabling them to produce fewer segments per polygon-side. They showed robust learning of the visuo-motor association, accompanied by a reduction in the ratio of out-of-line errors and pen-lifts. This enabled them to produce more correct polygon-sides on Day 2 than on Day 1, while maintaining their accuracy scores (no speed-accuracy trade-off). Differences in MC between kindergarteners and second graders did not account for this performance difference between the groups. Adults produced more sides that were correct and were more accurate than the two groups of children. In the adult group, both the number of correct sides produced and accuracy scores improved between days, while the number of correctly produced polygon-sides increased within-days as well. The main difference between the age groups involved between-day improvement, which increased with age.

Overall, children producing at least one correct polygon-side on Day 1 (23/38 children) were more likely to improve their performance between days. For these children only, performance on Day 2 was better than on Day 1 in terms of correct polygon-sides produced, with no reduction in accuracy. These data corroborate the notion that initial effective training experience that involves correct task solutions is needed in order to show gains in correct performance. Kindergarten children, or those who did not produce correct-sides on Day 1, improved in some aspects of their kinematic production, thereby indicating improvement with repeats. However, this improvement did not bring about a between-day increase in the number of correct polygon-sides produced. Possibly, these children had less practice opportunities due to the use of multiple strokes per side. Fewer strokes per side may offer more opportunities for movement corrections (e.g., via direction changes). Lack of success may indicate that the task was too difficult for some of the children. Future studies may test whether explicit instructions while performing the task (e.g., try producing longer lines, try staying within the double-line) may help children to solve the task.

In line with previous developmental studies of MD learning (Ferrel-Chapus et al., 2002; Lejeune et al., 2013; Finn et al., 2016), the findings of the current study suggest that MD learning matures with age. This finding is in line with results of other motor adaptation studies (Konczak et al., 2003; Contreras-Vidal et al., 2005; Bo et al., 2006; Kagerer and Clark, 2014). However, these findings do not indicate that for all tasks motor skill learning matures with age (Dorfberger et al., 2007; Ashtamker and Karni, 2013; Adi-Japha et al., 2014). A recent study of a grapho-motor task requiring the reproduction of a simple "Invented Letter", a dot-to-dot connecting task forming a two-segment pattern, indicated a similar learning profile within and between days in kindergarten children, second-graders, and adults (Julius and Adi-Japha, 2015). In the Invented Letter task, direct visual feedback exists. These data suggest that children's motor skill learning depends on the task's characteristics such task complexity and the affordance of visual feedback (Ferrel-Chapus et al., 2002). In simple tasks that do not require much attentional resources and on-line control, children improve in the same way as adults (Dorfberger et al., 2007; Adi-Japha et al., 2014; Julius and Adi-Japha, 2015), suggesting that efficient skill learning exists early on. The learning of complex tasks may require more controlled trail-and-error processes and more adaptation of performance solutions, which mature with age, in order to find an initial task solution/performance mode (Adi-Japha et al., 2008).

Furthermore, the findings of the current study show that performance indications for MD-learning in children, in terms of correctly performed sides, first emerge between days: in second-graders, performance on the second day of the study was significantly better than on the first (Vicari et al., 2005). In terms of accuracy, only adults showed significant improvement, also between days. In line with the similarity between motor and perceptual learning (Censor et al., 2012), training on different auditory perceptual tasks (Huyck and Wright, 2011, 2013) suggests late maturation of the learned skills, with indication of performance gains emerging between, rather than within, days. It has been suggested that inattention due to repeated experiences and fatigue may contribute to the finding of lack of improvement within sessions. Studies on fatigue suggest that learning could occur even after fatigue prevents any further gains in performance during acquisition. Fatigue build-up can also cause worsening in performance (Rickard et al., 2008; for a review on the difference between learning and performance, see Soderstrom and Bjork, 2015). Future studies may test whether older children and adolescents show within session MD learning.

The current study sought to test the source of difficulty kindergarten children have in MD learning, relative to older children. Kinematic analysis applied to the two groups of children studied here revealed that the kindergarteners lifted their pen more times per side, indicating that these children produced many segments. Overall, kindergarteners maintained a stable ratio of line crossings and directional changes (reversals) to polygon-sides produced. Second-graders produced fewer segments per side, but initially had more line crossings per side than kindergarteners had. These crossings emerged because of the many reversals second-graders produced due to the visual distortion. Initial rate of reversals was higher in second-graders than in kindergarteners. Furthermore, second-graders corrected their movement online by reversals, while most kindergarteners could not and preferred initiating new segments. Reversals were initially of a much larger magnitude in second-graders, but dropped with practice to a lower level than that of the kindergarteners. A drop in the rate of line crossings mirrored the drop in reversals. In both kindergarteners and second-graders, the number of pen-lifts per side was reduced across days. This indicates a decrease in the number of segments used per side, and suggests an increase in their length (covering the same trajectory length but with less segments). Possibly, the preference of kindergarteners to initiate many new segments while trying to stay within the double-line (see **Figure 1**) lowered their performance rate and prevented a significant increase in the overall number of sides produced (which increased insignificantly from 13 sides/session on Day 1 to 15 sides/session on Day 2).

On the whole, the kinematic analysis indicated that the 5–6-year-old kindergarten children exhibited a ballistic mode of control (rapid movements, followed by stopping for error evaluation that resulted in pen lifting). With practice, their motor control improved; therefore, they were able to reduce the number of segments used. They did not reduce their error rate, suggesting that in spite of an attempt to stay within the double-line, they repeatedly crossed the line and returned between the double-line. The 7–8-year-old second-graders in the current study also used ballistic movements, but to a lesser degree. Importantly, due to their better online movement control, second-graders were able to learn the visual-motor mapping, as indicated by the decrease in reversal rates with

practice. Our results concur with the classic motor control literature, suggesting that the performance at 7 years of age is characterized by the dominance of the visual guiding system (e.g., Hay, 1978, 1979; Chicoine et al., 1992; Adi-Japha and Freeman, 2001; Contreras-Vidal et al., 2005). These results contrast with the report of MD learning made by Ferrel-Chapus et al. (2002) who concluded that 5- and 7-year-olds used a similar, ballistic strategy. The difference between the studies may be related to the between-day design of the current study enabling a longer period of learning, or to the somewhat older age of the second-graders in the current study.

We also studied production kinematics differences within the 1 day, between those who did and those who did not produce correct sides on that day (9/19 kindergarteners and 14/19 second-graders produced correct sides on the first day). The analysis indicated similar within-day learning characterized by an increase in the overall number of sides produced, and a decrease in the number of line crossings and direction changes per side. Importantly, the group of children who produced correct sides on the first day, produced more sides overall (i.e., correct and incorrect) that day, and had less segments per side (suggesting that their segments were longer). Overall, these children had more experience in correcting their production through directional changes, which possibly enabled them to learn the MD visuo-motor representation. Taken together with the kindergarteners reduction in the number of segments produced between days, these data suggest that those not producing correct sides on the first day of the study understood the MD task and tried to the solve it, but did not succeed, probably because of a lower level of online movement control.

Limitations and Conclusions

The findings reported here must be considered within the limitations of the study. Only a small sample of participants of a very specific age-range per age group was studied. Only one simple MD shape was used that does involving diagonal lines. The kindergarten children showed a low success rate, resulting in low variability. Second graders differed in their motor profile, which may have contributed to greater performance variability in this group. Adults had an overall better performance with accompanying greater variability. Differences in performance variability may have inflated the Type I error, thereby increasing the probability for rejecting the null hypothesis. The low success-rate of the kindergarteners may also suggest that some of them did not understand the task. However, it should be noted that many children tried to peak at their hands in order to have a direct view, suggesting that these children were aware of the difficulty induced by the MD inversion. Although the children in the current study were typically developing, as reported by their parents and teachers, learning disabilities or attentional disorders may be diagnosed later. Lastly, the findings are of a correlational nature; therefore, causality may not be inferred.

Consistent with previous studies, the findings of the current study suggest that MD learning matures with age. Furthermore, similar to perceptual tasks, performance indications for learning in children first emerge between days. The findings support the notion of a minimal *correct* experience necessary for between-day improvement. In line with the literature, the findings support the notion of a qualitative difference in the underlying motor control strategy used in the MD task by kindergarten children, second-graders, and adults.

AUTHOR CONTRIBUTIONS

MSJ and EA-J conceived and designed the experiments, analyzed the data, and wrote the article. MSJ performed the experiments.

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REVIEW
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Psychosocial Modulators of Motor Learning in Parkinson's Disease

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Using the remarkable overlap between brain circuits affected in Parkinson's disease (PD) and those underlying motor sequence learning, we may improve the effectiveness of motor rehabilitation interventions by identifying motor learning facilitators in PD. For instance, additional sensory stimulation and task cueing enhanced motor learning in people with PD, whereas exercising using musical rhythms or console computer games improved gait and balance, and reduced some motor symptoms, in addition to increasing task enjoyment. Yet, despite these advances, important knowledge gaps remain. Most studies investigating motor learning in PD used laboratory-specific tasks and equipment, with little resemblance to real life situations. Thus, it is unknown whether similar results could be achieved in more ecological setups and whether individual's task engagement could further improve motor learning capacity. Moreover, the role of social interaction in motor skill learning process has not yet been investigated in PD and the role of mind-set and self-regulatory mechanisms have been sporadically examined. Here, we review evidence suggesting that these psychosocial factors may be important modulators of motor learning in PD. We propose their incorporation in future research, given that it could lead to development of improved non-pharmacological interventions aimed to preserve or restore motor function in PD.

Keywords: Parkinson's disease, motor learning, self-efficacy, task engagement, emotions, social interaction

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INTRODUCTION

In Parkinson's disease (PD) research much effort is devoted nowadays to the development of complementary, non-pharmacological interventions, which could help alleviate the symptoms and slow down the neurodegenerative progression of the disease. Physical exercise and motor training have the potential to be such alternative interventions (Fisher et al., 2008; Goodwin et al., 2008), yet, their success essentially depends on individual's capacity to acquire new motor skills, which is also affected by the disease. Finding new ways to boost motor learning capacity in the course of intervention will not only increase the efficacy of exercising and motor training in people with PD, but, more importantly, it will increase the likelihood of a successful intervention.

There is much evidence indicating that motor learning and performance can be improved in PD via additional sensory stimulation (visual or rhythmical) and task cueing, most likely through increased activity in basal ganglia and the cortico-striatal circuits (Nieuwboer et al., 2009; de Bruin et al., 2010). Yet, despite these advances, several

important knowledge gaps remain. (1) Most studies investigating motor learning in PD used laboratory-specific tasks and equipment, with little resemblance to real life situations. Thus, it is unknown whether optimal stimulation and task cueing can be achieved in more ecological setups and whether individual's task engagement can further improve motor learning capacity; (2) The role of social interaction in the process of skill learning in PD has not yet been investigated; (3) Despite evidence that individual's mind-set, such as self-efficacy, strongly impacts performance and learning capacity (Mak and Pang, 2008; Salanova et al., 2011; Wulf et al., 2012), this issue has been sporadically addressed in PD research; and (4) In real-life, motor learning involves not only task-related motor-cognitive processes, but also requires managing task-related emotions, which have also motivational consequences; thus, self-regulatory mechanisms could play a crucial role in motor learning process, especially in PD, which is characterized by motor and emotional dysfunction.

In the current review, we will first discuss the evidence for the potential benefits of developing new ecological approaches in motor learning research in PD, with special focus on the role of social context as external modulators. Then, we will analyze the role of psychosocial factors as internal modulators of motor learning capacity, specifically patient's self-efficacy and emotional state. Finally, we will link these findings with the context of PD neuropathology and potential for motor treatment regimes.

THE UNDERLYING LINK BETWEEN PD NEUROPATHOLOGY AND NEURONAL CORRELATES OF MOTOR SEQUENCE LEARNING

PD is a progressive neurodegenerative disorder, characterized primarily by motor symptoms including tremor, rigidity, slowness of movement (bradykinesia) and gait difficulties. Typical PD patients present not only nigrostriatal dopaminergic cell loss in the basal ganglia, but also disruptions in mesocortical dopaminergic, noradrenergic, and other systems (Jellinger, 2012). These affect motor program selection by the striatal circuitry, with widespread effects in the entire cortico-striatal system (Amano et al., 2013). Much evidence from motor learning research indicates that acquisition of new motor sequences is based on increased neuronal activity in the cortico-striatal and cortico-cerebellar circuits and on a dynamic interaction between them (Doyon et al., 2009). In fact, striatum is involved in all stages of motor sequence learning with different parts of it deemed essential in each stage (Doyon and Benali, 2005). This indicates a remarkable overlap between PD neuropathology and neuronal correlates of motor sequence learning (see **Figure 1**).

Given this overlap and the fact that cerebral plasticity is maintained or increased through repeated practice and enhanced stimulation from the environment (Hultsch et al., 1999; Vance et al., 2010), it is conceivable that practicing or learning motor sequences in a rich and stimulating context may increase the effectiveness of non-pharmacological interventions aimed to preserve or restore motor function in Parkinson's.

VALUE OF ECOLOGICAL EXPERIMENTAL SETUPS IN PD MOTOR LEARNING RESEARCH

A review of motor learning literature (Nieuwboer et al., 2009) indicates a relatively preserved acquisition and retention of motor skills in people with PD, despite reduced learning rates and efficiency as compared to controls. However, using additional sensory information and visual task cueing can optimize motor learning in PD with long-lasting effects (Nieuwboer et al., 2009; Sacrey et al., 2009; Anzak et al., 2011). For instance, some investigators have shown that the use of music as an external sensory cue helped performance in motor tasks in PD (McIntosh et al., 1997; Bernatzky et al., 2004; Sacrey et al., 2009; de Dreu et al., 2012). Importantly, patients benefited from rhythmical stimulation not only in terms of gait and postural control, but also in generating more complex motor sequences using upper limbs (Thaut and Abiru, 2010).

This research advanced significantly our knowledge about motor learning in PD, but the very fact that it was done mostly in controlled laboratory or clinical settings is, simultaneously, an asset and an important limitation, which may hamper its translation into real-life situations. While the setups used in these studies allowed for precise measurements and a good control of variables, they were also removed from the ecological context in which most daily living activities take place (i.e., at home, at work). For instance, implicit motor sequence learning is typically studied using the serial reaction time task (SRTT), in which participants have to respond to sequential or random cues by pressing buttons as fast and as accurate as possible (Muslimovic et al., 2007), a goal which is not ecological and may even be disengaging. In contrast, in real life, people learn sequences of key presses on a new phone, for example, while writing messages or playing games; thus the goal is to write or play, not to learn key sequences *per se*. In this context, learning the sequences of key presses represent the means by which an ecological goal is achieved. In addition, it is likely that PD patients are more susceptible to fatigue, anxiety, are less motivated and self-confident than their healthy peers, due to the compromised dopamine pathways. Therefore, it is possible that the laboratory setting might actually undermine their potential and the results would underestimate their actual motor learning capacity.

The above mentioned factors, such as the overall task engagement, reflecting individual's level of arousal, interest and energy put into a given task (Salanova et al., 2011), have rarely been measured in PD motor learning studies. One notable exception is the research investigating the impact of console gaming technology. A systematic review provides evidence that exercising using console videogames improved not only the motor performance, but also task engagement in people with PD (Barry et al., 2014). In fact, a randomized controlled trial showed that the benefits of console game exercising on

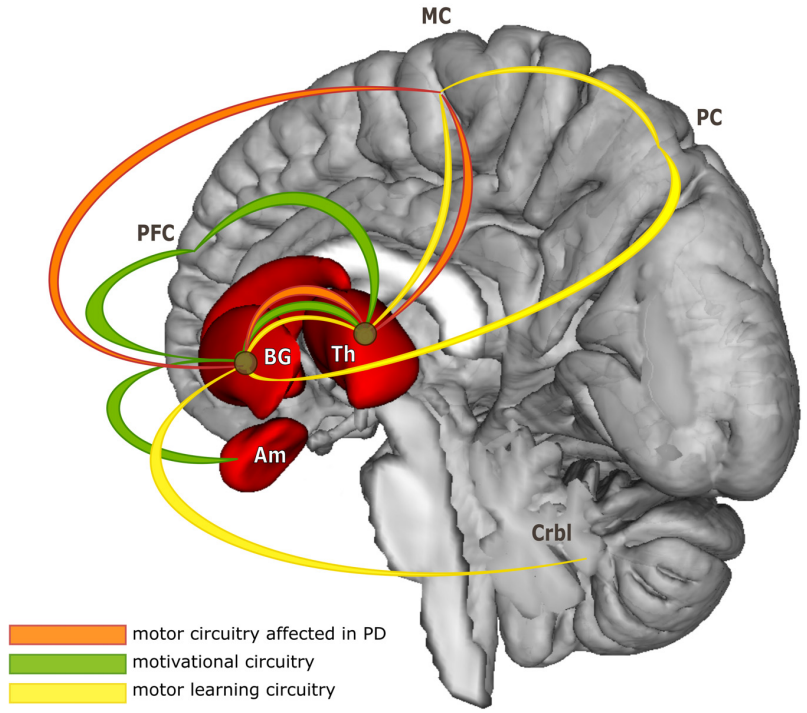

FIGURE 1 | Simplified model of functional circuitries of basal ganglia. The figure illustrates considerable overlap of neuronal pathways subserving three different functions targeted in the review. For more detailed model of cortico-striatal and cortico-cerebellar systems contributing to motor skill learning see for example Doyon et al. (2003); for basal ganglia functional organization in Parkinson's disease (PD) see Blandini et al. (2000) or Obeso et al. (2008); for basal ganglia motivational loop see Ikemoto et al. (2015). Abbreviations: BG, basal ganglia; Th, thalamus; Am, amygdala; PFC, prefrontal cortex; MC, motor cortex; PC, parietal cortex; Crbl, cerebellum.

balance, cognition and some motor symptoms were as good as the typical rehabilitation regimen in PD and lasted for up to 60 days post-intervention (Pompeu et al., 2012). Altogether, these results suggest that exercising in a more ecological context, with complex sensory stimulation and meaningful goals might increase task engagement, and consequently boost the effects of motor rehabilitation and non-pharmacological interventions in PD (Nieuwboer et al., 2009; Nombela et al., 2013; Barry et al., 2014).

Regarding the possible action mechanisms by which enriched environment may enhance learning and memory in PD, a study with rodents showed that the observed functional changes were also associated with numerous neuronal changes, including altered cortical weight and thickness or increased dendritic branching and synaptic strength (Nithianantharajah and Hannan, 2006). These findings suggest a neurophysiologic mechanism by which external sensory stimulation may facilitate the signal neurotransmission in the impaired cortico-striatal loop, thus improving some of the Parkinsonian motor symptoms, the gait, as well as the execution of movements that were previously automatic. However, given the lack of PD research employing ecological experimental setups, it is yet unknown to what extent these methods could also improve motor learning capacity and whether the underlying mechanisms of action are based on the above mentioned types of neuronal changes.

SOCIAL INTERACTIONS AND MOTOR SKILL LEARNING IN HEALTHY SUBJECTS AND PD PATIENTS

Another important aspect, largely ignored in motor learning research in general, and in PD in particular, is the fact that in everyday life people usually learn and perform motor actions together with others (i.e., sports, dance, etc.) and not in isolation. However, the vast majority of motor learning literature is based on tasks presented to single participants in settings removed from natural physical and social environments. In a recent study (Lungu and Debas, 2013), researchers increased the ecological value of the SRTT by manipulating the social context (i.e., doing the task in solitude vs. together with a partner). The results showed that cooperation with a partner boosted motor performance as compared to the solitude condition, suggesting that social interaction can influence motor learning capacity.

Although only a behavioral study, the above-mentioned findings have relevant bearings on the neural mechanism mediating the effects of social context on motor learning. For instance, performing a motor task in an ecological setup, in a familiar social context (e.g., in collaboration with a friend), can be stimulating and provide a social reward to the individual. In this context, it has been shown that social rewards activate

brain regions similar to those activated in response to monetary rewards, importantly involving the striatum (Izuma et al., 2008, 2010; Bhanji and Delgado, 2014). Rewards are generally associated with increased dopaminergic activity in the cortico-striatal system, which is known to play a key role in motor (sequence) skill learning and motor memory consolidation and automatization (Doyon, 2008; Yin et al., 2009; Debas et al., 2010; Sommer et al., 2014). In this context, it is noteworthy to mention that a study in which monetary values were used as reward (gains) or punishment (loses) in a procedural learning task indicated that reward improved motor learning through increased striatal activation, whereas punishment augmented motor performance (i.e., speed of execution), but not implicit sequence learning, through increased insular activation (Wachter et al., 2009).

Rewards stemming from social context (i.e., social interactions) can be seen as online modulators of motor learning and performance. Although in human research the rewards are typically operationalized as monetary incentives, there is evidence that social context can provide a benefit, above and beyond that of monetary reward itself. For instance, in the context of monetary games, earning a reward by cooperating with a partner evoked greater activity in ventral striatum as compared to gaining equivalent rewards in non-social condition (i.e., playing alone or with a computer partner), in addition to the fact that the mutual cooperation was valued as most satisfying by the participants (Rilling et al., 2002). In the motor domain, Sugawara et al. (2012) were the first to demonstrate the effect of social reward in form of praises given during initial training on offline motor skill consolidation, seen the next day, after a night of sleep. They showed that even when controlling for fatigue, alertness, duration and quality of sleep, the rate of offline improvements in motor sequence retention test were significantly higher in the "praised group" compared to individuals from the other two control groups, who received no self-related social feedback (Sugawara et al., 2012).

There is considerable overlap between the dopaminergic circuitry and the neuronal substrates affected by PD and those involved in reward-related information processing (Graef et al., 2010; van Wouwe et al., 2012; Balasubramani et al., 2015; see **Figure 1**). In some cases, PD patients are apathetic (Lawrence et al., 2011; Jordan et al., 2013), or have high level of impulsivity (Housden et al., 2010; Antonelli et al., 2014; Aracil-Bolaños and Strafella, 2016) often leading to depression or gambling addictions, respectively. Given that rewards, monetary or social, can be used to improve motor learning capacity in healthy individuals, it is imperative to investigate a similar approach in PD, too. There is evidence that despite impairments in dopaminergic circuitry involving striatum, PD patients possess compensatory mechanisms based on cerebellar and prefrontal cortex networks when processing rewards or feedback (Goerendt et al., 2004; Keitz et al., 2008). These mechanisms can be then used to modulate motor performance, as indicated by a study in which monetary rewards were found to speed up movement initiation and execution in PD patients with bradykinesia (Kojovic et al., 2014). However, there is a scarcity of research investigating facilitatory effects of rewards on restoring or improving motor learning capacity in PD and no study to assess the extent to which these effects can be elicited by rewards provided by the social context (i.e., social interaction). A better understanding of the effects of social rewards on motor skill acquisition and consolidation could lead to development of new intervention protocols that will incorporate and use social interactions to restore or improve motor functions and alleviate motor symptoms in PD. In line with this idea, we are currently conducting a study in which we aim to show that a console videogame with musical rhythms (Frets on Fire, a console videogame very similar to Guitar Hero), played in solitude or with a partner, will increase PD patients' engagement in the task, which, in turn, will increase fine motor coordination in the upper limb and motor sequence learning capacity. By using a more ecological design than the one typically employed in most motor sequence learning studies, we hope to demonstrate that people with PD can have many benefits from playing this type of low-cost and safe videogame. They can improve their motor learning skills and the hand motor coordination while finding pleasure in so doing, in addition to increasing the interaction with family and friends. In long run, if we can demonstrate that this type of gaming with a social component can preserve motor functions, we may employ it to slow down Parkinson's progression, the need for more medication and exposure to adverse side effects.

MINDSET AND MOTOR LEARNING IN PD

People's beliefs in their capabilities to produce given attainments are a psychological construct commonly known as self-efficacy (Bandura, 2001, 2004). High levels of self-efficacy were found to be consistently associated with increased performance in a variety of tasks in healthy individuals (Bandura, 2001, 2004; Clair et al., 2005; McAuley et al., 2006; Salanova et al., 2011). Unfortunately, this concept has not been fully integrated in PD research despite encouraging, but limited evidence. For instance, Mak and Pang (2008) were the first to demonstrate in PD patients that balance self-efficacy was an important determinant of their walking abilities. This findings suggests that increasing patients' confidence in their own skills, may have positive consequences on their motor function. Conversely, individuals' lack of confidence in their capabilities (i.e., low self-efficacy), may increase their psychological stress and anxiety while being engaged in motor activities, in turn having a detrimental effect on their performance. Yet, the extent to which self-efficacy can influence motor learning capacity in PD and its mechanisms of action remain unknown to date.

In addition to external factors that can impact motor skill learning, the individuals' internal mind set (i.e., the attitudes and beliefs with which a person approaches the task) may also play a role (Jourden et al., 1991). In particular, self-stereotypes or assumptions about loss of abilities may contribute to further decline in performance (Levy, 2003). This aspect could be especially relevant in people with PD who experienced visible losses in their motor skills, which may lead to an underestimation of their real, available capacity. The message transmitted to the public by the research community, based on the fact that most studies on motor learning in PD associate the deficit with the

disease and neurodegeneration, serves only to reinforce patients' expectation they should perform worse in these tasks. Thus, a vicious circle may form where PD patients become more anxious, feel under stress and less confident about their existing motor learning capacity when it comes to learning a new motor skill, which, in turn, will only hamper their learning process and performance. This is not to say that neurodegeneration is not real and it will not affect motor learning capacity in an objective manner, but just as the negative impact of neurodegeneration may be alleviated by medication, there is evidence that motor learning capacity may also be enhanced by breaking the negative self-beliefs and improving self-efficacy (Jourden et al., 1991; Mak and Pang, 2008; Barry et al., 2014).

There are two main approaches employed in interventions aimed to boost self-efficacy: providing individuals with a better sense of control over the task at hand through instructions or task setups and reinforcing the self-confidence through positive feedback on performance. In the context of the first approach, there is evidence that providing enhanced expectancies and support of autonomy during learning process in healthy individuals increased self-efficacy and these two factors were found to have an independent effect on learning (Hooyman et al., 2014; Wulf et al., 2014). In PD patients, it has been demonstrated that self-controlled practice enhanced not only individuals' self-efficacy, but also their motor performance and learning (Chiviacowsky et al., 2012). Specifically, PD patients in an experimental group, who had the choice to use or not a balance pole when learning a balance task (i.e., increased sense of control), experienced lower levels of nervousness and were less concerned about their body movements than patients in a control group whose use of the pole was yoked to the experimental group. In addition, the experimental group learned the task better than the control group (Chiviacowsky et al., 2012). The authors interpreted these results to indicate that learner-controlled practice plays not only a motivational function, but it may fulfill the basic psychological need for autonomy, which may be more important in people with PD than in their healthy counterparts.

The second approach in boosting self-efficacy is through the use of performance feedback, which can be provided by the social context. In real life, people usually learn skills in social contexts and interacting with others; as such, they tend to compare with others either explicitly or implicitly. For learners, the normative feedback (i.e., how other people perform in the same task) seems to be important; yet, only few studies have investigated, so far, the impact of social-comparative feedback on self-efficacy and motor learning capacity. For instance, positive social-comparative feedback was shown to increase performance in the retention test in a novel motor task in children (Ávila et al., 2012). In addition, individuals in the positive social-comparative feedback perceived themselves as being more competent as compared to the group with no social feedback. A similar effect was demonstrated in younger and older adults, where experimenters used positive social comparison to manipulate individuals' perceived competence (i.e., self-efficacy) when learning a novel balance task (Lewthwaite and Wulf, 2010; Wulf et al., 2012). The authors reported that positive social comparison not only decreased individuals' level of nervousness and concerns about performance during learning, but it had also a long-term impact on motor learning, as revealed by increased performance in the delayed retention test, when social feedback was no longer provided (Lewthwaite and Wulf, 2010; Wulf et al., 2012).

Given the power of the mindset to modulate, either positively or negatively, motor learning and performance, the scarcity of research investigating its effects in PD is peculiar and constitutes an important knowledge gap to be addressed by future studies.

THE ROLE OF EMOTIONS IN MOTOR LEARNING

A common factor underlying the facilitatory motivational effects on motor performance across the various domains we described above (task engagement, social interactions, self-efficacy) are the emotions associated with the learning process. During motor learning, many different processes are at play at the same time, including cognitive, social-cognitive and affective. Animal studies in rodents have provided evidence for a neurobiological model called "tag-and-capture", which postulates that initially weak memories are strengthened through subsequent activation that engages common neural pathways minutes to hours later, through a synaptic mechanism (Frey and Morris, 1997; Ballarini et al., 2009). This model explains how information is selectively consolidated following salient experiences and provides a mechanism by which emotions experienced during learning may influence memory consolidation. This type of learning also exists in humans and it is called emotional learning (Dunsmoor et al., 2015). However, rodent models of emotional learning, while providing direct neurophysiological evidence, use almost exclusively fear conditioning paradigms and tasks requiring navigation or other hippocampus-based information processing. Human models of emotional learning are more diverse in terms of experimental conditioning paradigms and tasks (i.e., investigating different types of memory).

In regards to how emotions influence motor learning, the evidence coming from these studies does not provide a clear picture. For instance, some authors found that emotional learning context did not improve procedural learning (Onal-Hartmann et al., 2012; Gorlick and Maddox, 2015), although it seemed to modulate sequence awareness in an implicit motor sequence learning task (Onal-Hartmann et al., 2012), whereas others found that negative emotional context during initial learning stage enhanced motor memory consolidation after a night of sleep (Javadi et al., 2011). In addition to being scarce, the research on the role of emotional context on procedural learning does not use ecological paradigms and relies on experimental manipulations "borrowed" from animal models (i.e., based on fear conditioning). Nevertheless, this area or research should be expanded to include emotional learning in PD patients given that PD is a neurological condition characterized by both motor and emotional dysfunction due to abnormal activation within the basal ganglia and limbic dopaminergic circuit.

A large number of PD patients have increased levels of anxiety, depression or apathy (McDonald et al., 2003). The emotions generated by these mood states, in interaction with the context in which tasks take place, may drastically affect motor performance and learning. For instance, there is evidence that higher levels of stress, nervousness or anxiety may impair motor performance (Masters, 1992; Wulf and Weigelt, 1997; Wulf et al., 2012), and these had been shown to have the same detrimental effect on procedural learning in healthy people as in individuals with PD (Chiviacowsky et al., 2012). Moreover, the more the learner is experiencing negative emotional responses during learning, the more extra energy is needed for self-regulation and attention, concurrently reducing learning capacity (Hooyman et al., 2014). The role of emotional context in PD is made very evident through a phenomenon known as paradoxical kinesia, which is a sudden, temporary improvement of motor functions, typically followed after some intense stimuli, for instance in a threatening situation (Glickstein and Stein, 1991; Anzak et al., 2011). This is a clear evidence that emotional context may directly affect motor performance in PD. In recent study, Naugle et al. (2012) demonstrated that presentation of positive emotional stimuli improved gait initiation in PD patients. This study probably provides first evidence that the mechanisms responsible for integration of affective and motor processes remain intact in medicated patients. Interestingly, this paradoxical facilitatory effect on motor movement was also demonstrated in PD patients both in ON and OFF medicated states, as well as in healthy age-matched counterparts, when arousing sounds were paired with visual cues triggering the movements (Anzak et al., 2011). These results indicate that the mechanism underlying this phenomenon might be independent of the disturbed dopaminergic pathways, therefore its exploration deserves attention in future research as a potential novel target for treatment of Parkinsonian symptoms, especially if these results could be replicated with more complex motor actions (e.g., motor sequences).

Summing up, more research is needed to investigate how the social context and social interactions may elicit positive or negative emotions, how individuals regulate them during learning and how these may impact individual's motor learning capacity. In **Figure 2** we provide a schema describing our view on how social context may provide goals and feedback to the individual and how these may affect the interplay between different motivational facets. Specifically, we propose that the goals (i.e., which activities to engage in, what tasks to choose from, etc.) arise from the interaction between the individual and his/her social context. Motivational processes include a volitional aspect manifesting as task engagement (i.e., how long to persist and how much effort to exert in the task or activity at hand), which, on the one hand, is shaped by the emotional and self-regulation aspects, and on the other hand, feeds into emotions and self-efficacy based on the feedback received from the task and social context. Exploring and better understanding of these phenomena will help design more effective motor rehabilitation interventions incorporating effective emotion regulation that will help performance not only in PD, but also in other movement disorders.

FIGURE 2 | Schematic model of psychosocial modulators of motor learning and its interactions. The model proposes that social context may provide both the goals (i.e., social or personal expectations) and feedback (i.e., how well individual's performance matches the set goals) to the learner. In turn these may affect the interplay between different motivational aspects, such as task engagement, perception of self-efficacy or experienced emotions while learning.

CONCLUSION

In the current work, we provided a brief overview of the psycho-social factors that may affect motor learning in general and its impact in PD, in particular. We argue for the adoption of a more ecological design in future research, closer to real-life situations, and for additional measures that will include the assessment of emotional, motivational states known to affect motor learning and performance. The conclusion of our review is that motor learning research in PD can only benefit from increasing the ecological nature of the context in which tasks are performed, thus augmenting its translational value, because evidence based on artificial laboratory setting may not always generalize to more complex natural environments, including social interaction. Moreover, motor learning in social context has the potential to be used as an intervention strategy to stimulate the motivational compensatory pathways, still intact in early PD, in order to overcome dopamine depletion and associated motor symptoms. For instance, providing positive task emotions and increasing self-efficacy can be used to improve not only motor performance, but also motor learning rates; thus these should be considered important online and offline modulators. However, the most important conclusion for the clinical practice is that each factor that makes motor training more joyful and increases individuals' motivation and engagement in the task, therefore has the potential to increase patients' compliance

with long term interventions, and prevent further physical decline.

AUTHOR CONTRIBUTIONS

Literature search for the study was managed by PZ and OL, first draft of the review was written by PZ, OL and MB critically revised and commented on the manuscript and figures. All the authors substantially contributed to and have approved the final manuscript.

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frontiers in Human Neuroscience

MINI REVIEW
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How Transcranial Direct Current Stimulation Can Modulate Implicit Motor Sequence Learning and Consolidation: A Brief Review

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The purpose of this review is to investigate how transcranial direct current stimulation (tDCS) can modulate implicit motor sequence learning and consolidation. So far, most of the studies have focused on the modulating effect of tDCS for explicit motor learning. Here, we focus explicitly on implicit motor sequence learning and consolidation in order to improve our understanding about the potential of tDCS to affect this kind of unconscious learning. Specifically, we concentrate on studies with the serial reaction time task (SRTT), the classical paradigm for measuring implicit motor sequence learning. The influence of tDCS has been investigated for the primary motor cortex, the premotor cortex, the prefrontal cortex, and the cerebellum. The results indicate that tDCS above the primary motor cortex gives raise to the most consistent modulating effects for both implicit motor sequence learning and consolidation.

Keywords: non-invasive brain stimulation, transcranial direct current stimulation, serial reaction time task, implicit motor sequence learning, memory consolidation

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Many of our everyday activities are organized into sequences, some deliberate, some simply by coincidence. Getting up and ready for work, writing a scientific paper, or doing leisure activities often follow repeated sequences of events. Many of these sequences are established incidentally rather than intentionally, that is, learning is implicit (Cleeremans et al., 1998). The implicit acquisition of sequences often involves a motor component and thus, it is termed implicit motor sequence learning (but see Meier and Cock, 2010; Weiermann et al., 2010 for non-motor implicit sequence learning tasks). After acquisition, performance can become resistant to decay, that is consolidated. In recent years, transcranial direct current stimulation (tDCS) has been used to enhance performance in a variety of learning and memory tasks in healthy participants, but the majority of the studies focused on explicit rather than implicit sequence learning tasks and on learning rather than consolidation (Coffman et al., 2014; Shin et al., 2015). Therefore, there is no clear consensus on how tDCS enhances implicit motor sequence learning and consolidation. The aim of this article is to review the evidence for modulating effects of tDCS on implicit motor sequence learning and consolidation.

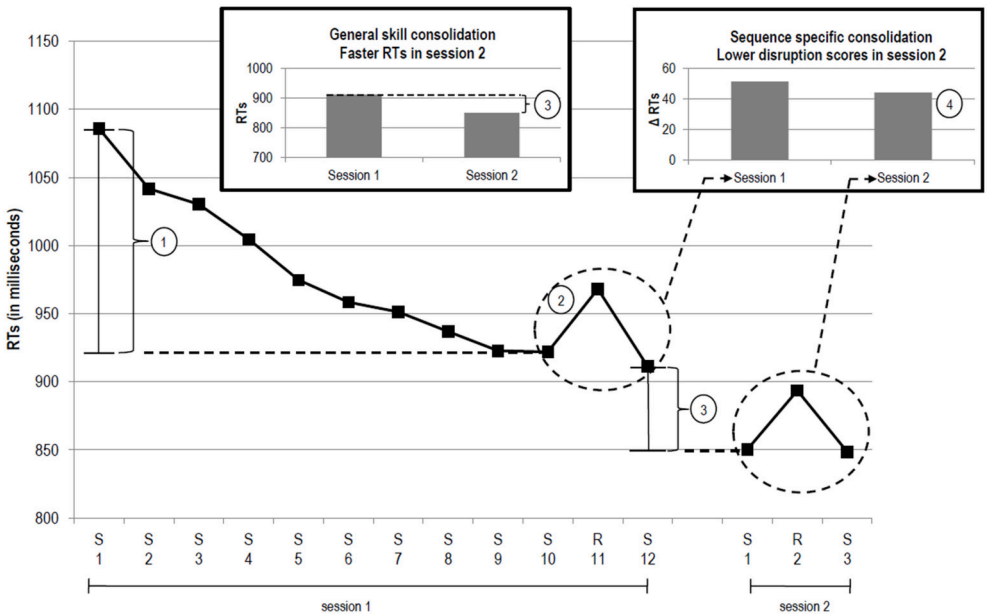

FIGURE 1 | Prototypical performance trajectory in the SRTT (adapted from Meier and Cock, 2014). The x-axis depicts RTs across blocks ("S" sequenced block, "R" random block). ① General motor skill learning (RT difference between S 1 and S 10). ② Sequence-specific learning (i.e., disruption score calculated as RT difference between R 11 and the mean of S 10 and S 12). ③ General motor skill consolidation (RT difference between S 12 of session 1 and S 1 of session 2). ④ Sequence-specific consolidation (RT difference between the disruption scores of the two sessions).

IMPLICIT MOTOR SEQUENCE LEARNING AND CONSOLIDATION

Implicit motor sequence learning is typically tested with the serial reaction time task (SRTT), originally introduced by Nissen and Bullemer (1987). In this paradigm, a sequence of correct response key presses follows the sequence of designated target locations. Unbeknownst to participants, the order of target locations follows a sequence predetermined by the experimenter. With practice, performance gets faster compared to a randomized control condition. If the sequence is switched to random, performance is slowed again. These changes are taken as evidence of implicit sequence learning.

Typically, two kinds of learning are involved, general motor skill (GMS) learning and sequence-specific (SS) learning (Meier and Cock, 2014; cf. Janacsek and Nemeth, 2013). GMS learning refers to the acquisition of expertise with the general requirements of the task[1]. It can be measured as the speed up of reaction times (RT) across blocks, see **Figure 1**①. SS learning can be measured as the RT difference between a random block that occurs after a sequence has been presented several times and the surrounding sequenced blocks. This disruption score is an indirect measure of SS learning, see **Figure 1**②.

With time passing and without further practice performance can become robust, resistant to decay and interference, that is, consolidated (Shadmehr and Brashers-Krug, 1997; Krakauer and Shadmehr, 2006). Memory consolidation can be conceptualized as performance improvement or maintenance across sessions (Robertson et al., 2004). Consolidation can be assessed by repeating the SRTT in a second session separated by a period of time in which participants are not engaged with the SRTT. GMS consolidation can be measured as the mean RT difference between the last sequenced block of session one and the first sequenced block of session two, see **Figure 1**③. SS consolidation can be measured as the mean difference between the disruption scores of the two sessions, as depicted in **Figure 1**④ (for reviews on consolidation see Doyon et al., 2009; Robertson, 2009; Siengsukon and Boyd, 2009; Song, 2009; Dayan and Cohen, 2011).

Transcranial Direct Current Stimulation (tDCS)

Through the application of a current between two electrodes (i.e., an anode and a cathode) tDCS can modulate cortical excitation (Nitsche and Paulus, 2000, 2001). Typically, anodal tDCS is thought to induce subthreshold membrane depolarization, and cathodal tDCS is thought to induce hyperpolarization, respectively (Nitsche et al., 2003a; Bikson et al., 2004; Ruffini et al., 2013). Moreover, it has been suggested that tDCS modulates mechanisms of cortical plasticity, which in turn modify the synaptic bonds between neurons (Fritsch et al., 2010; Stagg et al., 2011). As tDCS modulates cortical plasticity and cortical plasticity is generally involved in learning, the application of tDCS may have the potential to enhance or diminish learning (Rioult-Pedotti et al., 2000; Liebetanz

[1]When GMS learning is assessed as the speed-up of RTs across sequenced blocks it may also involve some implicit sequence learning. In contrast, when it is assessed as speed-up of RTs across random blocks it can be considered as pure measure.

et al., 2002). Particularly, anodal tDCS is thought to enhance learning and cathodal tDCS is thought to diminish it. The immediate effect of tDCS can outlast stimulation for more than 1 h dependent on parameters such as current strength, stimulation duration, electrode size, and inter-electrode distance (Shin et al., 2015). Reducing the electrode size increases the spatial resolution of stimulation, in other words a smaller electrode modulates a smaller area of the cortex underneath it (Nitsche et al., 2007; Bastani and Jaberzadeh, 2013).

The active electrode is placed on the scalp above the cortical area that is to be modulated with tDCS and the return electrode is placed above the contralateral side either on inactive or active regions. Inactive regions should not modulate cortical areas, for example the shoulder, while active regions should modulate cortical areas, for example the motor cortex. Placing the return electrode on an inactive region reflects a unilateral setting (i.e., only one hemisphere is stimulated). In contrast, placing the return electrode on an active region reflects a bilateral setting (i.e., both hemispheres are stimulated, see Nasseri et al., 2015 for an overview of tDCS settings). Importantly, for motor cortex stimulation the active electrode is usually placed above the motor cortex and the return electrode above the eyebrow (i.e., supraorbital region). This setting is considered bilateral because human head model studies show that the return electrode placed above the supraorbital region modulates cortical areas (Miranda et al., 2006; Laakso et al., 2015). In addition, two kinds of application can be distinguished. tDCS applied during the execution of a particular task (e.g., the SRTT) is termed on-line stimulation, tDCS applied before the execution of a particular task (e.g., the SRTT) is termed off-line stimulation. As a control condition, typically sham stimulation is used, during which current is delivered only for 30 s which has no effect on the neural population. Importantly, from a subjects' point of view, sham cannot be distinguished from real stimulation (Gandiga et al., 2006).

METHODS

We focus on studies in which implicit motor sequence learning and/or consolidation were addressed with the SRTT. The use of the SRTT was the critical criterion because the SRTT is the classic task for implicit motor sequence learning which gives reliable results that have been replicated consistently. In order to select the relevant studies, PubMed was used as a search engine, with "tDCS" and "implicit motor sequence learning," "tDCS" and "consolidation," and "tDCS" and "SRTT" as keywords. A total of six studies conformed to the search criteria. Five of them tested the influence of tDCS on frontal brain areas (in particular the motor and premotor cortex) and one of them tackled the cerebellum. **Table 1** shows an overview of these studies. Moreover, in order to make a comparison across studies possible, the critical learning and consolidation effects in milliseconds are also provided.

RESULTS

In a first study, Nitsche et al. (2003b) investigated whether *on-line* tDCS modulates implicit sequence *learning*. tDCS was applied to one of four brain areas of the left hemisphere, the motor cortex (M1), the premotor cortex (PM), the lateral, and the medial prefrontal cortex (PFC). Specifically, for M1 and PM stimulation the return electrode was placed above the right supraorbital region, and for both PFC stimulations it was placed above the right M1. The results showed that anodal tDCS above the M1 enhanced GMS learning, indicated by faster RTs in the sequenced blocks compared to sham. Furthermore, anodal tDCS above the M1 enhanced SS learning, indicated by a bigger RT difference between random and surrounding sequenced blocks compared to sham. tDCS above the other areas did not affect learning at all.

Kuo et al. (2008) investigated whether *off-line* tDCS also modulates *learning* with either anodal or cathodal stimulation. A further aim was to evaluate pharmaceutical interventions, however, here we focus on the placebo conditions. For the anodal montage, the active electrode was placed above the left M1 and the return electrode was placed above the right supraorbital region. For the cathodal montage the reverse setup was used. tDCS started and ended before the SRTT. The results showed that neither anodal nor cathodal tDCS affected SRTT performance. Thus, offline tDCS over M1 did not modulate sequence learning at all.

Kang and Paik (2011) investigated the influence of two *bilateral on-line* tDCS settings above the M1 on *learning and consolidation*. For the first setting, the anode was placed above the left M1 and the cathode was placed above the right supraorbital region. For the second setting, the anode was placed above the left M1 and the cathode was placed above the right M1. In a first session, stimulation started after three blocks, continued for 11 blocks, and ended before the last three blocks. The first and the last three blocks were composed of two random and one sequenced block which were used to calculate learning. After 24 h, another three blocks were used to test consolidation. Learning and consolidation was calculated as ratio between the RTs in the sequenced and the random blocks in session one and two, respectively. The results showed that at the end of session one, the ratio decreased for all conditions, indicating similar SS learning for all conditions. In session two, the ratio was maintained in the two tDCS conditions but not in the sham condition. These results suggest that tDCS enhanced SS consolidation. However, when SRTT components for session one were calculated as RT differences between random and sequenced blocks, the disruption score for tDCS conditions was already higher initially. This makes the interpretation of a specific SS advantage for the stimulation conditions somewhat equivocal.

Kantak et al. (2012) investigated the influence of *on-line* tDCS above the M1 and above the dorsal PM cortex on *learning and consolidation*. The anode was placed above the M1 or the dorsal PM of the right hemisphere. In both groups the cathode was placed above the left supraorbital region. In a first session, tDCS started after two blocks, continued for six further blocks, and stopped before the last two blocks. The first and the last two blocks were composed of a random and a sequenced block

TABLE 1 | Summary of the studies aiming to modulate implicit motor sequence learning and memory consolidation with tDCS.

Author	Hand tested	Sequence length	Sequence repetition (total number of trials per sequenced blocks)	Calculation	tDCS application	*N* (per condition)	Stimulation parameters	Electrodes size	Manipulation of electrode setting	Electrode setting		Effects in milliseconds (ms)				Significance			
												Learning		Consolidation		Learning		Consolidation	
										Anode	Cathode	GMS	SS	GMS	SS	GMS	SS	GMS	SS
Nitsche et al., 2003b	Right	12 items	720	RTs of each block were divided by the RTs of block one	On-line	20	1 mA; 15 min	35 cm^2	Between; stimulation type (anodal, cathodal, sham) manipulate within	Left M1	Right supraorbital region	(10) 15	(40) 60	**n.m.**	**n.m.**	↑	↑	**n.m.**	**n.m.**
										Left PM	Right supraorbital region	(25) 0	(55) 60			✘	✘		
										Left lateral PFC	Right M1	(10) 5	(50) 60			✘	✘		
										Left medial PFC	Right M1	(0) 0	(40) 50			✘	✘		
										Right supraorbital region	Left M1	(10) 5	(40) 60			✘	✘		
										Right supraorbital region	Left PM	(25) 10	(55) 50			✘	✘		
										Right M1	Left lateral PFC	(10) 5	(50) 50			✘	✘		
										Right M1	Left medial PFC	(0) 0	(40) 40			✘	✘		
Kuo et al., 2008	Right	12 items	720	"Standard" defined as in **Figure 1**	Off-line	12	1 mA; 10 min	35 cm^2	Between; stimulation type (anodal, cathodal, sham) manipulate within	Left M1	Right supraorbital region	(10) 10	(55) 50	**n.m.**	**n.m.**	✘	✘	**n.m.**	**n.m.**
										Right supraorbital region	Left M1	(20) 10	(45) 35			✘	✘		
Kang and Paik, 2011	Right	12 items	1200	Disruption scores measured as ratio between RTs in sequenced and random blocks	On-line	11	2 mA; 20 min	25 cm^2	Within	Left M1	Right supraorbital region	(80) 10	(60) 70	(−30) 5	(30) −15	**n.m.**	✘	**n.m.**	↑
										Left M1	Right M1	(80) 15	(60) 85	(−30) 10	(30) 10		✘		↑

(Continued)

TABLE 1 | Continued

Author	Hand tested	Sequence length	Sequence repetition (total number of trials per sequenced blocks)	Calculation	tDCS application	*N* (per condition)	Stimulation parameters	Electrodes size	Manipulation of electrode setting	Electrode setting		Effects in milliseconds (ms)				Significance			
												Learning		Consolidation		Learning		Consolidation	
										Anode	Cathode	GMS	SS	GMS	SS	GMS	SS	GMS	SS
Kantak et al., 2012	Left	10 items	600	Disruption scores measured as ratio between RTs in sequenced and RTs random blocks	On-line	13	1 mA; 15 min	8 cm^2 (Anode)	Within	Right M1	Left supraorbital region	(60) 70	(100) 160	(−80) 10	(10) 20	↑	✘	**n.m.**	**(↑)**
								48 cm^2 (Cathode)		Right dorsal PM	Left supraorbital region	(60) 60	(100) 140	(−80) −50	(10) 20	✘	✘		✘
Nitsche et al., 2010*	Right	12 items	720	RTs of each block were divided by the RTs of block one	Off-line	20(Experiment 1) 12(Experiment 2) 32(Experiment 3)	1 mA; 15 min	35 cm^2	None	Left PM	Right supraorbital region	(20) 0	(55) 55	(−50) 0	(45) −20	✘	**n.m.**	↑	**n.m.**
Ferrucci et al., 2013	Both hands	12 items	264	"Standard" defined as in **Figure 1**	Off-line	21	2 mA; 20 min	35 cm^2	None	Cerebellum (2 cm below inion)	Right arm	(200) 190	(25) 35	(−10) 150	(5) −5	**n.m.**	**n.m.**	↑	↑

*GMS, general motor skill; SS, sequence-specific. The effects in ms are measured as depicted in **Figure 1**, and they were extracted from graphs. For each effect in ms in brackets the ms for the sham tDCS groups, and outside the bracket the ms for the actual tDCS groups. If the method of calculation is different from **Figure 1** the calculation is described in the column "Calculation." ↑ , tDCS enhanced the respective SRTT component; ✘, no influence; n.m., not measured. * Because of the design complexity of this study, in **Table 1** only the tDCS condition that was significantly different from sham is described.*

and were used to calculate learning. After 24 h, another two blocks, one sequenced, and one random were used to calculate consolidation. The results showed that the decrease in RTs across the sequenced blocks was greater when M1 was stimulated compared to sham, indicating that anodal tDCS of M1 enhanced GMS learning. At the end of session one, the SS learning in the PM and M1 tDCS conditions was not statistically different from sham, even though there was a trend. To test consolidation, the ratio between RTs in sequenced and random blocks at the end of session one was compared to the according ratio after 24 h. This ratio was maintained in the M1 and sham groups but not in the PM group. Furthermore, in session two, the M1 group had a smaller ratio compared to PM and sham groups. Because the M1 and PM groups had already smaller ratios than the sham group at the end of session one, tDCS above the M1 may have enhanced GMS and SS learning initially and this was retained after 24 h.

Nitsche et al. (2010) investigated whether *off-line* tDCS above the PM cortex applied during sleep following learning could enhance *consolidation*. The active electrode was placed above the left PM and the return electrode was placed above the right supraorbital region. The study consisted of three experiments. In Experiment 1, two groups performed the SRTT and then went to sleep. One group was woken up during the night and was re-tested. The other group was re-tested the next morning. In Experiment 2, tDCS was delivered during an SRTT-like task that was composed of random blocks only. In Experiment 3, the same setting was used as in Experiment 1, but without sleep. In each experiment, the re-test consisted of three blocks, one random block followed by two sequenced blocks, which were used to assess consolidation. In Experiment 1, the results showed that anodal tDCS during sleep enhanced GMS consolidation, as indicated by smaller RTs in the sequenced blocks of the re-test compared to sham, but only when participants were re-tested during the night. When participants were re-tested the next morning there was no difference between the real tDCS and the sham conditions. In Experiment 2, tDCS had no effects on performance, indicating that tDCS did not influence GMS learning. In Experiment 3, tDCS had no effect on GMS learning, no effect on SS learning, and no effect on consolidation. Thus, this study provides further evidence that PM tDCS does not modulate implicit sequence learning or consolidation.

Finally, Ferrucci et al. (2013) investigated whether *off-line* tDCS of the cerebellum would enhance *consolidation*. The anode was placed above the cerebellum and the cathode was placed above the right arm. The results showed faster RTs for the tDCS group in the sequenced blocks post stimulation compared to pre stimulation. In contrast, for the sham group there was no difference. This indicates that tDCS enhanced GMS consolidation. Furthermore, post stimulation the disruption score was larger for the tDCS than for the sham group. This indicates that tDCS also enhanced SS consolidation.

DISCUSSION

Applying tDCS above the cortex of healthy individuals can modulate learning and memory. The purpose of this brief review was to evaluate how tDCS can be used to modulate implicit motor sequence learning and consolidation with the SRTT. So far, only six studies have addressed this question and most studies have tackled frontal brain areas.

For M1, bilateral anodal stimulation can enhance implicit motor sequence *learning* and probably also *consolidation* (Nitsche et al., 2003b; Kang and Paik, 2011; Kantak et al., 2012). This result is in line with previous studies which showed that M1 neurons are more responsive to tDCS than other cortical areas due to their morphology (Radman et al., 2009). Regarding consolidation, the results are not that clear yet and thus, further research is necessary to investigate the role of M1 for both GMS and SS consolidation. Nevertheless, as in both the studies by Nitsche et al. (2003b) and by Kang and Paik (2011), performance in the anodal or cathodal stimulation group was compared to the sham group separately rather than in a full ANOVA, the effects may have been overestimated. Importantly, future studies should also take SRTT parameters into account. Neurophysiological data have shown that the application of tDCS during an intense motor practice phase can impair motor performance while less intense practice can improve performance (Bortoletto et al., 2015). This suggests that the behavioral effects of tDCS are the result of an interaction between excitability changes induced by tDCS and by practice (Miniussi et al., 2013). Hence, the quantity of practice during the SRTT could influence tDCS effects.

For PM, there is not much evidence that tDCS might modulate implicit motor sequence learning (Nitsche et al., 2010; Kantak et al., 2012). If present, the effects seem to appear only immediately after tDCS (Nitsche et al., 2010; Kantak et al., 2012). Future studies should systematically vary tDCS parameters such as electrode size and shape, current length, and strength. This may be a promising avenue as neuroimaging studies have shown the involvement of PM in implicit motor sequence learning (Peigneux et al., 2000).

For PFC, only one study was available and this study did not find any modulating effects (Nitsche et al., 2003b). However, it is possible that more difficult sequence learning paradigms may be modulated by PFC stimulation. For example, there is evidence for the critical role of PFC in task sequence learning (Meier et al., 2013) Moreover, a recent study found that tDCS applied above the right PFC modulated performance in a probabilistic sequence learning task in which only every second element was sequenced (Janacsek et al., 2015).

For the cerebellum, there is initial evidence that off-line tDCS can enhance both GMS and SS consolidation (Ferrucci et al., 2013). This is in line with the hypothesis that the cerebellum is more responsive to tDCS compared to cerebral cortex areas (Rampersad et al., 2014).

So far, no study has evaluated the influence of supplementary motor area tDCS on implicit motor sequence learning and consolidation. This area can be easily tackled with tDCS and findings from neuroimaging and neurostimulation studies suggest its critical involvement in implicit motor sequence learning (Hazeltine et al., 1997; Kim and Shin, 2014). Therefore, future studies should also address the effect of supplementary motor area tDCS. Similarly, no study has evaluated the effects of parietal tDCS for implicit motor sequence learning and consolidation. Previous studies have shown that parietal cortex

tDCS can influence memory encoding (Jacobson et al., 2012). Moreover, parietal activation has been found in neuroimaging studies of motor learning and motor learning consolidation (Doyon et al., 2009; Albouy et al., 2015). In addition, because parietal tDCS may activate cortico-hippocampal networks, it could help to disentangle the role of these networks (Reber, 2013; Wang et al., 2014; Dudai et al., 2015). This may motivate future studies with parietal tDCS.

CONCLUSIONS

So far the most robust evidence for a modulating effect of tDCS on implicit motor sequence learning concerns the primary motor cortex (M1). Different studies have found that tDCS delivered on-line can enhance performance. There is also initial evidence for the modulating effect of off-line tDCS to the cerebellum. Evidence for PM stimulation is not robust, while evidence for PFC stimulation is negative. Further studies are required to address the effect of stimulation on different brain regions, different task parameters (e.g., number of sessions, see Meinzer et al., 2014), and different tDCS parameters (e.g., current intensity, see Cuypers et al., 2013). In any case, the investigation of the possibilities to modulate learning and consolidation with tDCS is still in its infancies and a more systematic examination of both task properties and stimulation parameters is warranted.

AUTHOR CONTRIBUTIONS

All authors listed, have made substantial, direct and intellectual contribution to the work, and approved it for publication.

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Declarative and Non-declarative Memory Consolidation in Children with Sleep Disorder

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Healthy sleep is essential in children's cognitive, behavioral, and emotional development. However, remarkably little is known about the influence of sleep disorders on different memory processes in childhood. Such data could give us a deeper insight into the effect of sleep on the developing brain and memory functions and how the relationship between sleep and memory changes from childhood to adulthood. In the present study we examined the effect of sleep disorder on declarative and non-declarative memory consolidation by testing children with sleep-disordered breathing (SDB) which is characterized by disrupted sleep structure. We used a story recall task to measure declarative memory and Alternating Serial Reaction time (ASRT) task to assess non-declarative memory. This task enables us to measure two aspects of non-declarative memory, namely general motor skill learning and sequence-specific learning. There were two sessions: a learning phase and a testing phase, separated by a 12 h offline period with sleep. Our data showed that children with SDB exhibited a generally lower declarative memory performance both in the learning and testing phase; however, both the SDB and control groups exhibited retention of the previously recalled items after the offline period. Here we showed intact non-declarative consolidation in SDB group in both sequence-specific and general motor skill. These findings suggest that sleep disorders in childhood have a differential effect on different memory processes (online vs. offline) and give us insight into how sleep disturbances affects developing brain.

Keywords: sleep deprivation, memory consolidation, declarative memory, skill learning, sequence learning, sleep-disordered breathing (SDB), implicit learning

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INTRODUCTION

Healthy sleep is critical for children's cognitive, behavioral, and emotional development. Unfortunately, sleep disturbances are common in childhood, including both primary (e.g., insomnia and sleep apnea) and secondary sleep disorders (other illnesses e.g., depression or bad/altered sleep hygiene results the sleep disorders; Anuntaseree et al., 2001; Rosen et al., 2003; Bixler et al., 2009). The prevalence of sleep disorders in childhood estimates vary from 0.7 to 13% (Brunetti et al., 2001; Bixler et al., 2009). Therefore clinical research and practice need to focus more on sleep disturbances in children. The current study focuses on the effect of childhood sleep-disordered breathing (SDB) on declarative and non-declarative memory consolidation.

Memory consolidation can be defined as a set of processes whereby the newly acquired and initially labile memory traces become more stable with the passage of time (Stickgold and Walker, 2007; Spencer, 2013; Urbain et al., 2013). Growing body of evidence indicates that sleep plays a crucial role in these consolidation mechanisms and leads to memory representation being more resistant to interference and forgetting (Dorfberger et al., 2007; Diekelmann et al., 2009; Rudoy et al., 2009; Diekelmann and Born, 2010; Diekelmann, 2014; Mednick et al., 2011; Born and Wilhelm, 2012; Schönauer et al., 2014, 2015).

The effect of sleep on declarative (e.g., remembering events or facts) and non-declarative/procedural memory (e.g., learning languages, learning to musical instruments and movement-based sports) domains is well explored in healthy adults (Fischer et al., 2002; Walker et al., 2002; Gais and Born, 2004; Gais et al., 2006; Song et al., 2007; Rickard et al., 2008; Nemeth et al., 2010), but only a few studies focused on children. These studies with typically developing children found that post-training sleep facilitates the consolidation of declarative memory processes (Gais et al., 2006; Backhaus et al., 2008; Wilhelm et al., 2008; Prehn-Kristensen et al., 2009) but the effect of sleep on non-declarative memory consolidation is still controversial. Some studies failed to find a facilitating effect of sleep on non-declarative memory consolidation (Wilhelm et al., 2008; Prehn-Kristensen et al., 2009), however some recent studies revealed that sleep impacts on non-declarative/procedural memory in children (Fischer et al., 2007; Wilhelm et al., 2012, 2013; Urbain et al., 2013). In contrast to these results, Fischer et al. (2007) demonstrated offline decrement after sleep in non-declarative memory in children compared to adults who showed offline improvement after sleep. In a recent study Borragán et al. (2015) clarified the picture by showing that sleep has a beneficial effect on the consolidation of motor skills but it has no influence on sequential skills. These results indicate that sleep-dependent non-declarative memory consolidation can depend on age (Fischer et al., 2007; Wilhelm et al., 2008) and the nature of the task (Wilhelm et al., 2008, 2012; Borragán et al., 2015). Less is known about how permanent sleep-disorder influences sleep-dependent consolidation of declarative and non-declarative memories in children.

In our study we examined children with SDB which is an ideal population to investigate the effects of sleep disorder on the consolidation of different memory systems. SDB is a spectrum disorder characterized by prolonged and intermittent partial (such as snoring) or complete upper airway obstruction (such as Obstructive sleep apnea, OSA) that disturbs normal ventilation and sleep pattern during sleep. Especially slow wave sleep and REM sleep are affected in SDB (Coleman, 2003; Li and Lee, 2009; Sinha and Guilleminault, 2010). OSA is the worst grade on this spectrum characterized by repetitive episodes of complete or partial upper airway obstruction during sleep resulting hypoxia and fragmented sleep (Banno and Kryger, 2007). The main cases of SDB in children is with adenotonsillar hypertrophy, but it also occurs with obesity, upper airway narrowing due to craniofacial or neuromuscular abnormalities or muscular coordination (Arens et al., 2001; Guimaraes et al., 2008; Katz and D'Ambrosio, 2008).

The neurocognitive consequences of SDB in children have not yet been fully evaluated. There is emerging evidence that cognitive deficits are most consistently apparent on tasks involving sustained attention and executive functions (Beebe and Gozal, 2002; Archbold et al., 2004; O'Brien et al., 2004b; Beebe, 2006). In addition, SDB is associated with deterioration of memory; for example, Gottlieb et al. (2004) revealed that children with SDB had significantly poorer performance on verbal (Narrative Memory) and visual memory tasks (Memory for Faces) compared to healthy participants. Kheirandish-Gozal et al. (2010) investigated the learning before sleep (acquisition) and delayed free recall performance after an overnight sleep (retention) in children with OSA compared with children without sleep disorder. They used pictorial-based memory task where the subjects required to learn and remember animal pictures. They found that both immediate (before sleep) and delayed recall performances (after sleep) were worse among OSA children compared to the control subjects. The authors suggested that this reduced performance may be caused by impaired ability to use adequate learning strategies which either leads to difficulties to learn new information or children with OSA suffer from impaired encoding or altered retrieval. In our recent study (Csábi et al., 2013), we investigated declarative and non-declarative memory performance in one learning session (without consolidation) and showed weaker declarative but intact non-declarative memory performance in children with SDB compared to the controls. These results suggest that the more attention-demanding declarative learning are more vulnerable to permanent sleep disorder than less attention demanding non-declarative learning.

The mechanisms causing these neuropsychological deficits have not been fully delineated. Previous studies suggest that the developing central nervous system in children may be relatively more vulnerable to the fragmented sleep and hypoxia, particularly the hippocampus and frontal lobe structures (Macey et al., 2002; Morrell et al., 2003; Bartlett et al., 2004; Halbower et al., 2006; Owens, 2009). Children with SDB can exhibit daytime behavioral regulation problems (such as inattention, hyperactivity, aggressiveness, social withdrawal) which might also imply frontal lobe dysfunction (Chervin and Archbold, 2001; Beebe and Gozal, 2002; Archbold et al., 2004; Archbold, 2006).

Previous studies examined memory encoding and consolidation before and after sleep in patients with sleep apnea in adults, and showed that declarative and some aspects of non-declarative memory performance is affected in patients with OSA (Kloepfer et al., 2009; Djonlagic et al., 2012; Csabi et al., 2014). Similarly to Borragán et al. (2015) we found dissociation in the effect of sleep (and/or sleep disorder) on offline changes of general motor skills and sequence-specific learning: adult OSA patients showed impaired consolidation of general motor but not on sequence-specific learning (Csabi et al., 2014). To our knowledge, the current study is the first to assess the effects of sleep disorder on declarative and non-declarative memory

functions before and after a nighttime sleep in children. Based on previous studies, we hypothesized that SDB in childhood has an adverse effect on the consolidation of declarative memory while it has less influence on non-declarative memory consolidation. Within the later one we expect differences in the consolidation of motor and sequence-specific aspects of the offline changes.

MATERIALS AND METHODS

Participants

Thirty two children participated in the experiment. Breathing events during sleep, Body Mass Index (BMI) and working memory (WM) measures of the SDB patients and healthy participants are listed in **Table 1**. All participants underwent an overnight polygraphy, which was performed with the Somnomedics Somnoscreen plus device (Randersacker, Germany) at the Sleep Disorders Laboratory of Heim Pál Children's Hospital, Budapest, Hungary. Patients who met the International Classification of Sleep Disorders criteria's (American Academy of Sleep Medicine, 2001) for SDB were included in the study. SDB was diagnosed by a board-certified sleep physician. The SDB group consisted of sixteen children with SDB (average age: 8.56 years [min: 6 to max: 11 years], *SD*: 2.31; 6 females/10 males) six of them with OSA and ten of them with primary snoring. The Apnea/Hypopnea (AHI) index of the OSA patients (M = 17.32, SD = 30.54, range 2–79) was significantly higher (all p's < 0.01) than that of the snoring patients (M = 0.11, SD = 0.19, range 0–1) as well as the controls (M = 0.11, SD = 0.20, range 0–1). Similarly, the snore index of the snoring patients (M = 55.10, SD = 54.95, range 6–155) was significantly higher (all p's < 0.03) than that of the OSA patients (M = 16.67, SD = 28.52, range 0–73) as well as the controls (M = 0.13, SD = 0.34, range 0–1). According to the literature, the neurobehavioral deficits is associated with snoring in children are similar to those found in children with OSA (Gozal and O'Brien, 2004; O'Brien et al., 2004a). Therefore we compared the performance of the SDB group to that of controls and did not intend to examine the OSA and snoring subgroups separately. All SDB patients were untreated prior to and during the experimental night in the sleep laboratory.

TABLE 1 | Means (standard deviations) of participants' data are presented in the table.

	Control (n = 16)	SDB (n = 16)	t (df)	p-value
Snore index events/hour	0.13 (0.34)	40.69 (49.52)	−3.28 (15.001)	**0.005****
AHI event/hour	0.11 (0.20)	6.56 (19.62)	−1.31 (15.003)	0.21
Max. desaturation (%)	92.31 (4.13)	90.56 (7.75)	0.80 (30)	0.43
Desaturation index (%)	0.56 (0.89)	11.25 (26.76)	−1.60 (15.003)	0.13
BMI kg/m^2	15.19 (1.22)	19.25 (5.17)	−3.06 (16.67)	**0.01***
Counting span	2.88 (0.72)	2.48 (0.55)	1.74 (30)	**0.09**$^+$
Listening span	2.40 (0.75)	2.16 (1.09)	0.72 (30)	0.48
Digit span	4.81 (0.65)	4.50 (0.89)	1.13 (30)	0.27

*Snore Index: snoring events per hour; AHI: Apnea-Hypopnea Index: apnetic and hypopnetic events per hour of sleep; Max. desaturation: ratio of oxihemoglobin to the total concentration of hemoglobin present in the blood; Desaturation Index: number of time/hour of sleep that the blood's oxygen level drops by 3% or more for baseline; BMI: body mass index kg/m². Listening Span Task: a working memory (WM) task in which the participants are required to listen to increasingly longer sequences of sentences and to recall the final word of all the sentences in each sequence in serial order (Daneman and Blennerhassett, 1984). Counting Span Task: a WM task in which participants are required to count a growing number of colored dots on the computer screen and remember the number of the dots of each sequence (Case et al., 1982; *$p < 0.05$, **$p < 0.01$, $^+p < 0.10$).*

The control group consisted of sixteen healthy participants (average age: 8.75 years, *SD*: 1.44 [min: 6 to max: 15 years]; 8 females/8 males). The control and the patient groups were matched on age ($t_{(30)}$ = 0.28, p = 0.78) and gender ($\chi^2_{(1)}$ = 0.51, p = 0.48) and parental education (mother education: $t_{(12.54)} < 0.001$, $p > 0.99$; father education $t_{(23)} = 0.61$, $p = 0.55$). They did not suffer from any developmental, psychiatric or neurological disorders, and were free of any sleeping disorders. Informed written parental consent and verbal assent of the children were provided, and participants did not receive any financial compensation for their participation. Ethics approval was obtained by the Ethics Committee at Heim Pal Children's Hospital, Budapest.

TASKS

Tasks

Story Recall-"The War of the Ghosts" Test

Declarative memory performance was measured by "The War of the Ghosts" test (Bartlett, 1932; Bergman and Roediger, 1999). This is a story recall test, which is widely used to measure declarative memory for episodes (Bartlett, 1932; Bergman and Roediger, 1999; Andreano and Cahill, 2006, 2008; Schwabe and Wolf, 2009; Hardt et al., 2010). In this test children are asked to listen and repeat the story after various intervals (immediately or after a determinate interval). The story consisted of 36 sentences; based on the standardized scoring, each sentence is allocated 1 point for the verbatim recalled sentences and 0.5 points for partly correct responses (gist of the sentences; Bartlett, 1932; Gauld and Stephenson, 1967; Csábi et al., 2013).

Alternating Serial Reaction time (ASRT) Task

We used a modified version of the original ASRT task in order to assess non-declarative/procedural learning performance. In the original version of this task, four open circles were displayed in the middle of the computer screen and subjects had to press the corresponding button when the circles were filled in with black (Howard and Howard, 1997). In our version, a dog's head appeared in one of the four empty circles on the screen and participants had to press the corresponding button (Nemeth et al., 2010). The computer was equipped with a special keyboard with four marked keys (Y, C, B and M on a QWERTZ keyboard; thus, compared to the English keyboard layout, the location of the buttons Z and Y were switched), each corresponding to one of the horizontally aligned circles. Before beginning the task, detailed instructions were read to the

participants. We emphasized that the aim was to try to respond as quickly and as correctly as possible. Session 1 (Learning Phase) consisted of 25 blocks, with 85 key presses in each block—the first five stimuli were random for practice purposes, then an eight-element alternating sequence (e.g., 2r1r4r3r, where numbers represent the four places on the screen, and r represents an event randomly selected from the four possible places) repeated ten times. This sequence structure is often described as non-adjacent second-order dependency (Remillard, 2008). Similarly to earlier studies (Nemeth et al., 2010), stimuli were presented 120 ms after the previous response (response-to-stimulus interval, RSI). Each block required about 1.5 min and the entire session took approximately 30–40 min. Between blocks, participants received feedback about their overall RT and accuracy on the screen and then rested 10–20 s before starting a new block. Session 2 (Testing Phase) consisted of 5 blocks; the number of key presses and the RSI were the same as in Session 1 and this Testing Phase took approximately 5–10 min to complete.

A different ASRT sequence was selected for each participant based on a permutation rule so that each of the six unique permutations of the four repeating events occurred. Consequently, six different sequences were used across participants.

As there is a fixed sequence in the ASRT task alternating with random stimuli (for instance 2r1r4r3r), some triplets or runs of three stimuli occur more frequently than others. For example, in the above illustration, triplets 2_1, 1_4, 4_3, and 3_2 would occur often because the third element could be derived from the sequence or could also be a random element. In contrast, 1_2 or 4_1 would occur less frequently because in this case the third element could only be random. Following previous studies (Howard and Howard, 1997; Song et al., 2007; Nemeth et al., 2010), we refer to the former as high-frequency triplets and the latter as low-frequency triplets. Out of the 64 possible triplets, the 16 high-frequency triplets occurred 62.5% of the time and the 48 low-frequency triplets occurred 37.5% of the time. Note that the final event of high-frequency triplets is therefore more predictable from the initial event compared to the low-frequency triplets.

Previous studies have shown that as people practice the ASRT task, they come to respond more quickly to the high- than low-frequency triplets, revealing sequence-specific learning (Howard and Howard, 1997; Howard et al., 2004; Song et al., 2007; Nemeth et al., 2010; Janacsek et al., 2012). In addition, general motor skill learning is revealed in the ASRT task by the overall speed-up due to practice, irrespective of the triplet types. Thus, using the ASRT task enables to measure both sequence-specific and general motor skill learning.

Procedure

There were two sessions in the experiment. The declarative and non-declarative performance was assessed at 7–9 PM prior to sleep (Learning Phase/Session 1) and 7–9 AM after sleep (Testing Phase/Session 2), thus the average interval between the Learning and Testing Phase was 12 h. The order of the administration of declarative and non-declarative tasks was counterbalanced in order to minimize the interference between declarative and non-declarative tasks (see Brown and Robertson, 2007).

Statistical Analysis

To facilitate data processing, the blocks of ASRT were organized into epochs of five blocks. The first epoch contained blocks 1–5, the second epoch contained blocks 6–10, etc. We calculated mean accuracy and median RT for correct responses only; separate for high- and low-frequency triplets and for each subject and each epoch. Note that for each response (n), we defined whether it was a high- or a low-frequency triplet by considering whether it was more or less predictable from the event n-2. For the analyses reported below, as in previous research (Howard and Howard, 1997; Song et al., 2007; Nemeth et al., 2010), two kinds of low frequency triplets were eliminated: repetitions (e.g., 222, 333) and trills (e.g., 212, 343). Repetitions and trills were low frequency for all participants and people often showed pre-existing response tendencies to them (Howard and Howard, 1997; Howard et al., 2004). By eliminating them we attempted to ensure that any high- vs. low-frequency differences are due to learning and not to pre-existing tendencies.

RESULTS

Story Recall Test

We conducted a mixed design ANOVA with SESSION (1–2) as a within-subject factor and GROUP (SDB vs. control) as a between-subject factor to assess offline changes in declarative memory performance. The main effect of GROUP was significant ($F_{(1,29)} = 6.155$, $\eta_p^2 = 0.175$, $p = 0.019$), indicating weaker story recall performance in the SDB compared to the controls (6.267 vs. 10.406, respectively). This weaker performance of the SDB group compared to the control group was evident both in Session 1 (6.87 vs. 10.38; $p = 0.03$) and in Session 2 (5.67 vs. 10.44; $p = 0.01$; **Figure 1**).

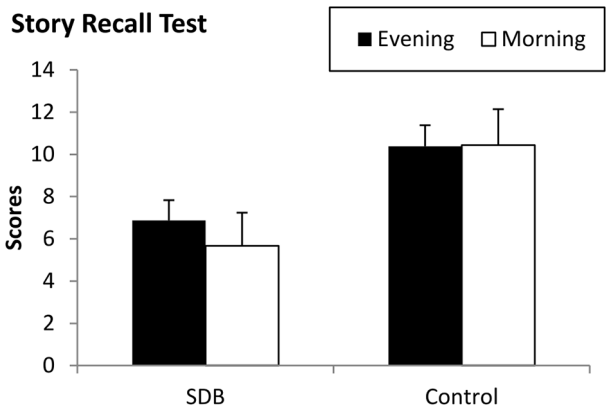

FIGURE 1 | Declarative memory performance in the evening and in the morning in the SDB and control groups. The dependent variable was the number of correctly recalled sentences. The overall declarative memory performance of the SDB group was significantly lower compared to the control group, but there were no offline changes in the memory performance in either group. Error bars indicate SEM.

The main effect of SESSION failed to reach significance ($F_{(1,29)}$ = 2.05, η_p^2 = 0.06, p = 0.16), suggesting no change in the performance during the offline period. Similarly, the SESSION × GROUP interaction was not significant either ($F_{(1,29)}$ = 2.53, η_p^2 = 0.08, p = 0.12), suggesting no differences in offline changes between the SDB and control groups.

Accuracy Analysis in the ASRT Task

Online Learning During Session 1 (Learning Phase)

A mixed design ANOVA was conducted on the 5 epochs of the data shown in **Figure 2** with TRIPLET (2: high vs. low) and EPOCH (1–5) as within-subjects factors and GROUP (SDB vs. control) as a between-subjects factor.

There was significant sequence-specific learning (indicated by the significant main effect of TRIPLET: $F_{(1,30)}$ = 61.26, η_p^2 = 0.67, $p < 0.001$), such that accuracy was greater on high- than on low-frequency triplets. SDB and control groups showed no differences in sequence-specific learning (TRIPLET × GROUP interaction: $F_{(1,30)}$ = 0.29, η_p^2 = 0.01, p = 0.59).

The main effect of EPOCH did not reach significance ($F_{(4,120)}$ = 2.58, η_p^2 = 0.07, p = 0.06), although accuracy decreased across epochs on a trend level. SDB and control groups performed at the same level (EPOCH × GROUP interaction: $F_{(4,120)}$ = 1.29, η_p^2 = 0.04, p = 0.28).

The TRIPLET × EPOCH interaction was significant ($F_{(4,120)}$ = 3.37, η_p^2 = 0.10, p = 0.01), but there were no significant differences between the groups (indicating by the TRIPLET × EPOCH × GROUP interaction $F_{(4,120)}$ = 0.41, η_p^2 = 0.01, p = 0.79; respectively), demonstrating that the pattern of learning was similar in the groups. The main effect of GROUP did not reach significance ($F_{(1,30)}$ = 3.91, η_p^2 = 0.11, p = 0.06), although the SDB group had lower accuracy on a trend level (SDB group: 88.6%, control group: 91.8%).

Offline Changes of Sequence-Specific and General Motor Skill Learning

To investigate the offline changes of sequence-specific and general motor skill learning we compared the accuracy from the last epoch of Session 1 (Epoch 5) and the epoch of Session 2 (Epoch 6) in both groups. These variables were submitted to a mixed design ANOVA with TRIPLET (2: high- vs. low-frequency) and EPOCH (2: last epoch of Session 1 and epoch of Session 2) as within-subject factors, and GROUP (SDB vs. control) as a between-subject factor. The data is shown in **Figure 2**.

There was significant sequence-specific learning (indicating by the main effect of TRIPLET; $F_{(1,30)}$ = 95.40, η_p^2 = 0.76, $p < 0.001$), such that accuracy was greater on high- than on low-frequency triplets. It was similar in the SDB and control groups (indicated by the non-significant TRIPLET × GROUP interaction: $F_{(1,30)}$ = 0.04, η_p^2 = 0.002, p = 0.82).

There was a significant offline changes of general motor skills (indicating by the main effect of EPOCH; $F_{(1,30)}$ = 13.40, η_p^2 = 0.30, p = 0.01), thus accuracy increased from evening to morning. SDB and control groups performed at the same level (EPOCH × GROUP interaction: $F_{(1,30)}$ = 3.26, η_p^2 = 0.09, p = 0.08).

The TRIPLET × EPOCH and TRIPLET × EPOCH × GROUP interactions were not significant ($F_{(1,30)}$ = 0.20, η_p^2 = 0.01, p = 0.65; $F_{(1,30)}$ = 0.28, η_p^2 = 0.01, p = 0.59; respectively), indicating that the pattern of sequence-specific learning was similar in the groups. The main effect of GROUP was not significant ($F_{(1,30)}$ = 1.31, η_p^2 = 0.04, p = 0.26), reflecting that all groups responded with similar accuracy rates (SDB group: 88.8%, control group: 91.2%).

Reaction Time Analysis in the ASRT Task

Online Learning During Session 1 (Learning Phase)

To investigate learning during Session 1, a mixed design ANOVA was conducted on the first 5 epochs of the data shown in **Figure 3**, with TRIPLET (2: high- vs. low-frequency) and EPOCH (5: 1–5) as within-subject factors, and GROUP (SDB vs. control) as a between-subject factor.

Our data revealed significant sequence-specific learning (indicated by the significant main effect of TRIPLET: $F_{(1,30)}$ = 64.33, η_p^2 = 0.68, $p < 0.001$), such that RTs were faster on high- than on low-frequency triplets. SDB and control groups showed no differences in sequence-specific learning (TRIPLET × GROUP interaction: $F_{(1,30)}$ = 0.59, η_p^2 = 0.04, p = 0.44).

There was also significant general motor skill learning (shown by the significant main effect of EPOCH: $F_{(4,120)}$ = 54.80, η_p^2 = 0.64, $p < 0.001$), such that RTs deceased across epochs. SDB and control groups performed at the same level (EPOCH × GROUP interaction: $F_{(4,120)}$ = 0.95, η_p^2 = 0.03, p = 0.38).

The TRIPLET × EPOCH interaction was significant ($F_{(4,120)}$ = 5.26, η_p^2 = 0.14, p = 0.003), suggesting that sequence-specific knowledge increased during practice. The TRIPLET × EPOCH × GROUP interaction was not significant $F_{(4,120)}$ = 0.49, η_p^2 = 0.013, p = 0.67), indicating that the pattern of learning was similar in the groups. In overall RT both group performed at the same level (main effect of GROUP: $F_{(1,30)}$ = 1.37, η_p^2 = 0.04, p = 0.25).

Offline Changes of Sequence-Specific and General Motor Skill Learning

To investigate the offline changes of sequence-specific and general motor skill learning we compared the RTs from the last epoch of Session 1 (Epoch 5) and the epoch of Session 2 (Epoch 6) in both groups. These variables were submitted to a mixed design ANOVA with TRIPLET (2: high- vs. low-frequency) and EPOCH (2: last epoch of Session 1 and epoch of Session 2) as within-subject factors, and GROUP (SDB vs. control) as a between-subject factor. The data is shown on **Figure 3**.

There was significant sequence-specific learning (indicating by the main effect of TRIPLET; $F_{(1,30)}$ = 125.76, η_p^2 = 0.80, $p < 0.001$), thus RTs were faster on high- than low-frequency triplets when analysing the two epochs together. The groups did not differ in overall sequence-specific learning (indicated by the

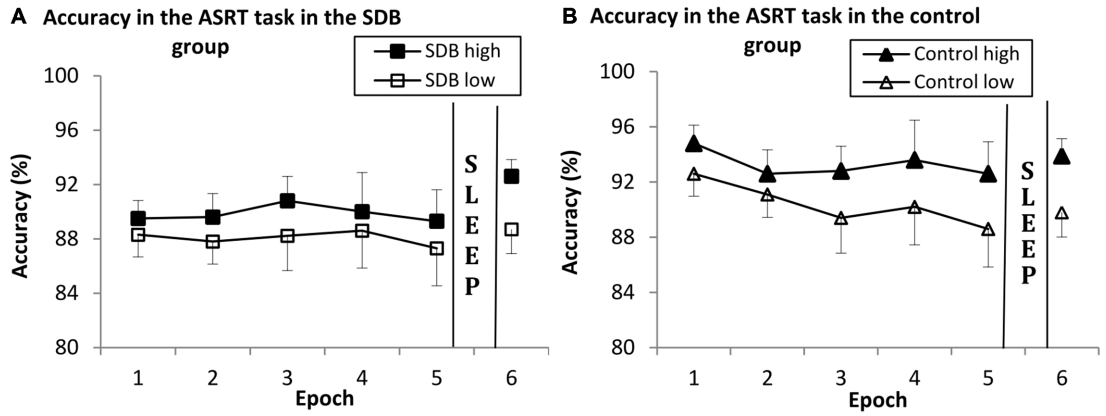

FIGURE 2 | Results of sequence-specific and general skill learning in the SDB (A) and control group (B) in Session 1 (Epoch 1–5) and Session 2 (Epoch 6) on accuracy measures. Both groups showed significant sequence-specific and general skill learning. There were no differences in learning and in offline changes between the groups; the pattern of learning was similar in the SDB and control groups. Error bars indicate SEM.

non-significant TRIPLET × GROUP interaction: $F_{(1,30)} = 0.42$, $\eta_p^2 = 0.01$, $p = 0.51$).

There was significant general motor skill learning during the offline period (demonstrated by the main effect of EPOCH: $F_{(1,30)} = 20.71$, $\eta_p^2 = 0.40$, $p < 0.001$), such that RTs were faster in the morning compared to the evening. The SDB and control groups showed similar level of offline general motor skill learning (EPOCH × GROUP interaction: $F_{(1,30)} = 0.24$, $\eta_p^2 = 0.01$, $p = 0.62$).

The TRIPLET × EPOCH and the TRIPLET × EPOCH × GROUP interactions were not significant ($F_{(1,30)} = 0.84$, $\eta_p^2 = 0.02$, $p = 0.36$; $F_{(1,30)} = 2.18$, $\eta_p^2 = 0.06$, $p = 0.15$, respectively), indicating that the SDB and the control group demonstrated no differences in the pattern of offline changes. There were no significant differences in the overall RTs between the SDB and control groups (main effect of GROUP: $F_{(1,30)} = 2.54$, $\eta_p^2 = 0.07$, $p = 0.12$).

DISCUSSION

Our goal was to investigate the consolidation of declarative and non-declarative memory in children with SDB. We believe our study to be the first to investigate the offline changes of these two types of memory processes in children with sleep disorder. We found no group difference in the consolidation of declarative memory; the SDB group, however, showed generally weaker memory performance in both sessions. We used the ASRT task to measure non-declarative learning processes. This sequence learning task allowed us to differentiate between two components of learning: general motor skill learning and

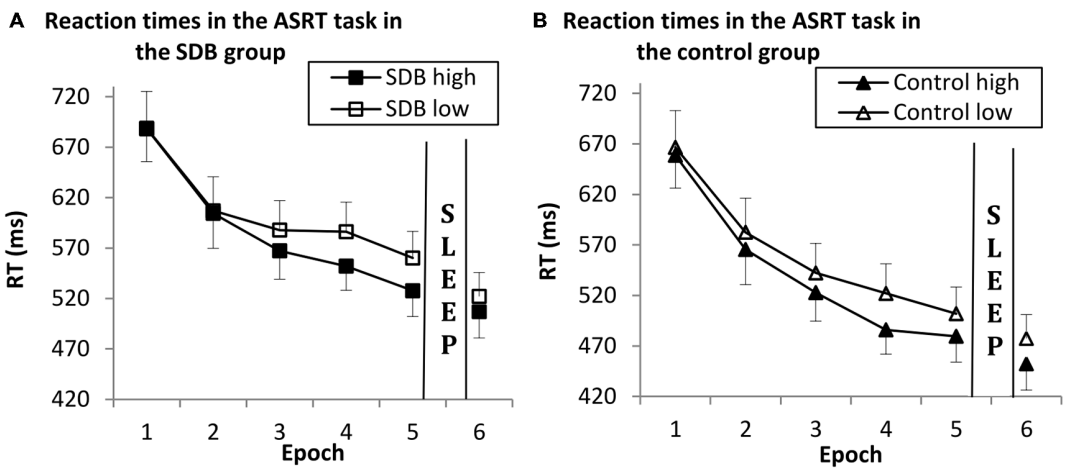

FIGURE 3 | Results of sequence-specific and general skill learning in the SDB (A) and control (B) group in Session 1 (Epoch 1–5) and Session 2 (Epoch 6) on reaction time measures. Both groups showed significant sequence-specific and general skill learning. There were no differences in learning and in offline changes between the groups; the pattern of learning was similar in the SDB and control groups. Error bars indicate SEM.

sequence-specific learning. We found that these two types of non-declarative learning and consolidation are intact in children with SDB.

Our results on online declarative memory performance are in line with previous studies that found weaker declarative performance in the SDB group in general (Blunden et al., 2000; Kaemingk et al., 2003; Gottlieb et al., 2004; Kennedy et al., 2004; Csábi et al., 2013). Gottlieb et al. (2004) found lower performance on verbal and visual memory tasks in children with SDB compared to healthy controls. The mechanism causing these neuropsychological deficits has not been fully explored. Results from previous studies suggest that sleep fragmentation and intermittent hypoxia could have negative influence on the development of the central nervous system resulting structural changes in brain circuits, particularly in the hippocampus and frontal lobe (Macey et al., 2002; Bartlett et al., 2004; Halbower et al., 2006; Owens, 2009). For example Bartlett et al. (2004) found that in the left hippocampal area, N-acetyl-containing/creatine-containing compounds was significantly increased in adult OSA patients using proton magnetic resonance spectroscopic imaging. In childhood OSA Halbower et al. (2006) showed also significant differences in the mean metabolite ratio N-acetyl in the left hippocampus and right frontal cortex compared to controls leading the conclusion that childhood OSA is associated with neuronal injury in the hippocampus and frontal cortex. It is important to note that we assessed only the breathing indices during sleep. Further investigations using polysomnography need to clarify the relationship between declarative memory functions and sleep stages or sleep deprivation in children with SDB.

In the case of the overnight consolidation of declarative memory, we failed to find differences between the SDB and control group. Although there was a general group difference in the overall performance, both groups showed intact consolidation. This result contradicts with the finding of Kheirandish-Gozal et al. (2010) who demonstrated decreased consolidation of declarative memory in children with OSA. The difference between the two studies might be explained by the type of materials to be remembered (verbal vs. nonverbal) and other task characteristics (e.g., number of repetitions). Another possible explanation might be that the SDB group in our study demonstrated a floor effect with no room to forget in the offline period. For example, compared to the healthy controls, sleep disturbances in the SDB group can lead to a greater fatigue effect, which can be more pronounced by the evening where the first session took place, and could lead to weaker memory performance in the SDB group. This explanation can be tested by controlling for circadian effects and comparing AM-PM vs. PM-AM designs. Future studies need to unravel how task characteristics and/or circadian factors affect sleep-related declarative memory consolidation in children.

In the case of non-declarative learning, we found similar performance between the SDB and control group in general motor skill and sequence-specific learning in the Learning Phase, both in accuracy and in RT. Our results are in line with our previous study in which the SDB group showed impaired declarative memory performance while the non-declarative learning remained intact compared to the healthy controls (Csábi et al., 2013). Nemeth et al. (2012) using the ASRT task also found intact non-declarative sequence learning in elderly adults with OSA. These results indicate that the relationship between online non-declarative memory formation and sleep is similar in children and adults with SDB. The performance difference between declarative and non-declarative tasks in session one can be explained by that the disrupted sleep pattern influences the more attention-demanding and cortical structure-guided explicit processes (story recall), while the less attention-demanding implicit processes (ASRT task) mediated by subcortical structures are preserved (Csábi et al., 2013).

In the overnight consolidation of non-declarative memory we found no differences in the offline changes of either general motor skill or sequence-specific learning between the two groups. We found offline improvement on general motor skill, while the sequence-specific learning remained on the same level and did not improved. To our knowledge, consolidation of non-declarative memory has not been tested in children with SDB yet. These results are in line with studies investigating the effect of sleep deprivation on non-declarative sequence learning in adults without sleep disorder (Genzel et al., 2009; Van Der Werf et al., 2011). There are a few studies investigating non-declarative memory consolidation in adults with OSA. For example, Kloepfer et al. (2009) found reduced overnight improvement on average RT performance in OSA patients using a very different task compared to ours (motor adaptation vs. sequence learning, respectively). Djonlagic et al. (2012) also examined adult OSA population and revealed that OSA and control groups showed almost identical performance in the initial training in the evening on a sequence learning task, but the control group exhibited significantly more overnight improvement. The authors suggest that this weaker offline performance was caused by sleep fragmentation in OSA. In our previous study with adult OSA patients, we revealed differences in the offline changes of general motor skill learning between the OSA and control group. The control group showed offline improvement on general motor skill learning from evening to morning, while the OSA group did not. In contrast, we did not find differences between the groups in offline changes in sequence-specific learning (Csabi et al., 2014). These results partly differ from our current findings and highlight the importance of developmental factors in the consolidation of non-declarative memory: sleep disordered breathing might affect the underlying neural network differently in childhood compared to adulthood.

It worth mentioning that our study have two important potential limitations. Firstly, the declarative and non-declarative tasks could be interfere to each other. For example Brown and Robertson (2007) found that declarative tasks can actually boost non-declarative learning. It is possible that our manipulation namely counterbalancing these two types of task is not enough to eliminate the interference. Secondly, it is possible that the actual story recall task is not sensitive enough to demonstrate sleep effect. Further studies need to clarify these issues by examining the declarative and non-declarative tasks separately in different experiments and using other type of declarative tasks as well.

In conclusion, our study found dissociation between the declarative and non-declarative processes in children with SDB. Similarly with Csábi et al. (2013) we found weaker declarative memory than non-declarative performance in the first Session (Learning Phase). Regarding the consolidation, we found intact consolidation in the case of declarative memory as well as sequence-specific and general motor skill aspects of non-declarative memory in SDB. These findings imply that actual and/or long-term disturbance of sleep has a differential effect on different memory processes (online vs. offline). Our findings underscore the importance of examining the effect of sleep disturbances on motor and cognitive functions in childhood. These studies can give us a deeper insight into the effect of sleep on the developing brain and memory functions and how the relationship between sleep and memory changes from childhood to adulthood. Since persistent sleep problems in childhood can lead not only to impaired cognitive functioning—consequently lower general intelligence and school performance—but also anxiety and depression disorders in adulthood (Gregory et al., 2005), these results can help us develop more sophisticated diagnostic tools, neuropsychological profile and more effective rehabilitation programs.

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Sleep-Related Offline Improvements in Gross Motor Task Performance Occur Under Free Recall Requirements

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Nocturnal sleep effects on memory consolidation following gross motor sequence learning were examined using a complex arm movement task. This task required participants to produce non-regular spatial patterns in the horizontal plane by successively fitting a small peg into different target-holes on an electronic pegboard. The respective reaching movements typically differed in amplitude and direction. Targets were visualized prior to each transport movement on a computer screen. With this task we tested 18 subjects (22.6 ± 1.9 years; 8 female) using a between-subjects design. Participants initially learned a 10-element arm movement sequence either in the morning or in the evening. Performance was retested under free recall requirements 15 min post training, as well as 12 and 24 h later. Thus, each group was provided with one sleep-filled and one wake retention interval. Dependent variables were error rate (number of Erroneous Sequences, ES) and average sequence execution time (correct sequences only). Performance improved during acquisition. Error rate remained stable across retention. Sequence execution time (inverse to execution speed) significantly decreased again during the sleep-filled retention intervals, but remained stable during the respective wake intervals. These results corroborate recent findings on sleep-related enhancement consolidation in ecological valid, complex gross motor tasks. At the same time, they suggest this effect to be truly memory-based and independent from repeated access to extrinsic sequence information during retests.

Keywords: sleep, enhancement consolidation, gross motor task, sequence learning, free recall

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INTRODUCTION

There is ample evidence by now that sleep (but not wake) after initial training of motor skills can produce significant improvements in performance at later retesting without any further physical practice (e.g., Fischer et al., 2005; Walker, 2005; Doyon et al., 2009). This phenomenon usually is referred to as "sleep-related offline learning", and has been associated with an "active system consolidation" process (Born and Wilhelm, 2012). Here, it is assumed that newly encoded skill representations are being actively reprocessed during slow-wave sleep, resulting in strengthening synaptic connections in the neocortex and in a qualitative reorganization of the respective memory representations. These processes are understood as a prerequisite for the sudden improvements in overt performance frequently observed. However, suchlike processes and effects appear to be closely related to certain motor task characteristics as well as to

specific learning procedures. That is, in general sleep-related offline learning seems to require some involvement of *declarative* memory processes. This is often associated with routines of *explicit* learning and awareness (Robertson et al., 2004). Enhancement of motor sequence memory supposedly pertains to an abstract spatial map of the sequence that represents the series of movements to perform during recall. This representation is supported by a distinct hippocampo-cortical neural network (Albouy et al., 2015), and is supposedly associated with declarative knowledge concerning the action's goal as well as the type of sequence elements and their temporal order. Moreover, performance improvements in motor adaptation tasks (i.e., precise sub-maximal force production; visuo-motor adaptation) have been found to be fairly small and rather *time*- instead of sleep-dependent (Blischke et al., 2008; Doyon et al., 2009; but see Huber et al., 2004). Thus, sleep-related EC should be most pronounced in *sequentially* structured motor tasks.

In most of the studies addressing sleep-related motor offline learning sequential-finger-tapping or thumb-to-finger movements were involved. Only a couple of years ago the question has been raised if the respective findings also apply to ecologically valid gross motor skills (Blischke et al., 2008). And it was only recently that these findings have successfully been extended to gross motor tasks involving multi-joint limb movements (Genzel et al., 2012; Kempler and Richmond, 2012; Morita et al., 2012; Al-Sharman and Siengsukon, 2013; Malangré et al., 2014). Moreover it has been shown that the degree of sleep-related motor enhancement consolidation in the elderly is modulated by the kinematic demands of the task. In one recent study, sleep-related performance improvements were observed in older age groups only when a classic sequence learning task requiring individuated finger movements was replaced by an adapted version of the same task. In this adapted version reaching movements were performed with the whole hand (Gudberg et al., 2015). This dissociation of specific mechanisms of sleep underpinning motor sequence consolidation in older adults is certainly of theoretical importance. And it emphasizes the potential of incorporating whole limb movements in research activities concerning the relation of sleep and motor memory consolidation.

Although criterion tasks incorporated in all these studies reporting gross motor sleep-related offline learning were of considerable variety and involved movements of the upper as well as of the lower extremities, again they were all *sequentially* structured. However, when motor *adaptation* was the prominent task requirement, sleep did not enhance, but only stabilize performance (Hoedlmoser et al., 2015). Thus, the above mentioned dissociation of motor sequence learning and motor adaptation with respect to sleep-related memory consolidation processes seems to hold also for gross motor skills. However, there are still some aspects of practical and theoretical importance waiting for closer scrutiny. One such aspect is the question as to whether sleep-related offline learning will also come into effect at retention even under *free recall* conditions at an early learning stage. This question is of particular importance in the applied field of movement studies (i.e., vocational training, sports, occupational therapy, and motor rehabilitation). Here trainees, athletes and patients initially are supplied with stimulus information and feedback while acquiring new motor skills at initial training sessions. But soon after initial training they are usually required to recall and execute those skills under "real-life" conditions in the absence of any augmented information.

Here as a first step we present an experiment set up to scrutinize if sleep-related offline learning was to be found at all in a gross motor task under *free recall* conditions with no extrinsic criterion information available. The criterion movement employed was a sequential motor task with high demands on precision and manual dexterity. This task incorporated a series of 10 unrestrained multi-joint reaching movements involving the whole non-dominant arm. Such a task bears good resemblance to a wide variety of sport skills and activities of daily living. Following a fixed spatial pattern, participants had to execute this movement sequence as rapidly as possible with as few errors as possible.

It was hypothesized that after initial learning *sleep*, but not wake, significantly facilitates performance (namely: sequence execution speed) at retention under free recall conditions when compared to post-training performance (i.e., free-recall performance assessed shortly after acquisition).

MATERIALS AND METHODS

Participants and Groups

Two groups of participants ($N = 12$ each) voluntarily participated in this experiment, which was conducted at the Saarland University (Department of Sport Science) in accordance with the ethical standards of the 1975 Declaration of Helsinki, and was approved by the Ethic Committee of the Faculty 5 Empirical Social Sciences of Saarland University. Subjects took part in the experiment in accord with the department's course regulations and gave their written informed consent before participation. Participation was accounted for as partial fulfilment of course requirements. For organizational reasons both groups were recruited from different courses, and were examined at different times about 6 months apart by different experimenters.

Six subjects did not complete the experiment, because they were unable to recall the criterion task under free recall conditions. These subjects were excluded from further analysis. Only the remaining 18 participants entered the final analyses reported in the following sections. As a consequence the first group (in the following labeled the Morning-Evening-Morning (MEM) group according to the experimental design; cf. "Design and Procedure" Section) comprised only 8 participants (22.1 $\pm$ 2.4 years, 4 females, one left handed, 4 males), while 10 participants (22.9 $\pm$ 1.5 years, 4 females, 6 males, one left handed) remained in the second group (labeled the Evening-Morning-Evening (EME) group accordingly).

There was no additional reward or remuneration. Participants were required to refrain from daytime naps, alcohol, excessive

caffeine-intake, and any other drugs from 24 h before initial training until the end of the experiment. Physical activity (e.g., sport practice) was permitted. All participants were naïve with respect to the criterion task and the research hypotheses.

Duration and quality of each subjects' sleep during the night of the experiment was assessed with a standardized sleep questionnaire (Goertelmeyer, 1986). There was no indication of poor sleep quality for any of the participants. Also, daytime activities during the wakening retention interval were assessed with a time-line protocol. Again, no peculiarities were observed with respect to any of the subjects.

Task and Apparatus

The criterion task required participants to repeatedly carry out a fixed sequence of 10 reaching movements with their non-dominant arm. Subjects were seated comfortably in a height-adjustable chair in front of a table-mounted electronic pegboard and a vertical computer screen with their upper trunk against the backrest. With their hand visible all the time, participants could freely move shoulder, elbow and wrist. On each trial, following a start signal they had to successively fit a small hand-held peg into the respective target-holes (depth: 22.22 mm; diameter: 12.7 mm) on the pegboard (see **Figure 1**). Thereby they followed a fixed pattern of end-point locations in the horizontal plane, which was void of any apparent regularity. Transport movements differed in amplitude (range: 3.83–33.75 cm) and direction. Precision requirements for all sequence elements amounted to an index of difficulty (ID) of 5.03 (±0.94) on average (Fitts, 1954). According to Fitts, the ID is determined by the equation Log_2 (2A/W), where A represents the movement amplitude measured from one target center to the other target center and W represents the width of the target area in the direction of the movement.

The sequence to perform was never presented entirely before or during execution. Rather, participants learned the sequence by repeated execution, similarly to a serial reaction time task. During acquisition, targets were visualized one after the other prior to each reaching movement on a computer screen. Correct execution of a sequence element was indicated by a color change of the respective target stimulus from red to green, while the next target symbol was illuminated red. In case of a reaching error, the symbol representing the target that had been missed turned green as well, while the next target was illuminated red. Thus, explicit error control always required participants to compare the peg's present position on the pegboard to the target position indicated on the screen. As soon as one sequence element was terminated, the next reaching movement had to be started immediately, until the sequence was completed. Once a sequence trial was finished, subjects had to place the peg back into the starting position and prepare themselves for the next trial. After announcing they were ready again participants received an oral start-signal about 1 s later, and then executed the next trial. This procedure was repeated until a block of 10 trials had been accomplished. During recall, no extrinsic information (neither stimulus information nor feedback) was provided. Sequence configuration, raw data assessment and screen display during sequence execution were controlled by means of LMD Software (Wagner: IAT Leipzig, Germany).

Dependant Measures

Acquisition and recall tests were organized in successive blocks of 10 trials, separated by 30-s resting periods. To prevent any build up of fatigue during acquisition, the resting period following block six was extended to 2.5 min. Performance measures were number of Erroneous Sequences (ES) per trial block (i.e., error rate), and Total Execution Time (TET) per sequence, with TET averaged for each subject across correct sequences in a trial block. TET thereby is inversely proportional to sequence execution speed. Participants were instructed to execute each single sequence-trial as rapidly as possible with as few errors as possible. They were also advised not to speed up performance at the expense of an increasing error rate. Instructions were followed by most of the participants,

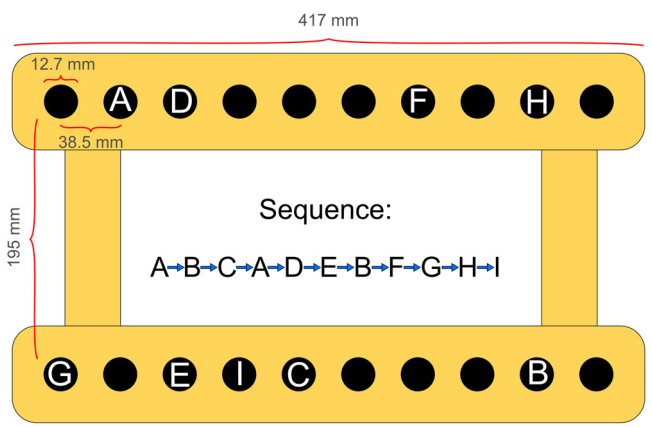

FIGURE 1 | Experimental apparatus and spatial locations to be reached for one after the other, defining the 10-element arm movement sequence.

resulting in marked skewness of the dependent variable ES (i.e., number of ES).

It should be mentioned here that this gross motor task was sufficiently complex and difficult to prevent performance reaching an asymptote within one single practice session. As had been shown previously in a pilot study with eight subjects (23.13 ± 2.1 years, 4 females, 4 males) extensively practicing this same criterion task on three successive days (600 trials altogether; two training sessions of 100 trials per day, stimulus information continuously provided), mean performance (i.e., sequence execution speed, operationalized via TET) continuously increased following a power function, and started to level off only after about 550 trials at about 5.7 s TET on average (unpublished data; Schmitz and Waßmuth, 2013). It also became clear from that study that more than 100 trials would be needed to fully memorize the spatial movement pattern.

Design and Procedure

After being shortly familiarized with the electronical pegboard and the peg-plugging procedure in general, both experimental groups received initial training of the criterion task (12 blocks of 10 trials each). Both groups then were retested three times in a free-recall condition, namely 15 min after end of practice (Post-Training), and again 12 h (Retest 1) and 24 h later (Retest 2), with each Retest comprising three blocks of 10 trials. The first group to take part in this experiment received initial training in the morning (7–9 a.m.) and was labeled the MEM group accordingly, while the second group practiced in the evening (7–9 p.m.), and was labeled the EME group respectively. Thus, subjects in the MEM-group had a regular night's sleep during their second 12-h retention interval, those in the EME-group during their first 12-h retention interval (cf. **Figure 2**). To prevent mental rehearsal of the criterion task during the 15-min retention interval directly following acquisition, participants were asked to read a series of comic stories combining pictures and text. They also were instructed to report on the stories' content at the end of the respective test session.

Statistics

Changes in performance during acquisition and retention were analyzed with reference to five different time points, namely "Start of Practice", "End of Practice", "Post-Training", "Retest 1", and "Retest 2". Time point-specific performance values were calculated as follows: first, for each subject ES- and TET-measures were averaged across trials per block. Then for each subject and dependent variable, average performance measures were calculated from the first three initial training blocks (Start of Practice, blocks 1, 2 and 3) and from the last three initial training blocks (End of Practice, blocks 10, 11 and 12), while retest measures were calculated from blocks 13, 14, and 15 (Post-Training), 16, 17, and 18 (Retest 1), and 19, 20, and 21 (Retest 2) respectively. Group mean values (medians) were calculated on this basis.

In the presence of small sample sizes and extreme skewness of the dependent variable ES for inferential statistics non-parametric procedures were applied. Accordingly, Friedman test and Wilcoxon test were used for within-group comparisons, while Mann-Whitney U test was applied when data were compared across groups. A significance level of $p < 0.05$ was used for all inferential statistics. In case of multiple testing Bonferoni-corrections were applied. As a rule statistical significance was assessed two-tailed, with exact p-values being reported. Effect sizes were provided in terms of Cohen's r

$$\left(r = \frac{|z|}{\sqrt{N}}\right) \text{ and } \Phi_c \left(\Phi_c = \sqrt{\frac{\chi^2}{\mathrm{N}(k-1)}}\right)$$

with respect to non-parametric tests (Fritz et al., 2012).

RESULTS

Descriptive Data

Performance data (i.e., number of ES and TET) achieved by each group at the respective time points are presented in **Table 1**.

Acquisition and Transfer to Free Recall

In a first step, changes in performance during acquisition and at transfer to the first free-recall test were determined for both

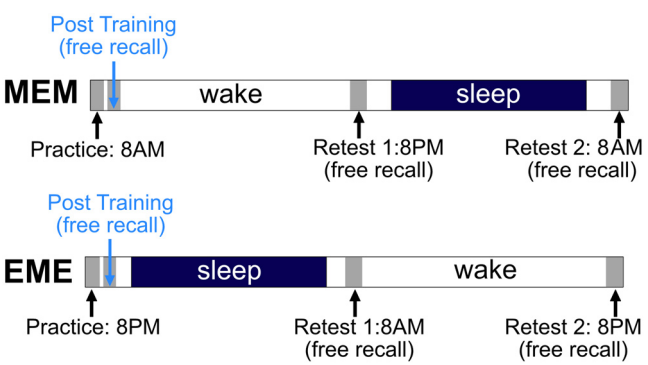

FIGURE 2 | Experimental paradigm. For details see text.

TABLE 1 | Behavioral data: number of Erroneous Sequences (ES) and Total Execution Time (TET).

	MEM-Group		EME-Group	
Time point	**ES [*n*]**	**TET [s]**	**ES [*n*]**	**TET [s]**
Start of practice	2.16 (0.7–3.5)	10.33 (9.8–11.2)	1.33 (0.3–2.9)	11.78 (11.2–12.7)
End of practice	0.33 (0.0–1.5)	7.64 (6.8–8.4)	0.83 (0.5–1.3)	9.39 (7.9–10.6)
Post training (free recall)	1.00 (0.0–1.2)	7.50 (6.7–8.6)	1.00 (0.5–2.3)	9.41 (8.4–11.3)
Retest 1 (free recall)	0.50 (0.3–2.0)	7.55 (6.4–8.5)	0.66 (0.3–1.5)	8.19 (7.0–10.5)
Retest 2 (free recall)	0.50 (0.0–1.2)	6.57 (6.1–7.5)	0.50 (0.0–1.3)	7.98 (7.1–9.8)

Reported are medians and lower and upper quartile values (in parentheses) for Number of Erroneous Sequences (ES) and Total Execution Time (TET) from each experimental group at each time point. MEM, Morning (Acquisition and Post-Training) – Evening (Retest 1)-Morning (Retest 2); EME, Evening (Acquisition and Post-Training) – Morning (Retest 1) – Evening (Retest 2). Shaded areas indicate sleep-filled retention periods.

groups. Throughout acquisition, number of *ES* was low on average (MD = 0.87) in the MEM-group, but even so from Start of Practice to End of Practice error rate significantly decreased ($Z = -2.527$, $p = 0.008$, Cohen's $r = 0.892$), as did *TET* ($Z = -2.521$, $p = 0.008$, Cohen's $r = 0.890$). However, when participants were subjected to the first free-recall test at Post-Training, compared to End of Practice both ES ($Z = -0.527$, $p = 0.688$) and TET ($Z = -0.280$, $p = 0.844$) statistically remained about the same. Also in the EME-group, error rate was low on average throughout acquisition (MD = 1.16). While number of *ES* this time did not change significantly from Start of Practice to End of Practice ($Z = -1.602$, $p = 0.129$), *TET* again significantly decreased ($Z = -2.521$, $p = 0.008$, Cohen's $r = 0.890$). When participants underwent the first free-recall test at Post-Training, compared to End of Practice both ES ($Z = -1.266$, $p = 0.258$) and TET (EME: $Z = -0.968$, $p = 0.375$) statistically remained about the same again.

Thus, both groups during acquisition significantly improved sequence execution speed and also somewhat reduced error rate, while transfer from an informational guided practice condition to free recall 15 min later did not yield any performance decrements. On the whole, error rate was real low throughout the whole experiment in either group, and there was no speed-accuracy trade-off across time points.

Retention (Free Recall Only)

In a second step possible performance changes during retention under free-recall conditions had to be determined. According to our theoretical considerations it was of specific interest, if possible performance changes during the sleep-filled retention intervals were any different from performance changes during the respective wake intervals. Considering the small sample sizes, and in order to achieve sufficient statistical power, we applied the following procedure: data of both experimental groups were combined and subjected to the respective statistical tests conjointly, so that pre- and post-wake performance data of all 18 participants could be compared directly, and pre- and post-sleep performance data of all 18 participants could be compared directly, too. Due to the circadian offset of 12 h between both experimental groups the combined pre- and post-wake interval and pre- and post-sleep interval data for each dependent variable had to be compared in two separate test runs. It has been argued that these two tests were conceptually related. Therefore the level of significance in these cases was and set at $p = 0.025$ (two-tailed) following Bonferoni correction.

The following results now refer to the combined data of both groups. According to the respective Wilcoxon tests, *error rate* (ES) remained the same across both retention intervals (wake retention interval: $p = 0.404$; sleep-filled retention interval: $p = 0.106$). However, *sequence execution time* (TET) *significantly decreased* during the *sleep-filled* retention interval ($Z = -3.245$, $p = 0.001$, Cohen's $r = 0.540$), but not so during the wake retention interval ($Z = -1.894$, $p = 0.060$, Cohen's $r = 0.315$). The respective TET-data are depicted in **Figure 3**.

Thus, regarding our total sample (N = 18) the following became evident: *TET significantly decreased* (i.e., sequence execution speed increased) during the *sleep-filled* 12-h retention interval, but remained statistically unchanged during the respective 12-h wake interval. *Error rate (ES)*, on the other hand, remained completely unaffected by the respective treatment conditions throughout retention. So also during retention there was no indication of any speed-accuracy trade-off. These findings were well in line with our theoretical expectations of sleep-dependent offline-gains in sequence execution speed. They were also corroborated by statistical analysis at the single group level (see "Supplementary Material").

DISCUSSION

The present study was intended to test the notion of sleep-related offline learning coming into effect in a sequentially structured gross motor task after only limited practice and under free recall requirements. These are conditions common to many applied areas in the motor learning domain. From a theoretical point of view, any offline improvements in performance observed at retention under these conditions can be attributed solely to an enhanced sequence memory, since continued online learning at retests is effectively prevented by the absence of criterion-related stimulus information. In traditional motor learning experiments, only terminal feedback is usually removed to prevent further learning. But as long as stimulus information is still present at retesting (like e.g., in the typical serial reaction time task), continued updating

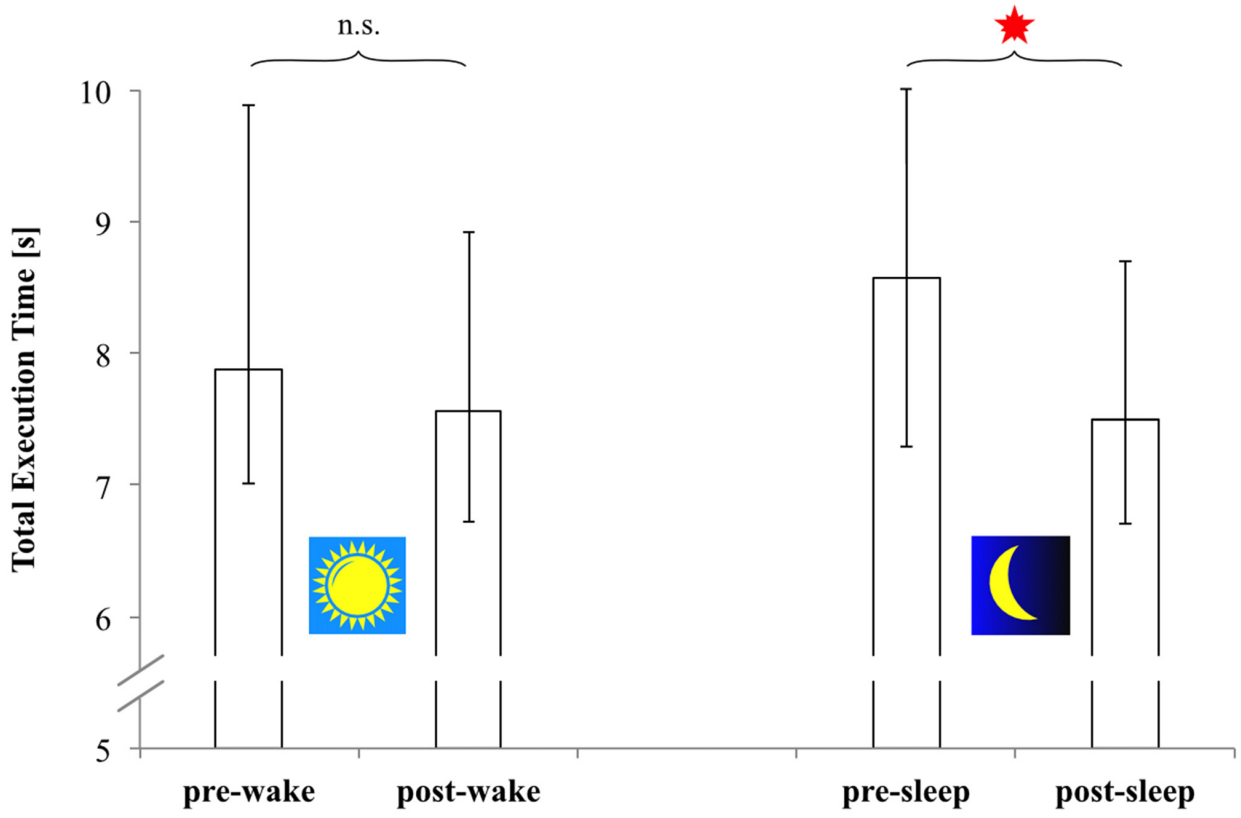

FIGURE 3 | Total execution time (seconds; correct sequences only) of all 18 subjects (Morning-Evening-Morning-group and Evening-Morning-Evening-group combined) at free recall. Presented are measures based on the combined data from both groups' pre- and post-wake retention tests (left panel), and from both groups' pre- and post-sleep retention tests (right panel). Open bars: medians; Error bars: upper and lower quartiles. *Significant difference of group medians ($p = 0.001$).

of sequence memory on grounds of externally provided information cannot be prevented. From an ecological point of view such testing conditions are not likely to reliably engage retrieval strategies relevant to many real-life situations in the field.

In the present study a 10-element sequence of reaching movements was used for a criterion task. Participants executed this sequence on an electronic pegboard with their unrestrained non-dominant arm, thereby following a fixed spatial pattern in the horizontal plain. The pattern had no apparent regularities. The sequence had to be carried out as rapidly and with as few errors as possible. Dependent variables were number of ES, and total sequence execution time. These performance measures thus represented error rate and sequence execution speed. Two groups of altogether 24 subjects initially learned this sequence for a total of 120 trials either in the morning (MEM-group) or in the evening (EME-group). Performance was retested 15 min post training, as well as 12 h and 24 h later. Thus, each group was provided with one sleep-filled and one wake retention interval. All three retests required free recall of the criterion sequence.

At the end of practice all subjects had more or less explicit knowledge of the sequence they had learned, and were using different retrieval strategies at (re)testing. This can be concluded from subjects' verbal reports given at the end of the experiment. However, to which extent participants used cognitive retrieval strategies or more procedural aspects of the motor task in question (cf. Hikosaka et al., 1999) cannot be decided. At any case, six subjects (four in the MEM- and two in the EME-group) were unable to reproduce the initially learned sequence under free recall conditions, even when they tried to explicitly remember the sequence. These subjects were excluded from further analysis.

In the remaining 18 subjects error rate was low right from the beginning and dropped to well below one erroneous sequence per block of 10 trials at the end of practice. Sequence execution speed improved significantly in both groups during acquisition. During retention error rate did not change any more (no group differences). Total sequence execution time during retention significantly *decreased* following sleep, but not following wake. This held true for the total sample, and could also be corroborated for each group separately (cf. "Supplementary Material"). Throughout the experiment there was no speed-accuracy trade-off.

It should be noted that sequence execution time at the end of practice in both groups was still well above (at least 2 s) asymptotic performance level. The performance asymptote for

this same task has been determined in a previous study after three days of continued practice by eight young subjects of comparable age (Schmitz and Waßmuth, 2013). Therefore it seems unlikely that global differences in sequence execution speed between experimental groups could have biased the sleep-related improvements in performance found at retention to any relevant extent. Also, this finding of sleep-related motor performance improvement was independent from retention interval duration and time of day of learning: the EME-group initially acquired the criterion sequence in the evening and was afforded sleep during the first 12 h retention period. The MEM-group to the contrary learned the sequence in the morning and slept during the second 12 h retention period. All in all these results corroborate recent findings of sleep-related motor offline learning in a very similar task, however with the same stimulus information provided at retention as well as during the initial learning phase (Malangré et al., 2014).

It should be mentioned that in the EME-group, following significant sleep-dependent offline improvement, sequence execution time also decreased somewhat during the second (the wake) retention interval. This effect is close to significance ($p = 0.064$, Cohen's $r = 0.597$; see "Supplementary Material"), and was not observed in the MEM-group. From this one might conjecture that sleep-dependent consolidation mechanisms are still in process during the following wakening period, while this is not the case during the wakening period prior to sleep. This aspect certainly requires closer consideration in the future.

In this context, also the following observation might be of particular interest: in a pilot study (unpublished data) we conducted in our laboratory preceding the experiment presented in this article, two randomized groups of participants (all students at the department of sport science) practiced the same criterion task as was used in our present study either in the morning (ME-group; 21.0 $\pm$ 2.4 years; 5 females; 4 males) or in the evening (EM-group; 21.0 $\pm$ 0.98 years; 4 females; 7 males) for 120 trials, and were retested under free recall conditions 12 h later, i.e., on the same evening or on the next morning respectively. Note that there was no early free recall test shortly following acquisition. During acquisition total sequence execution time significantly decreased in either group from 9.82 s on average to 7.53 s on average. But then in this pilot study at free recall seven out of the nine subjects in the ME-group were unable to reproduce the criterion sequence after their 12 h wakening interval. Obviously during a 12 h wake retention interval they had forgotten essential sequence components (i.e., certain elements and/or order of elements). To the contrary only two out of the eleven subjects in the EM-group failed to recall the sequence after their 12-h sleep-filled retention interval. Thus, sleep appeared to prevent sequence memory to deteriorate. Also, and different from our present results, in the absence of an early free-recall test in the remaining nine subjects of the pilot-study's EM-group sequence execution speed at free recall following a night of sleep appeared to be stabilized, but not improved as compared to performance at the end of acquisition. Thus, it could be argued that withdrawing stimulus information and feedback opportunity during testing might have hidden possible sleep-dependent performance improvements.

Thus, implementation of an early free recall test (Post-Training) in our present experiment not only provided for an appropriate datum point subjects' performance at the two later free recall tests could be related to i.e., transfer-appropriate processing; cf. Lee (1988). We conjecture that it also served as a means to effectively reduce the tendency for sequence representation to decay over a 12 h wakening period, and to provide a basis for subsequent enhancement of sequence memory during sleep. We assume that the necessity of free recall soon after acquisition stabilizes and even considerably elaborates the multifaceted sequence representation still intact at that point of time. This positive effect of early retesting on long term retention has recently been found for verbal material (Roedinger and Karpicke, 2006) as well as for effector transfer in motor sequence learning, which is indicative for the generalization of the abstract spatial sequence pattern (Boutin et al., 2013). Thus, testing conditions not only boosts memory when learners are allowed to practice between testing sessions as in the study of Boutin et al. (2013), but early testing under free recall conditions might also shape sequence memory so to enhance later retention.

All in all, while with the present experiment we successfully corroborated and extended recent findings on sleep-related offline learning in gross motor sequence learning tasks, there are also clear limitations to our study in that sample size was rather small, and subjects were not randomly assigned to the experimental groups.

AUTHOR CONTRIBUTIONS

AM and KB contributed extensively and equally to the work presented in this manuscript, developed the research topic and designed the experiment, prepared, analyzed data and discussed results, wrote the article and discussed and commented on the manuscript at all stages. AM supervised data collection.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fnhum.2016.00134/abstract

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Reconsolidation of Motor Memories Is a Time-Dependent Process

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Reconsolidation is observed when a consolidated stable memory is recalled, which renders it transiently labile and requires re-stabilization. Motor memory reconsolidation has previously been demonstrated using a three-day design: on day 1 the memory is encoded, on day 2 it is reactivated and experimentally manipulated, and on day 3 memory strength is tested. The aim of the current study is to determine specific boundary conditions in order to consistently degrade motor memory through reconsolidation paradigms. We investigated a sequence tapping task ($n = 48$) with the typical three-day design and confirmed that reactivating the motor sequence briefly (10 times tapping the learned motor sequence) destabilizes the memory trace and makes it susceptible to behavioral interference. By systematically varying the time delay between memory reactivation and interference while keeping all other aspect constant we found that a short delay (i.e., 20 s) significantly decreased performance on day 3, whereas performance was maintained or small (but not significant) improvements were observed for longer delays (i.e., 60 s). We also tested a statistical model that assumed a linear effect of the different time delays (0 s, 20 s, 40 s, 60 s) on the performance changes from day 2 to day 3. This linear model revealed a significant effect consistent with the interpretation that increasing time delays caused a gradual change from performance degradation to performance conservation across groups. These findings indicate that re-stabilizing motor sequence memories during reconsolidation does not solely rely on additional motor practice but occurs with the passage of time. This study provides further support for the hypothesis that reconsolidation is a time-dependent process with a transition phase from destabilization to re-stabilization.

Keywords: consolidation, reconsolidation, motor learning, sequence task, memory updating

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INTRODUCTION

Acquiring a novel task leads to a new but initially fragile memory (Duncan, 1949; Misanin et al., 1968; Dudai, 1996; McGaugh, 2000). This initial memory is highly susceptible to interference and is in need of consolidation. The process of consolidation makes the memory more robust and resistant against competing influences and stabilizes memory representations despite the absence of any further training (Brashers-Krug et al., 1996; Walker et al., 2002, 2003; Korman et al., 2003, 2007; Censor and Cohen, 2011). Consolidation has been demonstrated for several memory domains including motor memories that are formed when a new task is practiced repetitively (Brashers-Krug et al., 1996; Walker et al., 2002, 2003). Hallmark features of this process

are an increase in motor performance (often estimated by a shift in the speed-accuracy function, i.e., movements are performed faster and more accurately after training) and a decrease in motor variability (Reis et al., 2009; Shmuelof et al., 2012). One motor task often used for studying motor memory consolidation is sequence tapping. A short sequence of 5–8 elements (each representing one tap with a specific finger) is usually performed either by typing the sequence on a keyboard (Walker et al., 2002, 2003) or as a finger-to-thumb opposition task (Karni et al., 1998). Practicing this task triggers a process in which multiple elements of the movement are integrated into one single behavior which is typically reflected by an increase in both tapping accuracy (i.e., producing the correct sequence) and in speed (Walker et al., 2002, 2003). Previous research investigating motor memory consolidation used this task and showed that practicing a novel sequence over twelve 30 s trials results in significant performance gains which reached plateau at the end of training (Walker et al., 2002, 2003). Further increases in performance can be observed once the memory is consolidated and large "offline gains" have been consistently observed after one night of sleep (i.e., performance increases significantly relative to the plateau performance reached at the end of training; Fischer et al., 2002; Walker et al., 2002, 2003, 2005; Stickgold, 2005).

Considerable evidence indicates that consolidation is a time-dependent process, with memories only susceptible to enhancement or disruption when specific interventions are provided shortly (i.e., within hours) after initial memory encoding, nevertheless not once this critical time-window has closed (Davis and Squire, 1984; Brashers-Krug et al., 1996; McGaugh, 2000; Walker et al., 2002, 2003). These findings have led to the long-held view that once a memory is truly consolidated it is rigid and can no longer be modulated. However, when performing introspective analyses of personal memories it becomes apparent that memories are often not constant or rigid in terms of strength or content (Lee, 2009). Experimentally it has been shown that a consolidated memory can be disrupted when an amnesic agent is presented shortly after memory retrieval. This effect was not observed when the administration of the amnesic agent was not preceded by retrieval, or when retrieval was not followed by the amnesic agent (Misanin et al., 1968). This finding suggested that memory retrieval renders a seemingly consolidated memory fragile again and is in need of re-stabilization, a process known as reconsolidation. Nader et al. (2000) provided the first conclusive evidence that memory erasure can be caused by interference during reconsolidation. Particularly, they showed in rodents that a conditioned fear memory can be blocked by injecting a protein synthesis inhibitor (a "consolidation blocker") immediately after reactivation. These findings caused a rapid increase in animal research investigating the process of memory *reconsolidation* in further detail (Nader and Einarsson, 2010; Besnard et al., 2012).

Reconsolidation has been investigated in several memory domains in humans (for review see Schiller and Phelps, 2011) including the motor memory domain (Walker et al., 2003; Censor et al., 2010, 2014; Hardwicke et al., 2016). To do so, most studies used a three-day design and applied an interference approach involving: (i) acquisition of a new motor task A on day 1; (ii) reactivation and experimental manipulation of motor task A on day 2; and finally; (iii) assessment of potential changes in memory strength of motor task A on day 3 (Walker et al., 2003; Censor et al., 2010, 2014; Hardwicke et al., 2016). A seminal study by Walker et al. (2003) used this three-day design and showed that when motor sequence A was learned on day 1 but then physically reactivated and subjected to experimental interference on day 2 (by practicing a new sequence B immediately afterwards), the accuracy of sequence A on day 3 decreased significantly relative to that on day 2, indicating true memory degradation. Importantly, no such memory degradation was observed when reactivation of sequence A was not followed by training of the interfering sequence B, or when sequence B was trained without prior reactivation of sequence A (Walker et al., 2003). However, later studies reported difficulties in replicating the finding that memory can be degraded during reconsolidation even when the identical task design and protocol were used as in Walker et al. (2003), de Beukelaar et al. (2014) and Hardwicke et al. (2016). Other studies used non-invasive brain stimulation to interfere with memory formation in primary motor cortex (M1; Censor et al., 2010, 2014) and found that applying repetitive transcranial magnetic stimulation (1 Hz rTMS) over M1 on day 2 immediately after reactivation of sequence A did not cause performance to drop on day 3. It did, however, block further gains in performance typically observed after a night of sleep between day 2 and day 3.

These divergent results reflect an ongoing scientific debate concerning the functional role of reconsolidation in the modification of stored memories and gave rise to two competing hypotheses (Lee, 2009): first, the "destabilization theory" posits that in order to modify a memory it needs to be destabilized so that new information can be added. Subsequently the modified memory is "re-stabilized" in order to generate an improved memory trace for future recall. Importantly, this hypothesis predicts that causing interference during the destabilization phase results in memory loss. This concept is consistent with most animal work (Besnard et al., 2012) and several human studies showing that interference after reactivation can lead to significant deterioration of task performance when probed during a retention test (Walker et al., 2003; Kindt et al., 2009; Chan and LaPaglia, 2013). The "updating theory" on the other hand, postulates that reactivating a stable memory may indeed open a time-window for memory modification, but importantly, there is no initial destabilization phase. Several human studies support this notion, showing that interference only blocks performance gains that one would normally observe when memory formation is uninterrupted, but that the interference could not induce performance decrements (Rodriguez-Ortiz and Bermúdez-Rattoni, 2007; Censor et al., 2010; Hardwicke et al., 2016).

When comparing divergent results between human and animal work, it should be noted that in humans, memory interference is mostly induced using methods which target the neural basis of the memory in an anatomically and

mechanistically unspecific manner, e.g., by acquiring a competing task (Walker et al., 2003; Forcato et al., 2007; Hupbach et al., 2007; Chan and LaPaglia, 2013; de Beukelaar et al., 2014; Hardwicke et al., 2016), by orally administered drugs like propranolol (Brunet et al., 2008; Kindt et al., 2009; Soeter and Kindt, 2011) or by applying invasive (Kroes et al., 2014) and non-invasive brain stimulation (Censor et al., 2010). In animal work on the other hand, methods are being used that directly target the molecular underpinnings of memory formation, e.g., by injecting consolidation inhibiting proteins directly into the brain areas responsible for memory formation (Nader et al., 2000). Other factors might also contribute to divergent results, such as subtle boundary conditions that may constrain the extent to which a memory can be experimentally interfered with upon reactivation. For example, in animal research it has been shown that specific determinants should be precisely controlled, such as the age of the memory (Milekic and Alberini, 2002; Suzuki et al., 2004), intensity of training (Eisenberg et al., 2003; Suzuki et al., 2004; Wang et al., 2009), reactivation length (Eisenberg et al., 2003; Pedreira and Maldonado, 2003; Suzuki et al., 2004), and novelty of information provided during the reactivation session (Pedreira et al., 2004; Morris et al., 2006; Díaz-Mataix et al., 2013). In humans, however, these boundary conditions are currently not well understood (Schiller and Phelps, 2011; Auber et al., 2013; Sevenster et al., 2013; Sandrini et al., 2015).

In a previous study, we showed that the length of reactivation on day 2 (i.e., actively performing sequence A) is a crucial boundary condition to effectively show a degradation of the motor memory after interfering with the induced reconsolidation process (de Beukelaar et al., 2014). A clear relationship between the length of reactivation and motor memory degradation was found, indicating that the longer the reactivation phase, the minimal the decline in performance due to interference when retested 24 h later. However, it remains unclear whether the re-stabilization observed during prolonged reactivation (i.e., tapping sequence A for more than 60 s) is triggered by continuous physical practice, or whether re-stabilization would also occur automatically with the passage of time after a short reactivation. Here we test the hypothesis that increasing the delay between a standardized short reactivation and an interfering intervention reduces memory degradation when tested the next day, suggesting that even though reconsolidation destabilizes the memory initially, this state is maintained only for a limited time-window.

MATERIALS AND METHODS

Subjects

Forty-eight right-handed subjects ($n = 12$ per group; 17 men; mean age 23.1; range 18–32 years) volunteered for this study. None were practiced musicians nor had extensive gaming experience, as assessed by a self-report questionnaire. All subjects were naïve to the purpose of the study and gave written informed consent prior to participation. Experimental procedures were approved by the local Ethics Committee for Biomedical Research at Katholieke Universiteit (KU) Leuven and conformed to the Declaration of Helsinki.

Subjects were instructed to sleep for a minimum of 6 h per night prior to and after the experimental sessions to avoid general fatigue and ensure overnight consolidation. Subjects were instructed not to take daytime naps or consume alcohol, and not to practice motor sequences in between sessions.

Motor Task

Subjects were comfortably seated in front of a laptop in a quiet room free of visual distractions. Motor memory formation was probed with a sequence tapping task, adapted from Karni et al. (1998), that has been used previously in motor reconsolidation research (Walker et al., 2003; Censor et al., 2010, 2014; de Beukelaar et al., 2014). Participants performed the sequence tapping task with their left (non-dominant) hand to reduce the likelihood of a ceiling effect during learning. Key presses were recorded by four neighboring keys labeled 1, 2, 3 and 4, which corresponded to the little, ring, middle and index finger, respectively (**Figure 1A**). Two different 5-element sequences (A: 4-1-3-2-4 and B: 2-3-1-4-2) were used interchangeably throughout the experiment; one being the learning sequence (*SeqLearn*) and the other the interfering sequence (*SeqInterf*). Sequences were randomized and counter-balanced across subjects.

Subjects initiated the behavioral task themselves by pressing the spacebar key on the laptop. The required sequence was then shown on top of the screen (each number represented a finger tap as specified above). While performing the task, each key press produced a black dot underneath the number indicating which finger should have been used. Note that this feedback indicated only that a key press was registered, but not whether the correct key had been selected (**Figure 1A**). Once a sequence was completed (i.e., when 5 keys were pressed irrespective of whether they were correct or not) the screen was refreshed so that all black dots were removed, while the sequence of numbers remained visible. An experimental trial consisted of 30 s sequence tapping followed by 30 s of rest to prevent fatigue. During the rest period the screen turned white. The trials following the rest period started automatically and subjects were continuously motivated throughout the experiment to type the sequences as quickly and accurately as possible.

Protocol

For each subject the experiment was conducted at the same time of the day on three consecutive days to account for possible circadian rhythm effects. During the first day of the experiment (*training session*) subjects practiced the sequence for 12 trials (SeqLearn). On the second day of the experiment (*reactivation session*) subjects reactivated SeqLearn by tapping the sequence a total of 10 times (irrespective of whether they were correct or incorrect) and were motivated to do this as quickly and as accurately as possible. Reactivation was followed by the acquisition of a new interfering sequence for 12 trials (SeqInterf). Subjects were instructed before reactivation that a new sequence had to be learned after reactivation, however, they did not know which sequence this would be. On the third and final day (*retention session*) subjects performed three SeqLearn

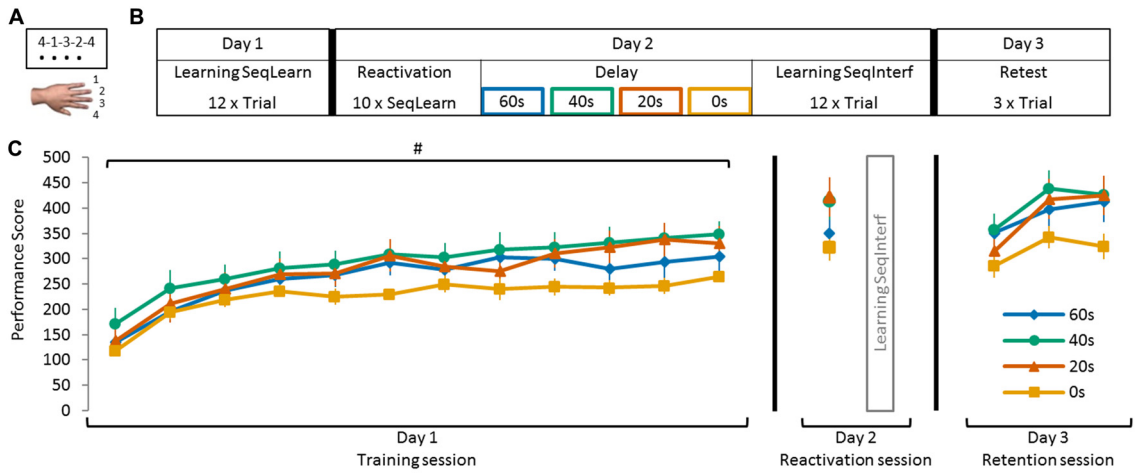

FIGURE 1 | Schematic representation of the sequence tapping task and experimental protocol. (A) The sequence tapping task was performed with the left non-dominant hand on a laptop keyboard. Two different 5-element sequences were used; a learning (SeqLearn) and an interference sequence (SeqInterf) respectively. An experimental trial consisted of 30 s of sequence tapping followed by a rest period of 30 s to prevent fatigue. Participants were instructed to type the sequences as quickly and as accurately as possible. **(B)** The experiment was conducted on three consecutive days. On day 1 (*training session*) subjects were trained on one sequence (SeqLearn) for 12 trials of 30 s. On day 2, the motor memory was reactivated by tapping SeqLearn 10 times (i.e., 50 key presses; irrespective of whether they were correct or not; *reactivation session*) and was followed by learning a new sequence (SeqInterf). Subjects were randomly assigned to groups with a different delay between the reactivation and the interference sequence, which were either 0 s, 20 s, 40 s or 60 s. On day 3 of the experiment (*retention session*), final performance levels on the SeqLearn (3 × 30 s) and SeqInterf (3 × 30 s) were measured. **(C)** Visualization of the performance data of all experimental groups (0 s, 20 s, 40 s, and 60 s) presented in the temporal order of the testing protocol. Performances are shown as collected in the separate sessions; on day 1 and day 3 subjects performed trials of 30 s (shown as $PerfScore_{all}$) while on day 2 they briefly tapped SeqLearn 10 times (shown as $PerfScore_{10}$). Significant main *trial* effect is indicated by $^{\#}(p < 0.001)$. Vertical bars indicate SEs.

trials, which were followed by three SeqInterf trials, to provide an indication of the final level of performance (**Figures 1B,C**).

Subjects were randomly allocated to one of the four experimental groups: in the first experimental group, reactivation was immediately followed by the acquisition of SeqInterf so that virtually no delay was present (0 s group). In the other experimental groups, the delay between reactivation and interference was 20 s, 40 s or 60 s (**Figures 1B,C**). Importantly, on day 2 all subjects received the identical instruction that they first had to tap SeqLearn, and subsequently learn a new sequence (SeqInterf) which would commence after the subject pressed the space bar. We did not inform the subject regarding the length of reactivation or the delay between reactivation and interference to minimize pacing strategies or other cognitive confounds. In the 0 s group the experimenter instructed subjects to press the space bar immediately after the 10 SeqLearn reactivation trials were completed. In the other groups, the delay (20 s, 40 s, and 60 s) was accurately timed by the experimenter and subjects were instructed on when to press the space bar. These delays were based on previous research showing that destabilization due to physical reactivation of SeqLearn only occurs for a duration <60 s of physical tapping (de Beukelaar et al., 2014). Accordingly, we chose 60 s as the maximum time interval in the present study even though we would expect analogous or even stronger re-stabilization effects for longer intervals. In summary, the experimental groups only differed with respect to the reactivation session on the second day. Specifically, the delay between reactivating SeqLearn and acquiring SeqInterf varied between 0 s and 60 s.

Data Analysis and Statistics

Subjects performed the sequence tapping task on a laptop where the key presses were registered by a custom data collection program (E-Prime Psychology Software Tools, Inc., Shapsburg, PA, USA). Performance measures consisted of both accuracy and speed. Accuracy was calculated as the percentage of correct sequences (i.e., sequences where all key presses corresponded to the temporal order of the elements) relative to the total number of sequences tapped per 30 s trial (i.e., number of sequences tapped within 30 s irrespective of whether the order was correct or incorrect). Speed was measured as the time between key presses (in s), i.e., the inter-tap interval (ITI). Based on the "speed-accuracy trade off", which indicates that for a given skill level accuracy is diminished when speed is increased, skill improvement is reflected by a shift in the speed-accuracy function (Reis et al., 2009; Shmuelof et al., 2012). de Beukelaar et al. (2014) reported a linear relationship between the accuracy percentage and ITI ($R = 0.94$). Therefore an overall *performance score* (PerfScore) was calculated for each subject and trial by dividing the percentage of accurately typed sequences by the ITI. A higher score indicates improved performance.

First we tested whether SeqLearn was acquired in a similar manner across groups on day 1. To do so, performance scores were calculated for the full 30 s tapping period ($PerfScore_{all}$) and an analysis of variance (ANOVA) model was conducted with the within subjects factor *trial* (1–12) and the between subjects factor *group* (0 s; 20 s; 40 s; and 60 s). Additionally we tested whether the initial $PerfScore_{all}$ measured during the first trial on

day 1 was similar across groups using an ANOVA with the factor *group* (0 s; 20 s; 40 s; and 60 s). Furthermore, we tested whether a plateau was reached at the end of training using an ANOVA with the factors *group* and *trial* (10–12).

Next we tested overnight performance changes of SeqLearn from day 1 to day 2 and from day 2 to day 3 to investigate consolidation and reconsolidation processes, respectively. Since reactivation on day 2 required subjects to tap only 10 sequences we calculated the performance score only for the first 10 sequences tapped within a given 30 s trial ($PerfScore_{10}$), thus increasing consistency of data analyses across the 3 days and minimizing confounds caused e.g., by fatigue (Brawn et al., 2010).

We first investigate performance changes due to offline consolidation between the end of training on day 1 and the reactivation on day 2. We calculated the *baseline* performance on day 1 as the mean $PerfScore_{10}$ of trials 10–12 (note that the last 3 trials were chosen to have a more reliable estimate of the baseline performance on day 1). We then tested offline learning from day 1 to day 2 in all four experimental groups with an ANOVA analysis including the within subjects factor *day* (day 1, day 2) and the between subjects factor *group* (0 s; 20 s; 40 s; and 60 s).

The reconsolidation effect was central to our research question and we performed an ANOVA analysis to specifically test whether the duration of the delay between reactivation and interference on day 2 has an influence on the extent of motor memory degradation on day 3. To do so, we conducted a repeated measures ANOVA on $PerfScore_{10}$ for the within subjects factor *day* (day 2, day 3) and the between subjects factor *group* (0 s; 20 s; 40 s; and 60 s). Note that we considered only the first trial on day 3 because our previous study has indicated that memory degradation due to reconsolidation can only be temporarily observed and is quickly compensated when additional training is provided (de Beukelaar et al., 2014). To visualize performance changes between two consecutive days, a ratio was calculated by dividing the $PerfScore_{10}$ of the latter by the former (i.e., D2/D1 and D3/D2). A ratio <1 indicates memory loss while a ratio >1 indicates further memory improvement overnight, i.e., offline gains (see **Figure 2B**).

One general concern is that individual differences in offline gains measured from day 1 to day 2 (note that all subjects have followed the same protocol up to this point) might have influenced performance changes measured from day 2 to day 3. In other words, larger offline gains from day 1 to day 2 might be followed by smaller gains from day 2 to day 3 consistent with the observation that learning curves follow a power-law. To consider this potential confound in our analysis, we first submitted the D3/D2 ratios and the D2/D1 ratios of each individual to a Pearson correlation analysis and estimated the strength of this potential association. Then, we performed a control analysis to ensure that the D3/D2 ratios differed across groups even if individual differences in D2/D1 offline gains are considered. To do so we submitted D3/D2 ratios to a general linear model with the between subject factor *group* (0 s; 20 s; 40 s; and 60 s) and included the D2/D1 ratios as a covariate of no interest. Based on our previous study we test the *a priori* hypothesis that there is a linear relationship between the different delay durations and performance changes from day 2 to day 3, more specifically shorter delays cause stronger memory degradation (i.e., no performance gains) than do longer delays between reactivation and interference. We tested the hypothesis directly via a preplanned comparison using the following contrast vector $[-3, -1, 1, 3]$ for the 0 s, 20 s, 40 s and 60 s groups, thus modeling that performance gains at D3 compared to D2 increase linearly with the

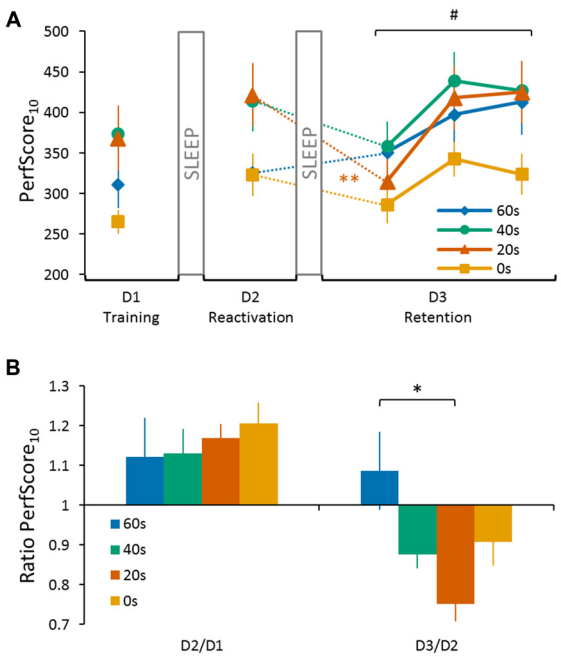

FIGURE 2 | Performance of the four experimental groups (0 s, 20 s, 40 s and 60 s) on the sequence tapping task over three consecutive days. (A) $PerfScore_{10}$ on day 1 (D1 Training) represents the average performance score (accuracy (%)/inter-tap interval (ITI; s)) of the first 10 tapped sequences (i.e., SeqLearn, irrespective of whether they were correct or incorrect) of the last three training trials. $PerfScore_{10}$ on day 2 (D2 Reactivation) represents the reactivation performance obtained from tapping SeqLearn 10 times. $PerfScore_{10}$ data on the third and final day (D3 Retention) represent the first 10 complete SeqLearn sequences of each trial. We found a significant *day* × *group* interaction ($F_{(3,44)} = 5.28$, $p < 0.01$) and a Tukey HSD *post hoc* analysis showed a significant drop in performance from day 2 to day 3 for the 20 s group ($p < 0.01$). The performance was only degraded during the first trial on day 3 and increased quickly during the subsequent two tapping trials (main *trial* effect $F_{(2,88)} = 28.31$, $p < 0.001$). **(B)** Performance ratios visualizing changes in performance between consecutive days. Performance ratios from day 1 to day 2 represent the change in the reactivation performance on day 2 (10 × SeqLearn) relative to the baseline performance level on day 1 (average of the first 10 tapped sequences of the last 3 training trials; D2/D1). Performance ratios from day 2 to day 3 represent the change in performance on day 3 (first 10 × SeqLearn) relative to reactivation performance on day 2 (10 × SeqLearn; D3/D2). A ratio <1 indicates memory loss while a ratio >1 indicates further memory improvement overnight, i.e., offline gains. We found a significant *group* main effect for the D3/D2 ratio (preplanned comparison with D2/D1 as a covariate of no interest; $F_{(3,40)} = 3.71$, $p = 0.03$, one-sided). A Tukey HSD *post hoc* analysis revealed a significant difference in performance from day 2 to day 3 for the 20 s group compared to the 60 s group ($p < 0.01$). Significant main *trial* effect is indicated by $^{\#}(p < 0.001)$. Significant Tukey HSD *post hoc* is indicated for the main *group* effect by $^{*}(p < 0.01)$, and the *day* × *group* interaction by $^{**}(p < 0.01)$. Vertical bars indicate SEs.

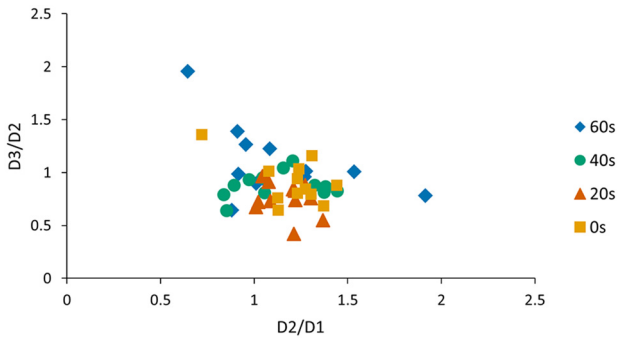

FIGURE 3 | Visualization of the correlation between D2/D1 and D3/D2 ratios. We found a significant negative association between the D2/D1 ratio and the D3/D2 ratio when pooled across groups ($r = -0.37$, $p < 0.01$). As a consequence, the D2/D1 ratio was included as a covariate of no interest when analyzing the D3/D2 ratio.

length of the delay (since we have strong prior evidence to expect a linear *increase* we report one-sided statistics for this comparison).

Finally, we tested whether the different delays between reactivating *SeqLearn* and acquiring the interfering sequence *SeqInterf* influenced PerfScore_{10} of the 3 tapping trials on day 3. An ANOVA model was conducted on the day 3 retention data with the between subjects factor *group* (0 s; 20 s; 40 s; and 60 s) and the within subjects factor *trial* (1–3).

Analogous analyses were performed for SeqInterf (see **Figure 5**).

The alpha level for all statistical tests was set to 0.05. *Post hoc* comparisons were performed with Tukey's HSD test.

RESULTS

Four experimental groups of subjects practiced the sequence tapping task and initial performance (i.e., performance on the first 30 s trail on day 1) did not differ between the groups (no main *group* effect $F_{(3,44)} = 1.11$, $p = 0.35$). Furthermore, all experimental groups significantly improved $\text{PerfScore}_{\text{all}}$ for SeqLearn over the course of training on day 1 (*trial* main effect $F_{(11,484)} = 68.91$, $p < 0.001$) and all groups exhibited similar learning gains (no *group* main effect $F_{(3,44)} = 1.51$, $p = 0.26$; no *trial* × *group* interaction $F_{(33,484)} = 1.30$, $p = 0.13$). The performance improvements leveled off at the end of day 1 such that $\text{PerfScore}_{\text{all}}$ changed only minimally across the last 3 trials (<10% of the overall learning gains) even though statistics revealed a trend towards a significant trial main effect ($F_{(2,88)} = 2.86$, $p = 0.06$). There was no significant *trial* × *group* interaction ($F_{(6,88)} = 0.31$, $p = 0.93$) nor main group effect ($F_{(3,44)} = 2.43$, $p = 0.08$) indicating that the plateau effect was not significantly different across groups (**Figure 1C**).

Successful consolidation was tested by reactivating the motor memory on day 2 (tapping SeqLearn 10 times). This reactivation revealed further overnight changes when quantified via PerfScore_{10} which ranged between +12.1% ± 3.4 and +20.6% ± 1.8 (so called "offline gains"; main day effect

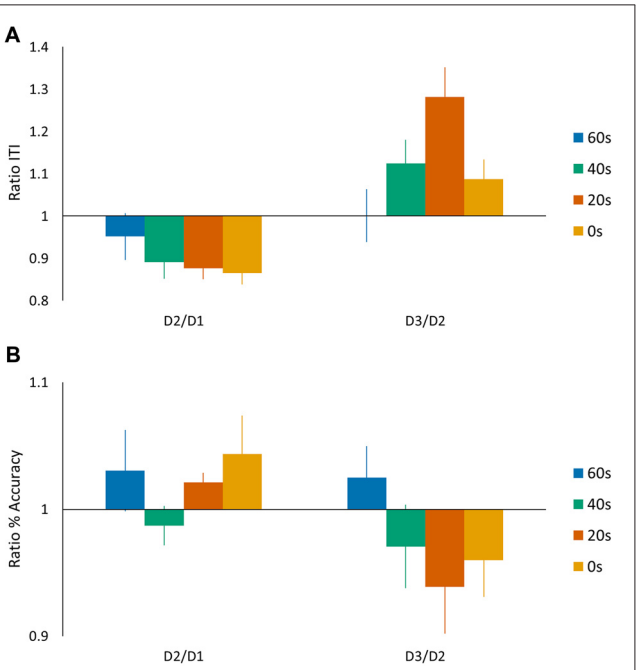

FIGURE 4 | Task performance represented by (A) speed (ITI (s)) and (B) accuracy (%) measures. The speed and accuracy data are presented in a similar manner as shown in **Figure 2B** for the performance scores. Vertical bars indicate SEs.

$F_{(1,44)} = 17.55$, $p < 0.001$; **Figures 2A,B**). Even though PerfScore_{10} differed across groups (indicating that some subjects were better tappers than others, main *group* effect $F_{(3,44)} = 3.06$, $p < 0.05$), there was no significant *day* × *group* interaction ($F_{(3,44)} = 0.95$, $p = 0.42$) indicating that offline gains did not significantly differ across groups.

Central to our research question, we next tested whether the duration of the delay between reactivating SeqLearn and acquiring SeqInterf on day 2 had a significant influence on retention performance on day 3 (**Figures 2A,B**). We found a significant main *day* effect ($F_{(1,44)} = 13.51$, $p < 0.001$) and the subsequent *post hoc* analysis showed an overall decrease in performance from day 2 to day 3 (Tukey HSD *post hoc*, $p < 0.001$). We did not find a main *group* effect ($F_{(3,44)} = 1.56$, $p = 0.21$) while, most interestingly, we found a significant *day* × *group* interaction ($F_{(3,44)} = 5.28$, $p < 0.01$). A Tukey HSD *post hoc* analysis showed a significant drop in performance from day 2 to day 3 for the 20 s group ($p < 0.01$).

One concern is that offline gains from day 1 to day 2 and performance changes observed from day 2 to day 3 are related. Therefore, we conducted an additional Pearson correlation analysis and found a significant negative association between the D2/D1 ratio and the D3/D2 ratio when pooled across groups ($r = -0.37$, $p < 0.01$; **Figure 3**) indicating that subjects who exhibited large offline gains from day 1 to day 2 tended to exhibit large losses in performance from day 2 to day 3. Since this association might have influenced our previous reconsolidation results we performed an additional control analysis and tested whether D3/D2 performance ratios differed across groups, even

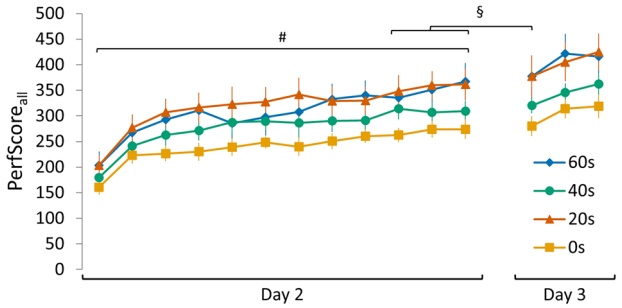

FIGURE 5 | Visualization of the SeqInterf PerfScore$_{all}$ data of all experimental groups (0 s, 20 s, 40 s and 60 s). A set of control analyses conducted on SeqInterf showed that: (i) initial PerfScore$_{all}$ (i.e., performance on the first 30 s trial on day 2) did not differ between groups (no *group* main effect $F_{(3,44)} = 1.092$, $p = 0.36$); (ii) a clear increase in PerfScore$_{all}$ over the course of training was evident (*trial* main effect $F_{(11,484)} = 50.83$, $p < 0.001$; no *group* main effect $F_{(3,44)} = 2.32$, $p = 0.09$; no *trial* × *group* interaction $F_{(33,484)} = 0.94$ $p = 0.57$); (iii) plateau PerfScore$_{all}$ was not significantly different between groups (no *group* main effect $F_{(3,44)} = 2.45$, $p = 0.08$; no *trial* main effect $F_{(2,88)} = 2.90$, $p = 0.06$; no *trial* × *group* interaction $F_{(6,88)} = 1.04$, $p = 0.41$); (iv) and similar over-night improvements in performance were seen comparing the averaged PerfScore$_{all}$ of the final three trials on day 2 with the retention PerfScore$_{all}$ obtained during the first trial on day 3 for each group (main *day* effect $F_{(1,44)} = 4.13$, $p < 0.05$; no main *group* effect $F_{(3,44)} = 2.52$, $p = 0.07$; no *day* × *group* interaction $F_{(3,44)} = 0.22$, $p = 0.88$). Significant main *trial* effect is indicated by $^{\#}(p < 0.001)$. Significant main *day* effect is indicated by $^{\S}(p < 0.05)$. Vertical bars indicate SEs.

if the individual offline gain (i.e., D2/D1 ratio) was added as a covariate of no interest. Our model revealed a significant group *effect* (preplanned comparison $F_{(3,40)} = 3.71$, $p = 0.03$, one-sided) and a Tukey HSD *post hoc* analysis revealed a significant difference in the D3/D2 ratio for the 20 s group compared to the 60 s group ($p < 0.01$). This finding indicates that the delay significantly influenced memory deterioration due to reconsolidation, an effect that was found over and above individual difference in offline gains exhibited from D2 to D1.

Note, however, that performance was only degraded during the first trial on day 3 but increased quickly during the subsequent two tapping trials (**Figure 2A**, main *trial* effect $F_{(2,88)} = 28.31$, $p < 0.001$). This performance increase was not significantly different across groups (no *group* main effect $F_{(3,44)} = 1.66$, $p = 0.19$; no *trial* × *group* interaction $F_{(6,88)} = 1.29$, $p = 0.27$).

In the above analyses we quantified tapping performance via a performance score based on a linear speed-accuracy function (de Beukelaar et al., 2014), whereas previous motor reconsolidation studies reported speed and accuracy measurements separately (Walker et al., 2003). We therefore repeated our main ANOVA analyses separately for the speed (ITI) and accuracy (%) measures (**Figures 4A,B**). For the speed measurement, we found a significant main *day* effect ($F_{(1,44)} = 12.98$, $p < 0.001$) and the subsequent *post hoc* analysis showed an overall decrease in performance from day 2 to day 3 (Tukey HSD *post hoc*, $p < 0.001$). We did not find a main *group* effect ($F_{(3,44)} = 1.42$, $p = 0.25$), while, most interestingly, we found a significant *day* × *group* interaction ($F_{(3,44)} = 3.09$, $p < 0.05$). A Tukey HSD *post hoc* analysis showed a significant drop in performance from day 2 to day 3 for the 20 s group ($p < 0.01$). For the accuracy measurement, we did not find any significant main effects (no main *day* effect $F_{(1,44)} = 3.58$, $p = 0.07$; no main *group* effect $F_{(3,44)} = 0.50$, $p = 0.68$) nor a *day* × *group* interaction ($F_{(3,44)} = 1.48$, $p = 0.23$). We also calculated the D2/D1 and D3/D2 ratios for both speed (ITI) and accuracy (%) measures. We submitted the D3/D2 ratios to an ANOVA analysis and included the D2/D1 ratio as a covariate of no interest. For these measures separately, we did not find main *group* effects (preplanned comparison ANOVA, Accuracy: no main *group* effect $F_{(3,44)} = 2.63$, $p = 0.11$; Speed: no main *group* effect $F_{(3,44)} = 0.97$, $p = 0.33$). These findings suggest that the differences in performance score observed on day 3 were mainly driven by changes in performance speed (longer ITI) since changes in accuracy were generally minimal (on average around 5%).

DISCUSSION

Here we explored the temporal dynamics of memory re-stabilization after reactivation, which represents an important experimental boundary condition for inducing motor memory degradation during reconsolidation. To do so, motor memory traces of a sequence tapping task were investigated using a well-established three-day design: on day 1 a novel motor memory was encoded, on day 2 the motor memory of the acquired sequence was reactivated and experimentally manipulated by learning an interfering sequence, and on day 3 the motor memory strength was retested (Walker et al., 2003; Censor et al., 2010; de Beukelaar et al., 2014; Hardwicke et al., 2016). We varied the duration of the delay between the brief reactivation of the previously acquired sequence and the interfering sequence in four experimental groups, so that in one group, the delay was 60 s, in the second group 40 s, in the third group 20 s and in the fourth group it was 0 s. Our results indicate that the duration between reactivation and interference critically influences the motor memory degradation process. These findings indicate that memory re-stabilization after reactivation is a dynamic process and that besides the length of reactivation also the delay between reactivation and interference constitutes a crucial boundary condition to test motor memory reconsolidation.

Subtle boundary conditions constrain whether a memory can be experimentally interfered with upon reactivation or not (Rodriguez-Ortiz and Bermúdez-Rattoni, 2007). While specific determinants of reconsolidation (e.g., age of the memory, intensity of training, reactivation length, and novelty of information provided during the reactivation session) have been identified in animal models (Milekic and Alberini, 2002; Eisenberg et al., 2003; Pedreira and Maldonado, 2003; Pedreira et al., 2004; Suzuki et al., 2004; Morris et al., 2006; Bustos et al., 2009; Wang et al., 2009; Auber et al., 2013; Díaz-Mataix et al., 2013), these remain less understood in humans (Schiller and Phelps, 2011; Auber et al., 2013; Sevenster et al., 2013). A previous study from our laboratory recently showed that the length of memory reactivation is a critical parameter

when interfering with human motor memory reconsolidation (de Beukelaar et al., 2014). In particular, a short reactivation (less than 60 s) renders the memory labile and susceptible to degradation through interference, while a longer reactivation does not. Moreover, the results showed a relationship between the length of reactivation and motor memory degradation: the longer the reactivation phase, lower the decline in performance due to interference when retested 24 h later.

In the present study, subjects reactivated the motor memory by tapping 10 repetitions of the previously acquired sequence (lasting on an average for 14.0 s ± 2.3). Our previous study showed that this brief period of motor reactivation rendered the motor memory most susceptible to degradation due to interference (de Beukelaar et al., 2014). Here we replicated these previous findings by showing that a brief reactivation followed by an interfering task degrades motor memories when retested 24 h later. In this study, we further explored the influence of the duration (or rest period) between reactivation and interference in four experimental groups with delay durations of 0 s, 20 s, 40 s or 60 s. Interestingly, the duration of this rest period directly influenced the extent to which the memory could be degraded. This was indicated by two main findings: first, delays between 0 and 40 s resulted in average memory degradation, while a delay of 60 s resulted in memory conservation and even caused an average performance gain. When directly compared by an ANOVA we found a significant *day* × *group* effect which was driven by differences between the 20 s and 60 s group (significant *post hoc* test). We further showed that only the 20 s group exhibited a significant performance decrease from day 2 to day 3 while the performance decrease of other groups (0 s and 40 s) as well as the increase of the 60 s group did not reach significance. However, one has to note that the reactivation period was rather short (10 sequences = 50 finger taps) most likely resulting in only potentially small offline gains from day 2 to day 3. Thus, in summary, our statistical analysis revealed clear group differences whereby the time delay between reactivation and interference was the only experimental parameter that was varied. Second, we performed an additional control analysis and tested a statistical model that assumed a linear effect of the different delays (0 s, 20 s, 40 s and 60 s) on the performance changes from day 2 to day 3. This model was hypothesized *a priori* based on a separate study that used the same overall paradigm but manipulated the length of reactivation (de Beukelaar et al., 2014) rather than the delay between reactivation and interference. This linear model revealed a significant effect consistent with the interpretation that increasing delays caused a gradual change from performance degradation to performance conservation across groups. Together with the results of our previous study (de Beukelaar et al., 2014), these findings suggest that memory modification is regulated by two time-dependent processes: first, the memory is destabilized due to a brief reactivation (note that our results tentatively suggest that destabilization might have been more complete in the 20 s than in the 0 s group) which is then followed by re-stabilization requiring that sufficient time has passed before subjects are exposed to an interfering intervention. This effect is observed irrespective of whether subjects rest or practice the previously learned sequence during this "re-stabilization period".

In accordance with de Beukelaar et al. (2014), performance of the sequence tapping task was quantified by calculating a linear speed-accuracy function; i.e., performance score. Since previous reconsolidation research using similar sequence tapping tasks often analyzed speed and accuracy measures separately (Walker et al., 2003), we also explored these measures in the current study. Taken together, our results indicate that interference was manifested as reduced speed (longer ITI), and to a lesser extent, reduced accuracy. These results are in line with previous findings since both parameters independently suggest that the current reconsolidation paradigm leads to degradation of the motor memory, however, specific parameters such as the length of the rest period influence the extent of degradation.

Overall, the results of the present study in combination with our previous work (de Beukelaar et al., 2014) support the *destabilization* theory, which states that that the reactivation of an existing memory leads to instability such that subsequent interference can induce memory loss or degradation (Nader et al., 2000; Walker et al., 2003; Kindt et al., 2009; Chan and LaPaglia, 2013). In both studies we showed that a short reactivation of an existing memory leads to instability of the memory and that interference early after reactivation (i.e., around 20 s) can induce degradation of the memory. When reactivation itself is prolonged by further practice or when the interfering intervention is presented outside the preferred time-window of destabilization (i.e., 14.0 s ± 2.3 tapping + 20 s rest), we show that limited or no degradation of the memory occurs. The most robust effect was found for the paradigms where short reactivations (≤30 s) were followed by interference after 20 s. It is worthwhile noting, however, that exact estimations of reactivation length or rest period are specific for the paradigm used in our studies, thus, the critical time-window for causing memory degradation via an inference approach is likely to differ across tasks and memory domains.

Although robust effects were found, we could not establish effective memory "deletion" without an additional fast recovery of performance when executed on day 3. Currently, it is not known whether interfering with reconsolidation causes a retrieval failure (retrieval theory) or an actual fractional erasure of the memory (storage theory; Tronson and Taylor, 2007). Importantly, previous motor reconsolidation research in animals (Peng and Li, 2009) and humans (Censor et al., 2010, 2014) indicate that interference only degrades but not effectively erases the formed motor memory. In human fear memory systems, however, a persistent erasure over a year has been established without relapse (Schiller et al., 2010; Björkstrand et al., 2015). It will be interesting for future researchers to investigate whether different protocols can potentially induce a more robust long-term drop in motor performance, for example, by more extensive interference learning, by repeating reactivation-interference sessions, or by applying other forms of interference (e.g., contextual interference).

To conclude, our data provide evidence that re-stabilizing motor sequence memories during reconsolidation does not necessarily require long periods of reactivation in order to

be resistant to memory degradation, but that the availability of a specified rest period between a short reactivation and interference is sufficient. The effect of interference, shown as a drop in performance when retested 24 h later, was only short-lived which implies that reconsolidation interference results in subtle behavioral changes and requires a well-controlled experimental protocol taking into account all possible boundary conditions. Future studies should aim for a better understanding of the underlying memory dynamics of reconsolidation so that its potential as a therapeutic target in patients with memory disorders can be optimized.

AUTHOR CONTRIBUTIONS

TTdB designed the study; collected, analyzed and interpreted the data; drafted and revised the manuscript; gave final approval. DGW and NW designed the study; interpreted the data; revised the manuscript; gave final approval. KA and SPS interpreted the data; revised the manuscript; gave final approval.

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Sensorimotor Memory Biases Weight Perception During Object Lifting

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When lifting an object, the brain uses visual cues and an internal object representation to predict its weight and scale fingertip forces accordingly. Once available, tactile information is rapidly integrated to update the weight prediction and refine the internal object representation. If visual cues cannot be used to predict weight, force planning relies on implicit knowledge acquired from recent lifting experience, termed sensorimotor memory. Here, we investigated whether perception of weight is similarly biased according to previous lifting experience and how this is related to force scaling. Participants grasped and lifted series of light or heavy objects in a semi-randomized order and estimated their weights. As expected, we found that forces were scaled based on previous lifts (sensorimotor memory) and these effects increased depending on the length of recent lifting experience. Importantly, perceptual weight estimates were also influenced by the preceding lift, resulting in lower estimations after a heavy lift compared to a light one. In addition, weight estimations were negatively correlated with the magnitude of planned force parameters. This perceptual bias was only found if the current lift was light, but not heavy since the magnitude of sensorimotor memory effects had, according to Weber's law, relatively less impact on heavy compared to light objects. A control experiment tested the importance of active lifting in mediating these perceptual changes and showed that when weights are passively applied on the hand, no effect of previous sensory experience is found on perception. These results highlight how fast learning of novel object lifting dynamics can shape weight perception and demonstrate a tight link between action planning and perception control. If predictive force scaling and actual object weight do not match, the online motor corrections, rapidly implemented to downscale forces, will also downscale weight estimation in a proportional manner.

Keywords: grasping, lifting, sensorimotor memory, weight perception, motor control

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INTRODUCTION

Perceiving and handling objects are inherently linked. To grasp, move or use an object accurately, its sensed physical properties must rapidly be integrated into the motor plan. For instance, while the grasp aperture is proportional to the size of the object (Jeannerod et al., 1995; Castiello, 2005), fingertip forces that are used to lift it are scaled according to the expected weight and frictional properties of the object in order to ensure a stable grasp and avoid slips (Johansson and Westling, 1984). Fingertip force planning based on an expectation of the object weight is crucial, as feedback mechanisms are generally too slow and will result in a

less smooth lift (Johansson and Westling, 1984, 1988). Previous lift experience with the object is used to build an internal model that can be used to predict the object weight and thus scale fingertip forces accordingly. In the absence of cues allowing weight prediction, it has been shown that force scaling is influenced by the object weight or frictional properties in the preceding lifts (Johansson and Westling, 1984, 1988). This effect of lift history on force scaling has been termed sensorimotor memory, can be found on a trial-by-trial basis (Johansson and Westling, 1988; Chouinard et al., 2005) and is also reflected in the corticospinal excitability (Loh et al., 2010). Precise force scaling is classically assessed by quantifying the force rate increase just after object contact and before lift-off (Loh et al., 2010; Baugh et al., 2012). For example, if a heavy object is lifted in the previous trial, force rates in the current lift will be larger compared to when a light object is previously lifted.

In return, acting upon an object provides additional sensory inputs that enhance perceptual information about its physical properties. Here, we refer to "perception" as explicit knowledge about an object property. Knowledge about the material, weight or inertia of an object can be acquired by touching and lifting it. Perception of weight has been studied extensively in psychophysical studies (Jones, 1986). Discrimination abilities follow Weber's law, that is, just noticeable differences depend on the intensity of the stimulus. Weight perception is, however, not always veridical, as shown by several weight illusions (Buckingham, 2014) of which the size-weight illusion (Charpentier, 1891) is the most notable and investigated one.

When objects vary in size, a weight expectation based on the size can be made if the object density is constant. In the size-weight illusion, smaller objects are perceived to be heavier than larger ones even though they actually weigh the same (Charpentier, 1891). In the first trials, fingertip forces are scaled to the "expected" object weight, based on the size (i.e., larger force scaling for the large object). The mere expectation based on object size is enough to create the illusion (Buckingham and Goodale, 2010). However, after a few trials, forces are accurately scaled to the actual object weight (i.e., equal force scaling for both objects), whereas the perceptual illusion remains (Flanagan and Beltzner, 2000; Grandy and Westwood, 2006). Flanagan and Beltzner (2000) argued that the grasping parameters are determined by sensorimotor memory. This separate adjustment of fingertip force scaling and illusionary perception suggests a dissociation between the control of action and perception. In the visual system, the dual-stream theory assumes a different neural processing of visual sensory input for action ("where/how") and perceptual related tasks ("what") in the dorsal and ventral stream, respectively (Goodale and Milner, 1992). Such a separation between brain areas for the processing of spatial and identity information has also been found in other modalities (Romanski et al., 1999; Reed et al., 2005; Dijkerman and de Haan, 2007).

Thus, if the control of action and perception is strictly separated, perceptual estimates should not be influenced by how an object is lifted. Previous research indicates that this is not true. For instance, if less grip force (GF) is needed to grasp an object due to a higher friction (Flanagan et al., 1995), or because more fingers or a higher contact area can be used (Flanagan and Bandomir, 2000) the object is perceived as lighter. Moreover, if a higher grip force (resulting in a larger safety margin) is consciously used to lift an object, the rating performance to differentiate between weights decreases compared to when a normal grip is used (Ellis and Lederman, 1999). It is noteworthy that these studies investigated whether an altered grip force throughout the lifting movement, i.e., during both the dynamic and static phases, can affect weight perception. Hence, it is still unclear whether changes in force scaling that only occur within the initial dynamic phase would bias perception. Here, we took advantage of sensorimotor memory effects in order to manipulate experimentally the force scaling. Sensorimotor memory gives rise to an implicit "expectation" about the upcoming weight and only influences the dynamic phase of the lifting motion. Since we know this specific phase reflects motor planning based on the expected weight, any effects of force scaling on weight perception would demonstrate a tight link between the planning of actions and perception, two systems that were long thought to be independent.

To address the influence of sensorimotor memory on weight perception, we compared lifts preceded by light or heavy objects and quantified force scaling and object weight rating for each trial (Experiment 1a). We hypothesized participants would assign different weight estimates for lifts of a given object that was preceded by a light compared to a heavy object. In a follow-up experiment (Experiment 1b), we experimentally increased the magnitude of sensorimotor memory effects by lengthening the same weight trial history. Here, we hypothesized that larger effects on force scaling would in turn lead to larger perceptual weight biases. In order to examine the effect of trial history on weight estimates in the absence of sensorimotor memory, we performed a passive weight perception task (Experiment 2) where forces were applied on the participants' resting hand. In this task, we expected no perceptual biases.

MATERIALS AND METHODS

Participants

A total of 28 healthy participants took part in the study. Ten participants took part in Experiment 1a with a mean age of 30.4 years (age range of 21–41 years, 6 females, all right-handed). Another 10 different subjects participated in Experiment 1b, with a mean age of 22.4 years (age range 19–27 years, 4 females, all right-handed). Finally, in Experiment 2, eight other participants took part with a mean age of 29.9 years (range 23–34 years, 4 females, 6 right-handed). Before the start of the experiments, they all provided informed consent. Experiments were performed in accordance with principles as stated in the declaration of Helsinki and were approved by the local ethical committee of the Faculty of Biomedical Sciences, Katholieke Universiteit Leuven.

Apparatus

A grip-lift manipulandum consisting of two 3D force-torque sensors (Nano17, ATI Industrial Automation, Apex, NC, USA) was attached to a custom-made carbon fiber basket in which different objects (cubes) could be placed (**Figure 1**, left). The weight of the basket underneath the manipulandum was perfectly balanced, using a slider. The graspable surface (17 mm diameter and 45 mm apart) of the force sensors was covered with fine sandpaper (P600) to increase friction. The forces in three directions were sampled at 1000 Hz. The objects were 3D-printed cubes of 5 × 5 × 5 cm, filled with different amounts of lead particles to create weights of 100, 300 and 500 g. Note that the loads the participants lifted also included the combined weight of the manipulandum and basket, which had a total weight of 120 g. To prevent visual cues, the cubes were hidden under a paper cover. The manipulandum was placed behind a transparent switchable screen (Magic Glass), which was either opaque or transparent.

The experimental set-up used in Experiment 2 is pictured in **Figure 1** (right). A Geomagic Touch X Haptic Device (3D systems, Rock Hill, SC, USA) was used to exert a normal force (∼1, 3 or 5 N) onto the palm of the participants' right hand. The end of the device arm was fixed to a plastic plate with a size of about 5 × 5 cm. The forces were applied instantly on the hand.

Experiment 1: Grip-Lift Task and Procedure

In the first experiment, participants were instructed to grasp and lift the manipulandum with the thumb and index finger placed on each force sensor. They had to lift it at a comfortable pace up to a height of 2 cm, hold it steady for a few seconds and then release it back on the table. The trial started when the switchable screen turned transparent, accompanied by a beep indicating participants could initiate the grasp. The screen remained transparent for 3 s and then turned opaque again indicating the object had to be replaced on the table for the next trial. Weight perception judgments were acquired using the method of magnitude estimation (Zwislocki and Goodman, 1980): just after object release, participants were asked to assign a number best representing the perceived weight, based on an arbitrary numerical scale (with no explicit upper or lower limit).

The objects were presented in a semi-randomized order. In Experiment 1a, sensorimotor memory was probed using the four possible two-trial sequences, namely a current *light* lift preceded by a light object (L*L*) or heavy (H*L*), or a current *heavy* lift preceded a light object (L*H*) or heavy (H*H*). These four conditions were presented 10 times each. In Experiment 1b, we sought to investigate the effect of a longer trial history. Block length was increased so as to include 10 times the following three-trial sequences: light-light-*light* (LL*L*), heavy-heavy-*light* (HH*L*), light-light-*heavy* (LL*H*) and heavy-heavy-*heavy* (HH*H*). The light object was 100 g, the heavy object 500 g. An intermediate object of 300 g was presented five times in Experiment 1a and 10 times in Experiment 1b as a dummy trial to make object weight presentation less repetitive (about 10% of the trials). The total lifted weight also included the mass of the manipulandum (120 g). The total number of trials was 51 and 100 in Experiments 1a and 1b, respectively. This number consists of 40 analyzed trials (four conditions repeated 10 times) and unanalyzed trials (dummy trials, trials directly after dummy trials and trials that only served as preceding lifts in longer sequences). To minimize the total trial number, a single trial could sometimes serve as a preceding lift as well as an analyzed lift. A trial lasted 3 s and participants had full view of the object during this time (i.e., the screen was transparent). Before the experiment, participants performed practice trials with an object of 200 g.

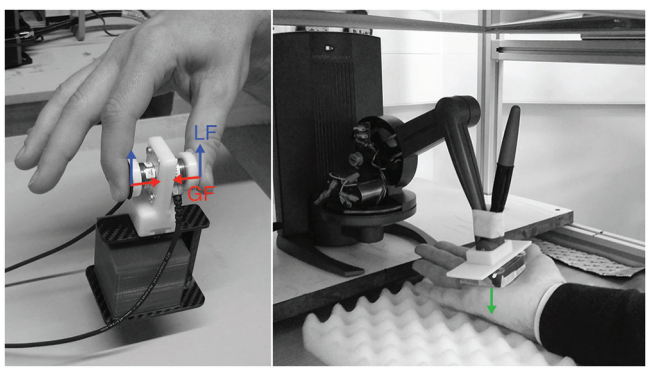

FIGURE 1 | Experimental set-up in Experiment 1 (left) and Experiment 2 (right). In Experiment 1, subjects had to grasp and lift a manipulandum with force sensors measuring the grip (GF, red) and load forces (LF, blue). A small carbon fiber basket was attached underneath the manipulandum to allow placement of different weights (100, 300 or 500 g). In Experiment 2, a force feedback robot (Geomagic Touch X) was used to apply forces (1, 3 or 5 N; green arrow) passively on the subjects' right hand.

Experiment 2: Passive Estimation Task and Procedure

In Experiment 2, a control experiment was performed where participants performed weight estimations, but without actively lifting the object. The goal of this experiment was to investigate the presence of a perceptual history effect in the absence of active force control. Participants were instructed to rest their right hand flat on the table, with the palm up. A haptic device exerted a normal force onto their hand palm for 3 s. They were told not to move their hand during the trial. Participants were asked to estimate the magnitude of the object weight on an arbitrary numerical scale, as in Experiment 1. The weight presentation sequence was the same as in Experiment 1a, where the four possible sequences of 2 weights (L*L*, H*L*, L*H* and H*H*) were presented 10 times each in a semi-randomized order. Forces of 1 N were used for light objects, 5 N for heavy objects and 3 N for dummy trials (10%). The start of force application was indicated by an auditory beep.

Force and Perceptual Parameters

In Experiments 1 and 2, participants' weight ratings were normalized by dividing each trial answer by the average of all perceptual estimates for each participant. Force parameters and perceptual ratings were averaged over trials in the four conditions: LL, HL, LH and HH (Experiments 1a, 2) and LLL, HHL, LLH and HHH (Experiment 1b). Dummy trials or trials that followed dummy trials were not analyzed. Three (0.75%), two (0.5%) and five (1.6%) trials were removed from analysis due to technical errors in Experiments 1a, 1b and 2, respectively.

In Experiment 1, baseline force sensor levels were measured before the experiment started when the manipulandum was placed stationary on the table. These baseline values were subtracted from the data to remove the offset and voltages were converted to Newtons. Force signals were filtered using a bidirectional 2nd-order Butterworth filter with a cut-off frequency of 10 Hz. The grip force (GF) was the average of the horizontal forces perpendicular to the graspable surface of both force sensors. The load force (LF) was defined as the sum of the vertical forces tangential to the graspable surface of both force sensors (**Figure 1**). The grip force rate (GFR) and the load force rate (LFR) were the differentiated GF and LF, respectively. GF and LF onsets were determined when force signals reached a threshold of 0.1 N after which a minimum of 0.8 N had to be reached to control for small non-meaningful force fluctuations. The variables of interest were the peak force rates (peak GFR and peak LFR), the GF value at peak GFR and the duration of the loading phase (LPD) and are illustrated in **Figure 2**. Because we were interested in the early stages of force planning, peak force rates were defined as the first peak that was higher than 70% of the maximal force rate. The LPD was defined as the time delay between LF onset and the first time LF overcame the static load. Static load force values were measured in a separate session for each weight by the first author (2.2, 4.2 and 6.2 N, for the light, intermediate and heavy object, respectively), which included the weight of the cube, manipulandum and basket. The GF value at peak GFR was calculated to quantify the sensorimotor memory effect on the actual force before the influence of feedback mechanisms. This value was used to compare the magnitude of the sensorimotor memory effect to the lifted weights.

Statistical Analyses

We analyzed the effect of sensorimotor memory on force and perceptual parameters by comparing trials preceded by either heavy or light objects. These analyses were performed separately for light or heavy lifts (Experiments 1a, b) or perceptual trials (Experiment 2). In other words, sensorimotor memory effects on a current *light* (*L*) lift were tested by comparing H*L* vs. L*L* (or HH*L* vs. LL*L* in Experiment 1b) conditions whereas sensorimotor memory effects on a current *heavy* (*H*) lift were tested by comparing L*H* vs. H*H* (or LL*H* vs. HH*H* in Experiment 1b) conditions. Comparisons were assessed using paired *t*-tests with an α-value of 0.05.

To evaluate the trial-by-trial relationship between force parameters and weight perception, peak force rates and perceptual estimates were correlated within each subject. In conjunction with these within-subject correlation analyses, covariance analyses were performed on the weight ratings, with peak GFR or peak LFR as covariates and participants as fixed factors. Again, these covariance analyses were performed separately for *light* (H*L* and L*L* in Experiment 1a or HH*L* and LL*L*

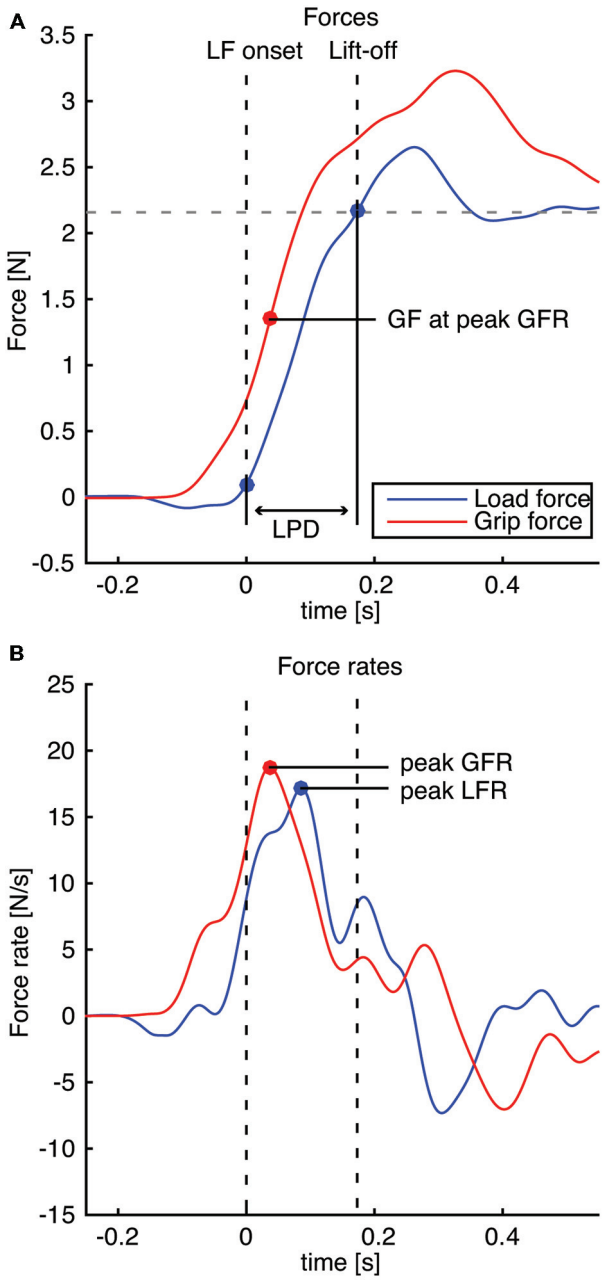

FIGURE 2 | Illustration of the measured fingertip force parameters: load phase duration (LPD), peak grip force rate (peak GFR), peak load force rate (peak LFR) and GF at peak GFR. Vertical dashed lines indicate LF onset and lift-off. **(A)** Red and blue solid lines indicate the recorded GF and LF respectively; the horizontal dashed line represents the static load force for the light object (including the basket). **(B)** Force rates. Note all traces are aligned to LF onset.

in Experiment 1b) and *heavy* lifts (L*H* and H*H* in Experiment 1a or LL*H* and HH*H* in Experiment 1b).

In a second between-subject analysis, we tested whether sensorimotor memory effects correlated with weight perception (**Figure 4**). Considering the low number of participants in Experiments 1a and 1b, data of these two experiments were pooled together. To do this, data of Experiment 1b were reanalyzed by calculating all variables grouped into the shorter two-trial sequences (e.g., L*L* instead of LL*L*). Then, we computed a force ratio (*X*-axis, **Figure 4**) by dividing GFR or LFR peak values of trials preceded by light lifts by trials preceded by heavy ones (i.e., L*L*/H*L* and L*H*/H*H*); a force ratio below 1 denoting an increasingly larger sensorimotor memory effect. Similarly, a perceptual ratio (*Y*-axis) was computed for weight ratings using the same formula as for force ratios (L*L*/H*L* and L*H*/H*H*). Here a ratio above 1 denotes a larger perceptual bias in weight estimation.

Finally, parameters measured in Experiment 1b were analyzed by grouping lifts in both two-trial and three-trial sequences in order to determine the effect of lengthening the trial sequence on the magnitude of sensorimotor memory and perceptual bias. For each variable (peak GFR, peak LFR, LPD, GF at peak GFR and weight ratings) and for light and heavy lifts separately, we quantified sensorimotor memory by computing ratios using the same formula as above, i.e., L*L*/H*L* and L*H*/H*H* for the two-trial sequences and compared them with ratios for the three-trial sequences (i.e., LL*L*/HH*L* and LL*H*/HH*H*, respectively). These ratios were compared with paired samples *t*-tests for light and heavy objects separately.

RESULTS

Experiment 1a: Sensorimotor Memory Biases Weight Perception

The goal of Experiment 1 was to test whether previous lift history (i.e., sensorimotor memory) influenced weight estimation of the currently lifted object. In Experiment 1a (**Figure 3A**), only the directly preceding lift was taken into consideration and sequences of two trials were compared. A systematic sensorimotor memory effect was found for both light and heavy lifts. When a light lift was preceded by a heavy object (condition H*L*), higher peak GFR ($t_{(9)} = -5.95$, $p < 0.001$), higher peak LFR ($t_{(9)} = -5.94$, $p < 0.001$) and shorter LPD ($t_{(9)} = 3.48$, $p = 0.007$) were observed compared to when it was preceded by a light object (L*L*). Similarly, when a heavy lift was preceded by a light object (condition L*H*), peak GFR was lower ($t_{(9)} = -4.41$, $p = 0.002$), peak LFR was lower ($t_{(9)} = -6.22$, $p < 0.001$) and the LPD was longer ($t_{(9)} = 8.07$, $p < 0.001$) than when it was preceded by a heavy object (H*H*). GF values at peak GFR followed the same pattern (L*L*: 1.80 ± 0.26, H*L*: 2.33 ± 0.26, L*H*: 2.17 ± 0.22, H*H*: 2.63 ± 0.31; mean ± SEM) with significant differences for lifts of light ($t_{(9)} = -6.83$, $p < 0.001$) and heavy objects ($t_{(9)} = -3.53$, $p = 0.006$). These results are in line with previous findings showing that sensorimotor memory can bias the predictive scaling of force parameters when lifting a series of objects. Here, we took advantage of this sensorimotor memory effect to test whether a change in force scaling during the loading phase will in turn influence perceptual estimates about the object weight.

Interestingly, we also found an effect of trial history on object weight perception. Perceptual estimates were significantly different for the light object ($t_{(9)} = 4.73$, $p = 0.001$), but failed to reach significance for the heavy object ($t_{(9)} = 0.86$, $p = 0.411$; **Figure 3A**, left). This indicates that the perception of light objects is influenced by the previous weight: the object feels lighter when a heavy object was previously lifted (H*L*) compared to when it was preceded by a light one (L*L*).

To estimate whether the peak force rates were correlated with the perceptual weight estimates on a trial-by-trial basis, a covariance analysis was performed. Here, a significant effect on the perceptual estimates was found for both peak GFR ($F_{(1,188)} = 6.2$, $p = 0.014$) and peak LFR ($F_{(1,188)} = 14.0$, $p < 0.001$) for the light lifts. The relationship between force and perceptual parameters was negative: lower weight estimations were associated with higher peak force rates. For the heavy lifts, no effect was found (peak GFR: $F_{(1,187)} = 0.12$, $p = 0.73$; peak LFR $F_{(1,187)} = 0.09$, $p = 0.77$). Individual relationships between force and perceptual parameters revealed mostly negative correlations with light lifts (8 out of 10 participants for peak GFR and 10 out of 10 for peak LFR). For heavy objects, correlation directions were more mixed (6 out of 10 participants negative for peak GFR and 7 out of 10 for peak LFR).

It is noteworthy that the absence of any perceptual bias for heavy lifts might be explained by the fact that the magnitude of the sensorimotor memory effect (GF at peak GFR rate difference: 0.53 and 0.46 N for light and heavy lifts, respectively) is drastically much smaller for heavy lifts (about 7%) vs. light lifts (about 24%) hence much less salient for inducing an effect on perception (see "Discussion" Section).

Experiment 1b: Larger Sensorimotor Memory Effects Increase Weight Perception Bias

The goal of Experiment 1b was to experimentally manipulate the magnitude of the sensorimotor memory effect and test its impact on the weight rating bias. We expected a larger sensorimotor memory effect with a longer sequence of same weight lifts in the preceding trials. Such trials were too few to be analyzed in the data of Experiment 1a. However, a preliminary analysis showed that differences between lifts preceded by two light and two heavy trials seemed to increase for the force parameters as well as for perceptual estimates. Motivated by this observation, we purposely designed a new experiment (Experiment 1b) with longer, three-trial sequences of light and heavy objects (e.g., LL*L*, HH*L* etc.). As can be seen in **Figure 3B**, the results of this experiment were similar to Experiment 1a. For the force parameters, a sensorimotor memory effect was observed for light as well as heavy lifts. Lifts preceded by two heavy objects (HH*L* or HH*H*) showed a higher peak GFR (light: $t_{(9)} = -7.85$, $p < 0.001$; heavy: $t_{(9)} = -4.82$, $p < 0.001$), a higher peak LFR (light: $t_{(9)} = -8.16$, $p < 0.001$; heavy: $t_{(9)} = -5.01$, $p < 0.001$) and a shorter LPD (light: $t_{(9)} = 6.38$, $p < 0.001$; heavy: $t_{(9)} = 6.31$,

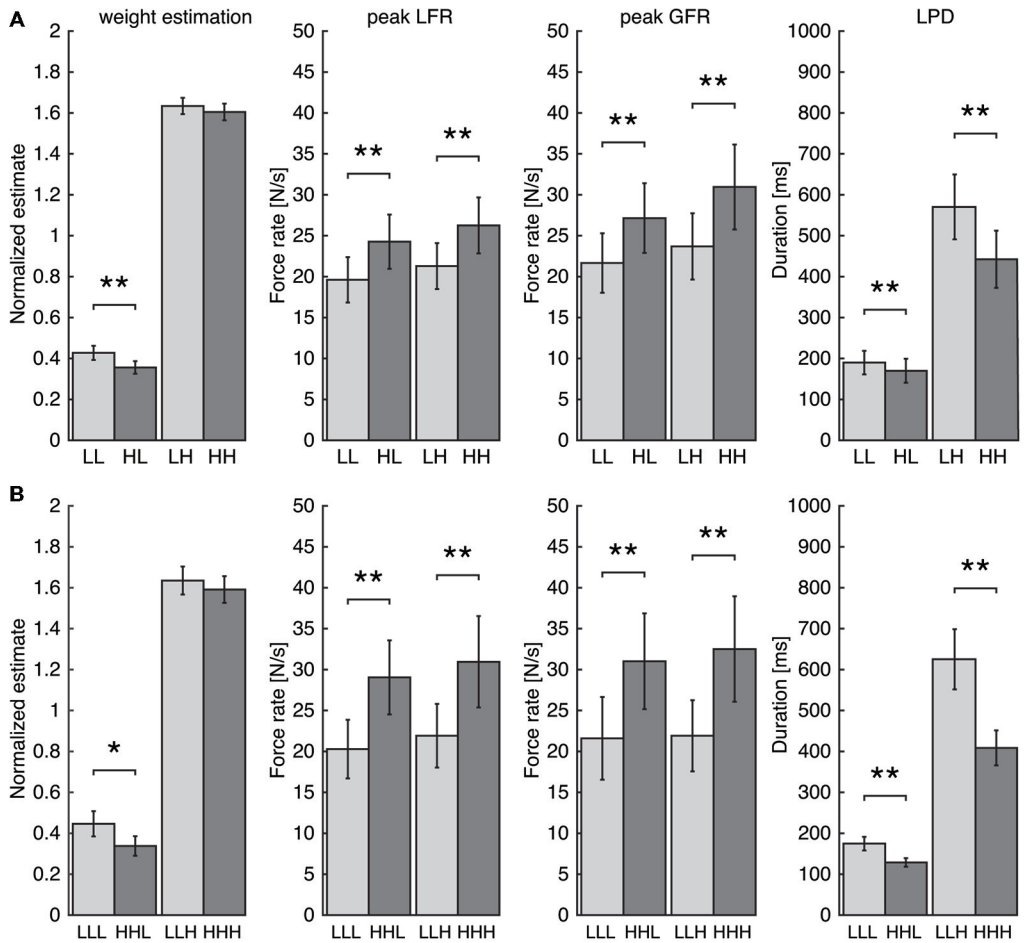

FIGURE 3 | Results for Experiment 1A (A) and Experiment 1B (B) for the normalized perceptual estimates, peak load force rates (peak LFR), peak grip force rates (peak GFR) and the load phase duration (LPD). Bars represent the average of trial groups based on the weight sequence (light: L, heavy: H). Error bars represent standard errors of the mean. Note the effect of the previous lift on force parameters for both light and heavy objects whereas weight estimation was only affected for light objects. $^{*}p < 0.05$, $^{**}p < 0.01$.

$p < 0.001$) compared to lifts preceded by two light objects. The GF at peak GFR showed similar effects (LL*L*: 1.61 ± 0.24, HH*L*: 2.33 ± 0.28, LL*H*: 1.92 ± 0.29, HH*H*: 2.71 ± 0.38; mean ± SEM) with significant differences for light ($t_{(9)} = -6.25$, $p < 0.001$) and heavy lifts ($t_{(9)} = -4.44$, $p = 0.002$). In addition, we replicated our effect of trial history on weight perception. Perceptual estimates were lower if a light lift was preceded by two heavy objects (HH*L*) compared to two light objects (LL*L*; $t_{(9)} = 2.96$, $p = 0.016$). No significant perceptual effect was seen for the heavy object ($t_{(9)} = 1.10$, $p = 0.30$).

Within-subject analyses were performed to investigate the trial-by-trial correlations between force and perceptual parameters. A covariance analysis revealed that the peak GFR was related to the perceptual estimate within participants (light: $F_{(1,188)} = 6.16$, $p = 0.014$; heavy: $F_{(1,188)} = 7.12$, $p = 0.008$). The same result was found for the relationship between the peak LFR and the weight ratings (light: $F_{(1,188)} = 18.6$, $p < 0.001$; heavy: $F_{(1,188)} = 7.49$, $p = 0.007$). Again, this relation was negative where lower perceptual estimates were seen for higher force rates. For the individual participants, 9 out of 10 had negative correlations between weight ratings and peak GFR and 8 out of 10 with peak LFR for light lifts. For heavy lifts, negative correlations of weight ratings with peak force rates were observed in only 6 out of 10 participants for both peak GFR and peak LFR.

The between-subject correlation of the sensorimotor memory effect and the perceptual bias is shown in **Figure 4**. This correlation was calculated for the pooled measurements of Experiments 1a and 1b. For light lifts, significant correlations were found between the perceptual ratios and the peak GFR ratios ($R = -0.55$, $p = 0.012$), but not for the peak LFR ratios. For heavy lifts, no significant correlation was found.

To test whether a larger sensorimotor memory effect was indeed produced with longer sequences of the same weight, two-trial sequences were compared with three-trial sequences. To do this, ratios of lifts preceded by heavy and light objects were compared within Experiment 1b, for *light* (L*L*/H*L* vs. LL*L*/HH*L*)

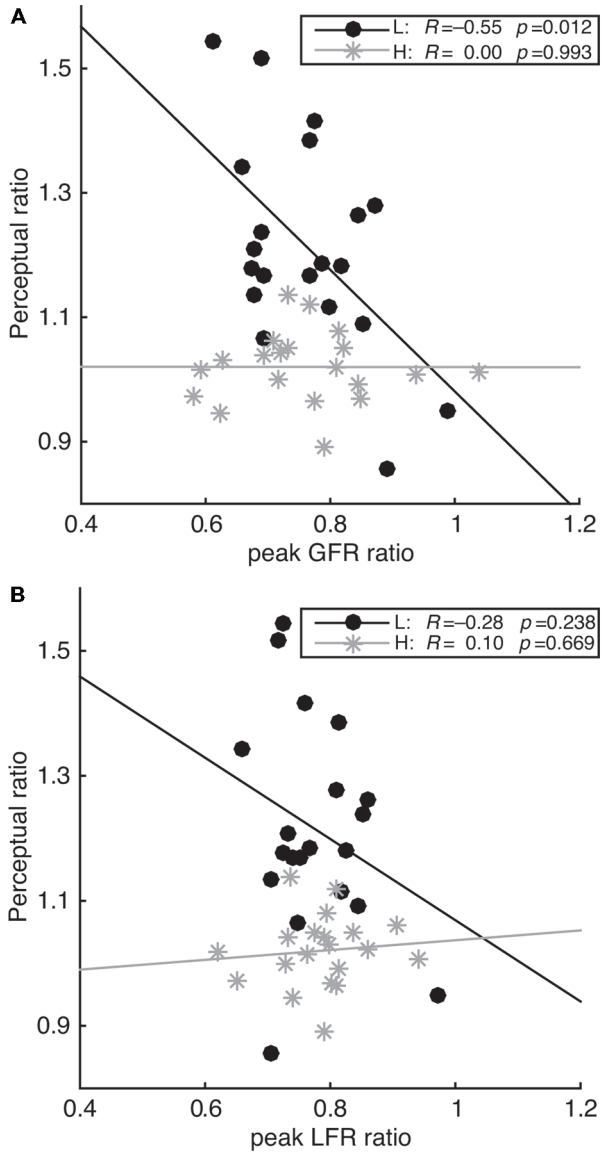

FIGURE 4 | Regression lines between the perceptual estimate ratio and the peak grip force ratio (A: peak GFR) and the peak load force ratio (B: peak LFR). Correlations are shown separately for light (L, black circles) and heavy objects (H, light gray asterisks). Correlation coefficients and *p*-values are indicated in the captions. Note that for light objects, but not heavy, the weight perception bias was larger as the magnitude of the sensorimotor memory effect increased.

and *heavy* (L*H*/H*H* vs. LL*H*/HH*H*) lifts separately. For light lifts, sensorimotor memory effects in the three-trial sequences were larger than in the two-trial sequences (peak GFR: $t_{(9)} = -3.46$, $p = 0.007$, peak LFR: $t_{(9)} = -4.64$, $p = 0.001$, GF at peak GFR: $t_{(9)} = -3.32$, $p = 0.009$), although this did not reach significance for LPD ($t_{(9)} = 2.13$, $p = 0.062$). Interestingly, the perceptual weight bias was also larger for the three-trial sequence compared to the two-trial sequence ($t_{(9)} = 2.57$, $p = 0.030$). For heavy lifts, the sensorimotor memory effects were also larger in the three-trial compared to the two-trial sequences (peak GFR: $t_{(9)} = -3.75$, $p = 0.005$, peak LFR: $t_{(9)} = -4.97$, $p = 0.001$, LPD: $t_{(9)} = 8.80$, $p < 0.001$, GF at peak GFR: $t_{(9)} = -2.68$, $p = 0.025$). However, no significant difference was found for perceptual estimates ($t_{(9)} = 0.67$, $p = 0.520$). Altogether, this experiment shows that larger sensorimotor memory effects on force scaling lead in turn to larger weight perception biases, which suggests a tight link between the action planning and perception.

Experiment 2: Weight History Does Not Affect Passive Weight Perception

When participants were presented with different forces (1 or 5 N) on their resting hand, no significant differences in perceptual estimates of the current object weight were seen when trials were preceded by heavy compared to light weights (light: $t_{(7.0)} = -0.69$, $p = 0.513$, heavy: $t_{(7.0)} = 0.31$, $p = 0.769$; **Figure 5**). The lack of effect in this control experiment highlights the lifting motion as the key component for biasing weight perception.

DISCUSSION

The aim of this study was to evaluate the interaction between object lifting and weight perception. Specifically, we investigated the relationship between sensorimotor memory effects and weight estimation in an object grip-lift task. We asked participants to lift light or heavy objects and estimate their weight. Importantly, the order in which light and heavy

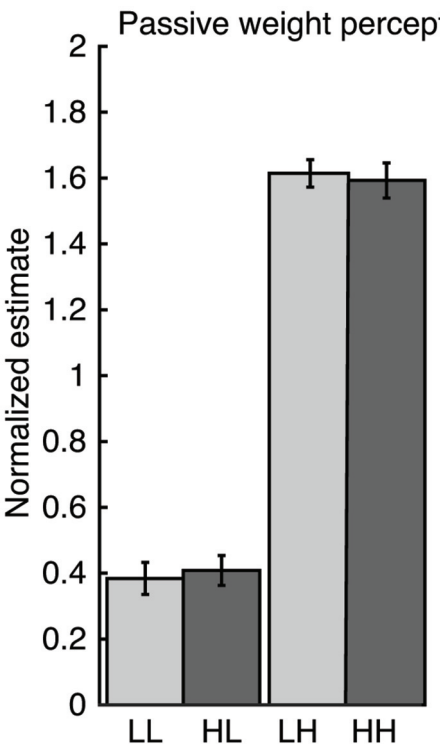

FIGURE 5 | Results of Experiment 2. Weight sequence did not affect normalized perceptual estimates when object weight was passively presented onto the subjects' right hand.

weights were presented was unpredictable. In short, we found that not only fingertip forces but also perceptual estimates were influenced by the previous lift. This finding indicates that action parameters and perception are intimately linked. Since sensorimotor memory has been considered as a fast 1-trial learning process (e.g., Fu et al., 2010), this shows how learning novel object dynamics can affect perceptual object representations. In accordance with previous studies (Johansson and Westling, 1988; Loh et al., 2010), we found a sensorimotor memory effect when participants had to lift a series of different objects. Fingertip forces were planned according to the previous lift and this effect was present for both light and heavy objects. This result indicates that no default strategy was used for lifting objects, but that forces were scaled based on recent experience. Importantly, besides the effect of the lifting order on force scaling, a bias was also found for perceptual weight estimations in both Experiments 1a and 1b. When a light object was lifted after a heavy object, it was perceived to be lighter than when lifted after a light object. To test whether the perceptual bias did not merely result from a cognitive contrast effect independent of active lifting or force scaling, a control experiment was performed in which a force was passively exerted with a haptic device on the hand at rest. In this case, we did not find any perceptual bias depending on the previous felt force.

The observation that the perceptual bias is only present when actively lifting an object suggests that the estimation bias is related to force scaling. Indeed, this relationship was found both within individual subject data and across all participants. This correlation was negative: lower weight estimations were associated with higher force rates. In addition, increasing the magnitude of sensorimotor memory effects by lengthening the trial history (Experiment 1b) also enhanced the perceptual bias. Although the within-subject correlations showed a significant relationship between perceptual estimates and both grip and load forces, the between-subject correlations were only significant for grip but not load force. This stronger relationship for grip force could be explained by a dissociable neural control of grip and load forces (Davare et al., 2006, 2007), likely to have different impacts on brain areas involved in perception.

The bias for perception was only seen for light lifts but not heavy ones, while a sensorimotor memory effect was present for lifts of both object weights. Although the peak force rates were found to be a significant covariate for the perceptual estimates with the lifting of a heavy object in Experiment 1b, there were still few within-subject relationships and no between-subject correlations between sensorimotor memory and perceptual biases. There are two possible explanations for the absence of perceptual bias for heavy objects. First, the force rate differences might not be large enough to produce perceptual differences in heavy weights. Perceptual differences of weight behave according to Weber's law. This means that the just noticeable difference is related to the intensity of the stimulus. Consequently, larger weight differences are needed with higher values to be able to be perceived. The magnitude of the sensorimotor memory effect was similar for both heavy and light objects, as seen in the difference in grip force at peak GFR (around 0.5–0.8 N). However, this difference is relatively much larger compared to a light (2 N) than to a heavy (6 N) object. Therefore, the magnitude of the sensorimotor memory effect might not have been salient enough to bias perception of a heavy weight, which was therefore perceived as having the same weight independent of the lifting history. A second explanation relies on the loading phase being much longer for the heavy object. When lifting a heavy weight after a light one, the planned forces are too small and lift-off does not occur when expected. Consequently, forces keep increasing at the same rate as for a light lift until lift-off takes place, a process during which feedback loops are heavily involved (Johansson and Flanagan, 2009). These recurrent feedback loops over the course of a longer loading phase might also influence weight perception and minimize the estimation bias. When a light object is lifted after a heavy one, feedback mechanisms are also used to correct the force overshoot and stabilize the object. However, in this case the stabilization process occurs after lift-off and might be less influential on the weight perception.

All in all, these results show that both perceptual and force parameters are affected by previous object lifts and that these parameters are also correlated. The finding of the association between perceptual estimates and force scaling appears to contrast studies on the size-weight illusion, where these two control systems seem to be dissociated. In previous research, it was found that perception of object weight was influenced by object size, whereas force scaling was not (Flanagan and Beltzner, 2000; Grandy and Westwood, 2006). In those studies, sensorimotor memory did not affect weight perception. **Figure 6** provides a schematic explanation for both of these findings. Online sensory information from the current object provides inputs to control forces applied by the fingertips and perceptual weight estimation. Furthermore, online information is also used in feedback loops to build up sensorimotor memory and priors. These loops reflect short and long-term learning processes of object representations. The sensorimotor memory is the representation that is build up from previous experience with the

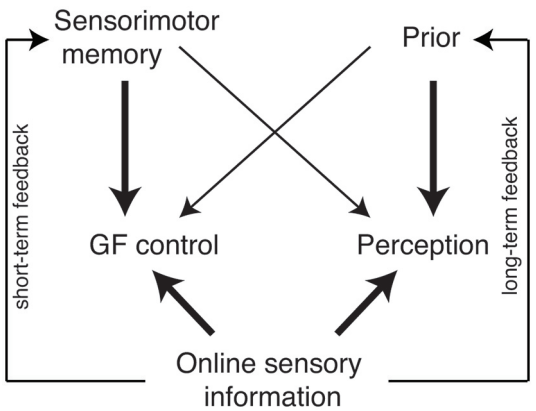

FIGURE 6 | Schematic diagram of the influence of sensorimotor memory, priors and online sensory information on grip force (GF) control and perceptual estimation. The arrow thickness reflects the importance of the gain of one input. Two feedback loops represent the input of sensory information used to build up the sensorimotor memory and the prior.

current or a similar object. This can be formed after a single trial and is therefore a short-term feedback loop. A prior is a long-term learned association between two object properties, which requires more time to develop, but also lasts longer. For example, a size-weight prior states that large objects are heavy assuming a constant density. In the diagram, sensorimotor memory and priors both influence the control of grip force and weight perception, but to a different extent. Sensorimotor memory has a stronger influence on force control than on perceptual weight estimation. Conversely, the prior has a stronger influence on perception than on force control. In the current experiment, no sizes cues or other priors are available, so only the sensorimotor memory influences the grip force control as well as the perceptual estimates. In the case of the size-weight illusion, a prior influences the grip force and perception. For the first trials in a size-weight illusion setting, no sensorimotor memory is build up yet and grip force scaling is affected by the prior (Gordon et al., 1991; Flanagan and Beltzner, 2000). After a few lifts, the sensorimotor memory of the object weights dominates the grip force control. For the perceptual estimation, the prior dictates weight estimation and produces the persisting size-weight illusion.

The effects of sensorimotor memory or priors on force control and perception do not only have a different gain, but also influence force and perception in opposite directions. Whereas force rates are scaled according to a weight prediction, weight perception changes based on the motor correction required when there is a mismatch between expected and actual weight. In other words, the force and perceptual parameters are affected by trial history in an opposite way: although *higher* force rates are used to lift an object after a heavy lift, it is actually perceived to be *lighter*. This negative relationship between forces and estimates suggests that a sense of effort is perceived. When lifting a light object after a heavy lift, less effort is needed than originally planned. The correction of the predicted weight compared to the actual weight makes the object to be perceived lighter than expected. Note that the weight expectation is an implicit phenomenon in the present experiments, and only results from sensorimotor memory-driven changes in force scaling. This is in contrast to explicit expectations that can also lead to different weight perceptions (e.g., in the size-weight illusion, Buckingham and Goodale, 2010).

Previous research relating grip forces and weight perception also point to the perception of a sense of effort. These findings reflect the sense of effort needed in the static phase of lifting, where a higher needed grip force (more effort) is associated with a higher perceptual estimate (Flanagan et al., 1995; Flanagan and Bandomir, 2000). In contrast to these studies, the objects lifted or the way they were lifted did not differ between conditions in the present experiments, but only the history and the planning of the action. If weight ratings would be estimated based on the static holding phase, where grip and load forces were the same in all cases, perception should then be the same. However, the dynamic phase of the lift differed according to lifting history. Hence, weight estimation seems to be formed early in the lift or is at least influenced by this phase. This is the first study showing an effect of the dynamic lifting phase, i.e., GFRs, on perception. Since the GFRs in the dynamic phase of the lift reflect the planning of the lift, this indicates that the action plan has an impact on the perception of an object.

The effect of force control on weight perception can generate several other predictions based on other findings related to sensorimotor memory. For instance, it has been found that sensorimotor memory is only partly disrupted by an isometric contraction (Cole et al., 2008), affecting grip force but leaving load force unchanged. It is therefore plausible to assume that perception of object weight should be altered by an isometric contraction, similar to a conscious grip force increase (Ellis and Lederman, 1999). Another interesting study found that with a series of increasing weights, force prediction does not depend on the last lift, but is extrapolated from the series (Mawase and Karniel, 2010). Given this extrapolation-driven increase in force scaling, we expect even larger changes in perception of object weight. Finally, as sensorimotor memory can be transferred between hands (Gordon et al., 1994; Nowak et al., 2005b), perceptual biases might also be found when alternating lifts with the two hands.

Future research should aim to find the neural substrate underlying the effect of sensorimotor memory on weight perception. It is plausible that this effect does not stem from a single brain area, but involves a network of areas; the primary motor cortex (M1), cerebellum and lateral occipital complex (LOC) are likely to be the key nodes in this network (van Polanen and Davare, 2015). The role of M1 in building up sensorimotor memory has previously been demonstrated (Chouinard et al., 2005; Nowak et al., 2005b; Loh et al., 2010). However, it has recently been shown that M1 also plays a role in sense of effort (Takarada et al., 2014). It is believed that a sense of effort is formed through both peripheral (Luu et al., 2011) and central (Morree et al., 2012) inputs. In our study, the discrepancy between the anticipated sensory consequences and perceived signals seems to have an impact on perceptual responses. In fact, this effect is proportional to the amount of force correction required. The cerebellum is proposed to play a role in predictive motor control and in the comparison between predicted and actual motor states (Nowak et al., 2007). The sensory consequences are predicted based on internal models (Wolpert and Flanagan, 2001) which are believed to reside in the cerebellum (Kawato et al., 2003). This structure is also involved in the control of fingertip forces and sensorimotor memory (Nowak et al., 2005a). In addition, Jenmalm et al. (2006) have found that processing of weight switches was different for light lifts preceded by heavy objects than for heavy lifts preceded by light objects. Increased BOLD signal was found in M1 for conditions with an increase in weight (light to heavy switch) and in the cerebellum for conditions with a decrease in weight (heavy to light switch). Interestingly in our study, we have only found perceptual biases for heavy to light switches, suggesting a possible role of the cerebellum in mediating this effect. Finally, it has recently been discovered that object weight representations are also found in the LOC (Gallivan et al., 2014). The role of LOC in the multimodal recognition of objects (Amedi et al., 2001) makes this area a possible site for perceptual weight estimation.

To summarize, we used sensorimotor memory as a tool to manipulate implicitly subjects' expectations about the weight of an object. Importantly, we found that the previous lift biased weight perception and this effect was negatively correlated with the magnitude of the planned force parameters. This highlights a key role of the action plan in modulating perception: if there is a mismatch between predicted and actual object weight, the implementation of online force corrections will also influence weight perception in a proportional manner.

AUTHOR CONTRIBUTIONS

VVP and MD designed the experiment. VVP performed the data acquisition. VVP and MD analyzed and interpreted the data. VVP and MD drafted and revised the manuscript and approved the final version. VVP and MD agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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frontiers in Human Neuroscience

REVIEW
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Endurance Exercise as an "Endogenous" Neuro-enhancement Strategy to Facilitate Motor Learning

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Endurance exercise improves cardiovascular and musculoskeletal function and may also increase the information processing capacities of the brain. Animal and human research from the past decade demonstrated widespread exercise effects on brain structure and function at the systems-, cellular-, and molecular level of brain organization. These neurobiological mechanisms may explain the well-established positive influence of exercise on performance in various behavioral domains but also its contribution to improved skill learning and neuroplasticity. With respect to the latter, only few empirical and theoretical studies are available to date. The aim of this review is (i) to summarize the existing neurobiological and behavioral evidence arguing for endurance exercise-induced improvements in motor learning and (ii) to develop hypotheses about the mechanistic link between exercise and improved learning. We identify major knowledge gaps that need to be addressed by future research projects to advance our understanding of how exercise should be organized to optimize motor learning.

Keywords: neuromodulation, endurance exercise, motor learning, brain, neuroplasticity, lactate, motor cortex, acute

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INTRODUCTION

The optimization of motor learning is of particular relevance in many sport-related settings such as competitive sports, disease prevention, rehabilitation after neurological or orthopedic injury as well as physical education. A huge body of literature in movement and training science proposes strategies to optimize motor skill learning with a strong emphasis on practice distribution (for example massed vs. distributed practice), scheduling (blocked vs. random practice), variation of motor tasks (constant vs. variable practice) as well as movement feedback or attentional focus (Magill, 2011; Schmidt and Lee, 2014).

From a more mechanistic perspective, strategies to improve motor learning may benefit from a deeper understanding of the underlying neurobiological mechanisms of skill acquisition, stabilization and retention in the brain. Thereby, targeted strategies can be developed to specifically modulate learning-related mechanisms with the aim to augment motor learning.

For example, transcranial electric or magnetic stimulation can be used to modulate brain function and behavior through external application of weak electric currents or magnetic fields throughout the scalp (Nitsche and Paulus, 2000; Reis et al., 2008; Dayan et al., 2013). One widely used technique is transcranial direct current stimulation (tDCS). TDCS of the primary motor cortex (M1) has been shown to increase long-term potentiation-like (LTP-like) plasticity or improve motor memory retention (Reis et al., 2008, 2009). Such external stimulation techniques

allow for focal modulation of cortical excitability and offer intriguing possibilities for example in stroke rehabilitation (Nowak et al., 2009).

Here we propose physical exercise as a more "endogenous" neuromodulation strategy to improve motor learning and brain plasticity. Mounting evidence demonstrates that physical exercise affects brain structure and function from the molecular to the systems level of brain organization (Voss et al., 2013b). Physical exercise facilitates long-term potentiation (LTP)-like plasticity in M1 (Singh et al., 2014b) and increases the level of learning-related neurotrophins (Rojas Vega et al., 2006). These and other mechanisms of physical exercise are discussed to potentially modulate motor learning (Fabel et al., 2009; McHughen et al., 2010; Cantarero et al., 2013).

Roig et al. (2013) provided a comprehensive summary about the behavioral effects of exercise on declarative and procedural memory processing. Here, we will focus on motor learning and review, in particular, the existing neurobiological evidence to generate hypotheses about the causal relationship between exercise and online/offline motor learning. We acknowledge that physical exercise may modulate motor performance via processes outside the central nervous system such as increases in muscle strength and flexibility (Schmidt and Lee, 2014). While we did not ignore these peripheral sources, we believe that modulation of brain function and structure through exercise constitutes largely unexplored mechanisms to optimize motor learning. Physical exercise is a natural and "endogenous" neuro-enhancement strategy potentially relevant for disease prevention, rehabilitation and education. To understand how exercise may enhance motor learning and neuroplasticity, it is important to characterize the neural correlates of motor learning.

ONLINE AND OFFLINE MOTOR LEARNING

Online motor learning is expressed as gain or loss in motor performance within a motor practice session (motor memory acquisition) while offline motor learning reflects performance changes between subsequent practice sessions (Dayan and Cohen, 2011). Hence, offline learning is thought to depend also on neuroplasticity within the period after task performance (Muellbacher et al., 2002). Subjects may reside in the resting-state, sleep or perform other tasks in this period which may positively or negatively influence offline learning-related neuroplasticity. For example, studies have shown abolished offline learning (motor memory consolidation and retention) through learning an interfering motor task (negative transfer, Brashers-Krug et al., 1996) or enhanced offline learning through sleep (positive transfer, Walker et al., 2003).

Here we propose that physical exercise induces positive transfer effects on online and offline motor learning through facilitation of the underlying neural processes of motor memory acquisition, consolidation and retention. This implies that certain aspects of exercise-induced brain changes are causally linked to the performance gains seen during online and offline periods as well as its neurobiological correlates in the brain. At present, this causal link has not yet been established and our hypothesis is based on independent evidence from (i) behavioral studies showing positive effects of exercise on motor learning as well as (ii) neurobiological studies on exercise-induced brain changes. Therefore, we will first review these separate pieces of behavioral and neurobiological evidence before hypotheses are generated about the causal link between exercise, motor learning and their underlying mechanisms.

After reviewing the neurobiological evidence, we will highlight behavioral studies showing improved motor learning through physical exercise scheduled before (acute or habitual exercise) or after (post-practice period) a motor practice session.

NEURAL CORRELATES OF MOTOR LEARNING

We will now briefly highlight some of the existing evidence on motor learning-related changes in the brain. With this, we would like to inform readers about relevant brain changes that are important for a better understanding of the effects of exercise on motor learning. For a more detailed review on the neural correlates of motor learning, the reader is referred to review articles by Doyon and Benali (2005), Monfils et al. (2005), and Dayan and Cohen (2011).

In brief, both online- and offline motor learning are associated with distinct changes in brain activation in typical sensorimotor networks (e.g., motor cortex, basal ganglia, cerebellum) and higher-order associative networks (e.g., prefrontal, parietal and temporal cortices). Online motor learning in the early practice period engages prefrontal, parietal and partly hippocampal brain regions in addition to sensorimotor cortical-striato-cerebellar networks (Karni et al., 1995; Honda et al., 1998; Floyer-Lea and Matthews, 2005; Albouy et al., 2008) while the prefrontal contributions decrease in the later practice period (Poldrack et al., 2005) and the motor memory seems to be stabilized in sensorimotor cortical and subcortical (striatum and cerebellum) networks. More extensive periods of motor practice induce structural changes in cortical gray matter and underlying white matter tracts (Scholz et al., 2009).

The structure of white matter fiber tracts regulate the timing and speed of action potentials across axons which are critical for the occurrence of learning-related neuronal plasticity ("neurons that fire together, wire together") (McKenzie et al., 2014). Training-induced plasticity in M1 may occur through lasting modulations in synaptic transmission (Butefisch et al., 2000; Rioult-Pedotti et al., 2000; Donchin et al., 2002; Antonov et al., 2003) including synaptogenesis (Xu et al., 2009) and the coordinated strengthening (e.g., LTP) and weakening (e.g., LTD) of synaptic connections (Mayford et al., 2012; Lee et al., 2014). In this respect, LTP and long-term depression (LTD) are considered as the cellular analog of motor learning (Rioult-Pedotti et al., 2000; McConnell et al., 2009; Cantarero et al., 2013). LTP and LTD reflect sustained changes in synaptic efficacy in response to the correlated arrival of action potentials between neurons (Hebb's learning rule, "neurons that fire together, wire

together," Hebb, 1949). In humans, neurophysiological studies showed that motor learning (i) requires LTP-like plasticity in M1 (Cantarero et al., 2013), (ii) increases the size of the movement representation of trained limbs in M1 (Pascual-Leone et al., 2005) and (iii) enhances motor corticospinal excitability (Muellbacher et al., 2002), although the relationship between cortico-spinal excitability and motor learning is complex (Tunovic et al., 2014).

In animals, LTP induction is linked to cellular structural changes (Toni et al., 1999; Harms et al., 2008). These structural changes rely on *de novo* protein synthesis (Lu et al., 2008) and injecting protein synthesis inhibitors in M1 results in a loss of previously learned skills as well as an impairment in new motor skill acquisition (Kleim et al., 2003). Worsened motor skill learning was correlated with reduced synapse number and size (Kleim et al., 2003) and learning a new motor skill rapidly increases the number of new synaptic spines in M1 (Xu et al., 2009). While the overall spine density returns to initial values soon, the newly formed spines are preferentially stabilized through subsequent practice and outlast the end of the training period (Xu et al., 2009). Further studies reported synaptogenesis after a few weeks of motor learning (Black et al., 1990; Kleim et al., 2002b) that was specific to the cortical representation of the trained limb and accompanied by an increase of motor map size (Kleim et al., 2002b, 2004). Such changes were not observable in the untrained limb representation and occur as a consequence of skilled motor activity instead of repetitive limb use (Kleim et al., 1996; Plautz et al., 2000; Tyč et al., 2005) or even strength training (Remple et al., 2001; Jensen et al., 2005). The prevailing and generally accepted view is that motor learning reorganizes neuronal and synaptic connections, whereas endurance exercise mainly influences the supportive vascular components (Churchill et al., 2002).

At the molecular level, motor learning reduces the concentration of the inhibitory neurotransmitter γ-*aminobutyric acid* (GABA) in M1 (Floyer-Lea et al., 2006; Stagg et al., 2011). Furthermore, the neurotrophic factor BDNF (*brain-derived neurotrophic factor*) is linked to functional plasticity in the human motor system. Subjects carrying the Val66Met polymorphism of the BDNF gene, which is known to affect activity-induced BDNF secretion (Egan et al., 2003), show reduced corticospinal excitability and reduced motor map reorganization in response to motor learning (Kleim et al., 2006). The Val66Met polymorphism also negatively affects online and offline learning of a complex motor tracking task (McHughen et al., 2010) but had no effect on learning a serial reaction time task (Freundlieb et al., 2012; Morin-Moncet et al., 2014). In Mice with BDNF mutations show diminished responses to excitability-enhancing brain stimulation of M1 (Fritsch et al., 2010). Not last, the loss or critically low levels of BDNF are associated with motor system dysfunction, for example with severe neurodegenerative diseases (Teixeira et al., 2010; He et al., 2013).

The central question that runs through this article is how endurance exercise influences these motor learning-induced brain changes at the systems-, cellular, and molecular level to create a productive neural environment for neural plasticity during online and offline periods of motor learning.

NEURAL CORRELATES OF EXERCISE

Like motor learning, physical exercise elicits neural changes from the systems- to the molecular level of brain organization. We will restrict the following review to exercise-induced brain changes that are potentially important to influence motor learning-induced neuroplasticity.

Systems-Level

To investigate exercise-effects at the *systems-level*, research in humans was performed using, e.g., transcranial magnetic stimulation (TMS) or magnetic resonance imaging (MRI). For TMS and functional MRI (fMRI), exercise-induced changes were found for corticospinal excitability, LTP-like plasticity and functional connectivity immediately or some minutes after the exercise interventions while structural MRI studies assessed lasting changes in gray and white matter after weeks to months of exercise. Such acute and lasting effects may contribute differently to improvements in online and offline motor learning.

TMS and fMRI

Transcranial magnetic stimulation is a non-invasive technique to focally stimulate superficial cortical brain regions across the scalp. Application of a single, suprathreshold TMS pulse over the primary motor cortex (M1) activates peripheral target muscles that can be recorded via electromyography. This response is referred to as motor evoked potential (MEP). The most commonly used TMS measures that characterize motor learning-related changes are (i) the size of cortical area from which an MEP could be evoked (movement representation), (ii) the lowest stimulus intensity to evoke an MEP (motor threshold) and (iii) the size of the MEP at a defined stimulation intensity (1 mV MEP). In general, motor learning increases motor map size, decreases the motor threshold, and increases MEP amplitudes (Pascual-Leone et al., 2005; Adkins et al., 2006). More recently, these indices have also been recorded in response to endurance exercise. Exhaustive exercise lowers the motor threshold indicating increased cortico-spinal excitability (Coco et al., 2010). Also, increased cortical excitability was found in very active compared to sedentary subjects (Cirillo et al., 2009). More recent studies, however, were not able to replicate the exercise-induced increase in cortico-spinal excitability and instead found evidence that exercise enhanced neuroplasticity in M1 (please see below McDonnell et al., 2013; Mang et al., 2014). Paired pulse TMS (ppTMS) allows to specifically examine local inhibitory and facilitative mechanisms within M1 (intra-hemispheric excitability) as well as between M1 and distant brain regions in the ipsi- and contralateral hemisphere (intra- and interhemispheric excitability). PpTMS pairs two TMS pulses over M1 with particular inter-stimulus intervals to target inhibitory (5 ms or less) or facilitatory (10–25 ms) mechanisms. A decrease in intracortical inhibition, which seems to be dependent on the level of the inhibiting neurotransmitter GABA, is generally assumed to reflect a favorable environment for the induction of neuroplasticity and therefore motor skill learning (Singh and Staines, 2015). Reductions in local GABA concentrations in M1 are correlated with motor learning in a serial-reaction time task

(Stagg et al., 2011), a sensorimotor adaptation paradigm (Kim et al., 2014) as well as a motor tracking task (Floyer-Lea et al., 2006). Using acute bouts of exercise, Yamaguchi et al. (2012) reported a decrease in short-interval intracortical inhibition (SICI) of the leg area (tibialis anterior and soleus muscles) after just 7 min of low-intensity cycling (Yamaguchi et al., 2012). Similar effects were observed for the upper extremity (first dorsal interosseous muscle) after 30 min of low–moderate or moderate–high intensity cycling (Smith et al., 2014). Likewise, 20 or 30 min of continuous biking with moderate intensity decreased SICI measured in the extensor carpi radialis and abductor policis brevis muscles (Singh et al., 2014a; Snow, 2014). Moreover, a rodent study showed that exercise upregulates genes associated with the excitatory glutamatergic system and downregulates genes related to the inhibitory GABA system in the hippocampus (Molteni et al., 2002). Taken together, these studies provide evidence that exercise at low, moderate or even high intensities rapidly reduces intracortical inhibition and that this effect is not limited to the exercised limbs. This may be beneficial for online motor learning. It must be mentioned that an increased intracortical inhibition in lower extremity M1 representations (vastus lateralis muscle) was observed during fatiguing cycling exercise (Sidhu et al., 2013) indicating that exercise at very high intensities may attenuate learning if motor practice involves similar effectors.

Finally, more recent studies examined the effect of endurance exercise on TMS protocols aiming to experimentally induce changes in synaptic efficacy using paired-associative stimulation (PAS; LTP- or LTD-like plasticity). Basically, this technique induces cortical LTP-like plasticity by first stimulating a peripheral nerve electrically, followed by a TMS pulse of the corresponding M1 area several milliseconds later. This enables researchers to study synaptic plasticity *in vivo* and to reduce the influence of numerous boundary conditions normally affecting behavior and associated brain changes (e.g., inter-individual differences in motor learning). Cirillo et al. (2009) tested the effect of regular physical activity on PAS-evoked neuroplasticity. Participants were divided into two groups dependent on self-reported physical activity level. The sedentary group performed physical activity less than 20 min per day on no more than 3 days per week, whereas the active group performed moderate-to-vigorous aerobic activity more than 150 min per day on at least 5 days per week. Active subjects showed increased LTP-like plasticity, as measured by the MEP amplitude of the abductor pollicis brevis (APB) muscle (hand muscle). Notably, similar effects were also registered in other experiments focusing on the effects of a single bout of exercise. For example, enhanced LTP-like plasticity in the APB muscle was observed after 20 min of moderate-intensity cycling (Singh et al., 2014b). This beneficial effect applies for higher exercise intensities as well, since PAS-effects were also noted after 20 min of high-intensity interval cycling (Mang et al., 2014). However, LTP-like plasticity was not enhanced in the soleus muscle (lower extremity) of endurance athletes but pronounced in skill athletes (Kumpulainen et al., 2015). The reason for the diminished plasticity in lower limbs and the enhanced plasticity in upper limbs in endurance athletes remain speculative.

Beyond M1, acute exercise has been shown to change large-scale brain network connectivity in the resting-state. Rajab et al. (2014) compared 20 min of moderate-intensity exercise (70% of age-predicted HR_{max}) with a resting control group ($n = 15$). Functional connectivity was tested before and immediately after the exercise bout and increased connectivity was found in sensorimotor and thalamic-caudate networks (Rajab et al., 2014).

To sum up, acute exercise induces facilitative effects on early neuroplasticity (within the first hour after exercise). However, the dose-response relationship between exercise parameters, especially exercise intensity, and TMS indices is not clear to date (Singh and Staines, 2015) and long-term intervention studies on corticospinal excitability or PAS-induced plasticity are still missing.

Structural MRI

An excellent method to observe brain morphology changes in humans is MRI. MRI can be used to non-invasively assess the shape and size of brain regions and to compare these measures between participants and across time within single individuals. Morphological measures such as gray matter volume/density or cortical thickness are derived from segmentation of individual brain images into distinct tissue classes (e.g., gray matter, white matter and cerebrospinal fluid). In recent years, a considerable amount of studies demonstrated structural changes in, e.g., gray matter density after complex motor learning (Draganski et al., 2004; Taubert et al., 2010). The cellular correlates of gray matter changes observed with MRI are still unknown and recent studies combining MRI with histological assessment in animals provide new insights into how MRI changes are correlated with alterations at the cellular level (Lerch et al., 2011; Hamaide et al., 2015).

First, cross-sectional studies in humans have found associations between brain structure and motor behavior. Schlaffke et al. (2014) directly compared grey matter density (GMD) between long-distance endurance athletes, martial artists and a non-sport control group not reporting participation in any regular physical activities. The idea of comparing endurance vs. martial artists is based on their differing metabolic profile (mainly aerobic vs. mainly anaerobic). In comparison to controls, statistical analysis of GMD across the whole brain showed higher GMD in the supplementary motor area/dorsal premotor cortex (BA 6) in both athlete groups. Endurance athletes additionally revealed higher GMD in medial temporal lobe. The authors conclude that structural differences in these regions in the athlete groups may be related to motor control and motor skill acquisition (Dayan and Cohen, 2011; Tomassini et al., 2011).

Longitudinal studies with repeated MRI measurements before and after training provide further insight into potential causes of brain differences since the aforementioned variations in gray matter may be attributed to physical activities or alternative factors such as genetic predispositions. In a longitudinal study with elderly humans, aerobic exercise for 1 year reversed the age-related decline in gray matter and increased hippocampal volume of approximately 2% (Erickson et al., 2011). Besides the hippocampus, the prefrontal cortex is also vulnerable

for exercise-induced gray matter changes in elderly humans (Colcombe et al., 2006). Both the hippocampus and the prefrontal cortex are relevant for learning, memory and cognitive control and the fMRI literature on motor learning suggests that both brain regions are involved in the early period of motor skill learning (please see Neural Correlates of Motor Learning). Also, motor learning induces structural GMD changes in the hippocampus and prefrontal cortex (Boyke et al., 2008; Taubert et al., 2010; Sehm et al., 2014). Therefore, long-term exercise may exert beneficial effects on motor learning by priming brain regions implicated in motor skill acquisition such as the hippocampus and/or prefrontal cortex.

With respect to motor learning, however, the aforementioned MRI studies have tested exercise effects using relatively long observation periods (6–12 months). Interestingly, two MRI studies with rodents (Sumiyoshi et al., 2014; Cahill et al., 2015) recently demonstrated that exercise over 1–4 weeks affects brain regions well known to be involved in motor function and learning (please see Neural Correlates of Motor Learning). In one of these studies, Cahill et al. (2015) exposed male mice to 4 weeks of voluntary exercise and compared alterations in brain structure to inactive controls using high resolution MRI. The authors registered exercise-induced gray matter changes in several brain structures, amongst them hippocampus, dentate gyrus, stratum granulosum of the dentate gyrus, cingulate cortex, olivary complex, inferior cerebellar peduncle and regions of the cerebellum. Furthermore, Sumiyoshi et al. (2014) examined gray matter changes in response to a period as short as 1 week of voluntary wheel-running. Analyses revealed gray matter changes in widely distributed regions of the cerebral cortex, including motor, somatosensory, association and visual areas but not the hippocampus or prefrontal cortex. Structural changes were kept up for a period of at least 1 week and correlated positive with the total running distance. Collectively, these results indicate that exercise-induced structural gray matter plasticity may shift from sensorimotor to prefrontal and limbic regions during the time course of physical exercise. Interestingly, such a shift from sensorimotor to prefrontal and limbic structural plasticity has already been observed during the course of practice of a complex whole-body balance task (Taubert et al., 2010, 2012; Sehm et al., 2014).

Below the cortical sheath, white matter tracts interconnect distant cortical regions to allow information processing within large-scale networks (Fields, 2008). In addition to changes in gray matter, aerobic fitness in cross-sectional studies as well as endurance exercise interventions have shown to affect white matter tract structure as well (Voss et al., 2013a; Chaddock-Heyman et al., 2014; Herting et al., 2014). A longitudinal study involving 33 patients with schizophrenia and 48 healthy controls (age 18–48 years, 60 males/21 females) randomly assigned the subjects to either a physical exercise or control condition (Svatkova et al., 2015). The intervention lasted 6 months and contained 1 h training sessions conducted twice weekly. The proportion of aerobic (for instance cycling, rowing, treadmill running) to anaerobic exercises (weight-based strengthening exercises) was 2:1. Using diffusion-tensor imaging (DTI), a method that assesses the diffusion properties of water molecules to infer microstructural white matter changes, Svatkova et al. (2015) found that 6 months of exercise training alters white matter microstructure specifically in fiber tracts implicated in motor functioning such as the corpus callosum, corticospinal tract and superior longitudinal fascicle. This effect was comparable for patients and healthy subjects.

Taken together, the aforementioned studies demonstrate that endurance exercise leads to structural adaptations in motor-related brain regions and associated fiber connections. Nonetheless, longitudinal MRI studies examining the relation between exercise-induced brain changes and subsequent motor learning-induced brain changes were not conducted yet. Furthermore, conclusions about the practical significance of macroscopic brain changes are hindered since the MRI-observable changes could be driven by very different cellular changes (Zatorre et al., 2012). To gain more insight about that, the next section will focus on neurobiological adaptations on a more fine-grained level of observation.

Cellular Level

As already mentioned, motor learning is associated with changes in synaptic efficacy (LTP/LTD) (Sanes and Donoghue, 2000; Lee et al., 2014) which depends on structural changes at the synaptic level (Toni et al., 1999) and is linked to changes in the size of movement representations in M1. In contrast to motor learning, Kleim et al. (2002a) showed that endurance exercise (wheel running) did not alter forelimb movement representations which is in line with earlier findings that fail to show synaptic structural changes (e.g., synaptogenesis) in response to endurance exercise (Black et al., 1990) but instead a greater density of blood vessels in layer V of the forelimb motor cortex (Kleim et al., 2002a). Also, exercise-induced blood vessel density increases were reported in other rodent studies using histological methods (Black et al., 1990; Isaacs et al., 1992) as well as MRI (Swain et al., 2003). Thus, endurance exercise does likely not lead to neuronal adaptations (except of neurogenesis in the hippocampus) but exercise-induced vascular changes might contribute to subsequent learning-related neuroplasticity (Adkins et al., 2006) since memory formation and consolidation are energy-demanding processes (Tononi and Cirelli, 2014). Thus, an improved supply of oxygen and other fuels to motor regions might be of relevance.

Molecular Level

BNDF and Lactate

On a molecular level, a concerted action of key neurochemicals is required for the occurrence of motor learning- and exercise-related physiological and structural changes (Korte et al., 1995; Boulanger and Poo, 1999; Monfils et al., 2005; He et al., 2013). Acute endurance exercise has been shown to enhance the levels of many memory-related trophic factors like BDNF, VEGF, and IGF or neuromodulatory transmitters like dopamine, epinephrine or norepinephrine in peripheral blood circulation (Rojas Vega et al., 2006; Rojas Vega et al., 2012a; Phillips et al., 2014).

Among the abovementioned neuromodulators and neurotrophic factors, BDNF is likely the best investigated and maybe the most important one. Using BDNF-mutant mice,

Korte et al. (1995) first demonstrated that BDNF contributes to LTP expression. In the same year, it was reported that rats exposed to 7 days of voluntary wheel running exercise showed increased BDNF gene expression in the hippocampus and certain layers of the caudal neocortex (Neeper et al., 1995), providing first evidence that growth factors may be responsible for the beneficial effects of exercise on cognition and the brain. These observations led to a series of studies examining the effects of exercise on growth factor signaling, brain structure and function (Voss et al., 2013b).

BDNF is involved in all steps of memory formation from neuronal excitation to the induction and maintenance of early and late forms of LTP (Korte et al., 1995; Vaynman et al., 2003; Bekinschtein et al., 2008; Gómez-Pinilla and Feng, 2012). Importantly, this not only applies for the hippocampus but also for the motor system (He et al., 2013). BDNF and its receptor TrKB are important molecular intersections of exercise and motor learning (Klintsova et al., 2004).

Because the exogenous administration of BDNF is problematic in humans (for discussion see Fumagalli et al., 2006), natural ways to elevate BDNF levels and other neurochemicals are a promising way to counteract motor dysfunction and to enhance motor learning in healthy people. In this respect, intrahippocampal injection of BDNF enhances cognitive learning in mice (Alonso et al., 2002) and acute exercise-induced increases in peripheral BDNF levels correlate with behavioral parameters of motor skill learning (Skriver et al., 2014) although the exact relationship between central and peripheral BDNF is unclear (Di Lazzaro et al., 2007). Knowing that values of BDNF as well as other trophic factors and neuromodulatory transmitters typically increase through endurance exercise (Knaepen et al., 2010; Skriver et al., 2014) indicates that exercise may represent a promising and natural enhancement strategy for key factors involved in motor learning. It is unclear how long BDNF levels remain elevated after the exercise session. In general, exercise-induced increases in peripheral BDNF return to baseline levels within several minutes after cessation of exercise (Rojas Vega et al., 2006). However, animal research provides evidence for elevated cortical BDNF levels 5 h after completion of exercise, with the 5 h values exceeding those obtained immediately after exercise (Takimoto and Hamada, 2014). In contrast to this, many human studies examining BDNF levels in the resting state after a long-term exercise intervention report just small increases of circulating BDNF levels (Rojas Vega et al., 2012a; Szuhany et al., 2015), whereas higher exercise intensities might elicit a more pronounced BDNF increase (Baker et al., 2010). Furthermore, regular exercise may also enhance the BDNF response to an acute bout of exercise (Szuhany et al., 2015). Noticeably, a cross-sectional study examining the link between habitual physical activity and resting BDNF levels report even a negative correlation (Currie et al., 2009). This discrepancy might be explained by an increased BDNF clearance and uptake in other tissues like the brain (Knaepen et al., 2010; Rojas Vega et al., 2012a) as well as the different ways of how peripheral blood was analyzed for BDNF. Peripheral BDNF values are significantly influenced by analysis kits and BDNF determination in blood plasma, serum or whole-blood (Knaepen et al., 2010; Klein et al., 2011). While exercise immediately increases BDNF levels in the brain and periphery, their dwell time remains speculative.

Besides the changes in neurochemicals, exercise influences the energy supply of the brain. For example, recent investigations highlighted that lactate, elevated in response to exercise-induced anaerobiosis in the muscle cells (Robergs et al., 2004), is increasingly used as energy source for the brain and becomes the preferred fuel if arterial lactate values exceed the lactate values in the brain (Dalsgaard et al., 2004; Kemppainen et al., 2005; Boumezbeur et al., 2010). This fact is of particular importance since high lactate levels increased motor cortex excitability (Coco et al., 2010). Moreover, the availability of lactate plays a crucial role in long-term memory formation because the blockade of the expression of monocarbocylate-transporters (MCT), which catalyze the diffusion of lactate, reduces the transfer of lactate to astrocytes and neurons *in vitro* and impairs long-term memory in rats (Suzuki et al., 2011). Given this, the finding that an acute bout of exercise near or above the lactate threshold results in an elevated expression of MCT's is potentially relevant (Takimoto and Hamada, 2014). However, it remains to be determined how regular exercise affects brain energetics and whether this might relate to motor function and memory. Maybe most important, lactate is directly involved in growth-factor signaling in response to exercise.

BEHAVIORAL EVIDENCE

This section reviews studies involving chronic or long-term endurance exercise and studies involving acute exercise to enhance motor learning. In contrast to cognition and declarative memory, only few studies have been published examining endurance exercise-induced improvements in motor learning. Acute protocols comprise endurance exercise activities on a single day that are intense enough to evoke a systemic physiological response. Typically, acute exercise takes place immediately before (think of classical warm-up) or immediately after a motor skill practice session. Long-term exercise includes studies examining the effects of endurance exercise over longer time periods (days, weeks, months) before motor skill learning. While both types of interventions have certain neurobiological mechanisms in common, they represent disparate strategies to affect memory. In general, long-term exercise aspires to enhance the responsiveness of the brain to new environmental stimuli through enhancement of learning-induced neuroplasticity. While this is also true for acute exercise prior to motor skill practice, this type of exercise additionally targets an optimal preparation for high performance in the upcoming training session, for example by increasing arousal. On the contrary, exercising after a practice session selectively impacts motor memory consolidation (Snigdha et al., 2014). This is especially relevant from a research-methodological perspective, since the effects of acute exercise likely outlast the practice session, thus not just affecting acquisition, but also consolidation (Roig et al., 2013). Therefore, the effects of acute exercise depend on its temporal positioning in relation to motor skill practice (Roig et al., 2012). Note that it is not possible in all

cases to draw conclusions on motor learning defined as relatively permanent changes in motor behavior, since many studies lack delayed retention tests (Kantak and Winstein, 2012; Schmidt and Lee, 2014).

Acute Exercise Before Learning

Generally, warm-up aims to prepare the central nervous, neuromuscular, cardiovascular and respiratory systems for the upcoming training session and therefore ensures high performance and reduction of injury risk (Shellock and Prentice, 1985). If training sessions target motor learning, the conditions for memory encoding should be optimized as well. From a psychological perspective, this may be induced by an optimal level of arousal that is in turn dependent on the nature of the task to be practiced (Schmidt and Lee, 2014). Likely, increased arousal is enabled by an exercise-induced elevation of catecholamines like dopamine, epinephrine or norepinephrine (Winter et al., 2007; Skriver et al., 2014). Additionally, as stated in the previous section, an upregulation of neurotrophic factors like BDNF may benefit subsequent learning-induced synaptic plasticity (Winter et al., 2007; Mang et al., 2014; Skriver et al., 2014). Furthermore, endurance exercise has shown to alter cerebral blood flow (Ogoh and Ainslie, 2009; Dietrich and Audiffren, 2011), reduce intracortical inhibition in exercised (Yamaguchi et al., 2012) as well as non-exercised limbs (Singh et al., 2014a; Smith et al., 2014) and to improve the conditions for the induction of synaptic plasticity (McDonnell et al., 2013; Mang et al., 2014; Singh et al., 2014b). What is the behavioral evidence with reference to the effects of acute endurance exercise prior to motor skill performance and learning?

One study specifically examined the role of acute exercise on motor skill acquisition and long-term motor memory (Roig et al., 2012). In an experimental design with 48 healthy young male subjects split into three groups, interval cycling was conducted either before (PRE) or after learning (POST) a visuomotor tracking task, whereas controls (CON) rested. The dependent variable was the absolute retention performance of a visuomotor tracking task (RMSE) after 1 h, 24 h, and 7 days. While no between-group differences regarding the rate of motor skill acquisition were registered, it was found that both exercise groups showed better retention compared with controls 24 h and 7 days after practice. The same working group published an association study correlating the peripheral blood plasma levels of several biomarkers with skill acquisition and retention of the tracking task (Skriver et al., 2014). Blood samples were drawn immediately after exercise (PRE condition as introduced above) or rest (CON). Interestingly, lactate ($r = 0.877$) and norepinephrine ($r = 0.636$) were associated with an improved rate of skill acquisition during practice. For skill retention 7 days after acquisition, correlations were found for norepinephrine levels in PRE ($r = -0.584$), with noticeable trends toward significance for earlier retention time points (1, 24 h). Likewise, plasma BDNF levels were associated with improved skill retention 1 h ($r = -0.672$) and 7 days ($r = -0.608$) after practice. An intriguing finding of Skriver's study is the significant correlation of lactate with better skill retention at all measurement points (1 h: $r = -0.658$, 24 h: $r = -0.715$, 7 days: $r = -0.672$). We will discuss the potential role of lactate in motor learning more detailed in the next section (see Hypothetical Mechanisms for Exercise-Induced Improvements in Motor Learning). In controls (CON), none of the examined biomarkers correlated with neither skill acquisition nor retention with the exception of norepinephrine, which showed, somewhat surprisingly, the opposite pattern as observed in PRE, since higher blood plasma values at each measurement point indicated higher error values at skill retention (1 h: $r = 0.530$, 24 h: $r = 0.535$, 7 days: $r = 0.529$). Significant associations with skill acquisition and retention were not found for dopamine, IGF-1 and VEGF in neither group.

Inspired by Roig's study, Mang et al. (2014) examined the effects of an acute bout of high-intensity exercise on PAS-induced LTP-like plasticity and on learning of an implicit visuomotor tracking task. A motor tracking task had to be acquired under different conditions and memorized approximately 24 h later. Subjects received either exercise or a resting control period before acquisition of a learning sequence. Serum BDNF blood samples were collected immediately before and after exercise. While the spatial task component of the tracking task was not affected by exercise, the temporal components improved from early to late practice and this improvement was preserved 24 h after practice in the exercise condition. Given the exercise-induced improvement especially of the timing-related task component, the authors hypothesized that exercise specifically affected cerebellar function (Mang et al., 2014). Despite the marked 3.4-fold increase in serum BDNF following exercise, significant correlations between normalized BDNF change and any behavioral data were not found. Note that a positive effect of an acute bout of exercise on skill acquisition was also observed by Statton et al. (2015). Using 30 min of moderate-intensity exercise prior to motor practice of a sequential motor task, Statton et al. (2015) observed improvements in skill acquisition but not skill retention which is in contrast to the above mentioned results of Roig et al. (2012) and may be induced by the different exercise intensities (high- vs. moderate-intensity).

As an intermediate result, the reported studies conducted in laboratory settings observed beneficial effects of an acute bout of high-intensity exercise prior to skill acquisition on motor learning as objectified with delayed retention tests (Roig et al., 2012; Mang et al., 2014) and enhanced performance improvements during initial practice (Statton et al., 2015). However, despite of the similar structure and intensity of exercise, a significant association of the behavioral data with BDNF was not consistently reported (Mang et al., 2014; Skriver et al., 2014).

Given the facts that the mastering of comparably simple skills like tracking does not require high amounts of practice and that it is a part-body movement questions the ecological validity of such findings, especially with respect to whole-body movements (Wulf and Shea, 2002). To gain insight into more complex motor learning processes, a recent meta-analysis focused exclusively on the performance of whole-body, psychomotor tasks following any type of moderate and strenuous acute conditioning exercise (endurance, resistance, balance) (McMorris et al., 2015). The results obtained from 28 studies involving 570 participants

revealed a slightly positive effect size for moderate ($g = 0.15$), and a considerably negative effect size for high-intensity exercise ($g = -0.86$). These results are contrary to the view that moderate, and even more high-intensive, warm-up improves performance.

The reasons why especially resistance and high-intensity endurance exercise might have detrimental effects on performance are not examined systematically to date. Theoretically, this effect could be based on reduced cortical excitability (Takahashi et al., 2011) or increased intracortical inhibition (Sidhu et al., 2013). Notably, studies registering a positive effect of high-intensity exercise on motor learning used lower limb exercise to promote skills performed with the upper extremity (Roig et al., 2012; Mang et al., 2014). This suggests that a local peripheral and/or central fatigue mechanism may affect exclusively the pre-strained muscle groups, but not the non-exercised limbs (note that this might just apply for endurance and not for resistance exercise, c.f. Takahashi et al., 2011). In line with this, increased PAS-induced synaptic plasticity after 20 min high-intensity interval cycling was observed in the non-exercised abductor pollicis brevis muscle (Mang et al., 2014). Also remarkably, studies using low to moderate intensity endurance exercise showed facilitative effects on complex motor skill performance like shot putting (Anshel and Novak, 1989) or soccer dribbling (McMorris et al., 1994). This suggests that the facilitative effect of exercise prior to motor skill practice is effector-dependent and not limited to simple skills like tracking.

To sum up, evidence indicates that acute exercise improves motor skill learning but further research is required to disentangle the effector-specificity of this improvement. Based on the existing evidence, a negative effect on motor skill performance and learning might be expected if warm-up exercise is potentially fatiguing and involves at the same time the main effectors that are important for the execution of the skill to be practiced in succession.

Acute Exercise After Learning

Immediately after motor practice, the motor memory trace is thought to be in a fragile state and practice-induced skill improvements need to be transformed into a persistent state (McGaugh, 2000; Robertson et al., 2004). This applies for both declarative and procedural memories (Mayford et al., 2012) and for the latter, incremental learning can be viewed as an ongoing cycle of consolidating fragile memory traces (Trempe and Proteau, 2012). This is relevant for the entire motor learning period because already stabilized memories may become partly labile through reactivation in a subsequent practice session, and thus need to be re-stabilized again (Alberini, 2005; Dudai, 2012).

While one promising possibility to facilitate consolidation is sleep, another lately discussed option might be a bout of endurance exercise immediately after practizing a motor skill. The theoretical basis of this strategy is that the neurobiological machinery of memory formation remains active after the termination of motor practice. In the first hours after practicing a motor skill, molecular blockade (Kleim et al., 2003) or downregulation of corticospinal excitability (Muellbacher et al., 2002) of M1 or learning a motor interference task (Brashers-Krug et al., 1996) can disrupt motor memory consolidation to a significant degree (reviewed in Robertson et al., 2004; Krakauer and Shadmehr, 2006). With the passage of time after initial practice cessation, the susceptibility to interferences gradually descends (Krakauer and Shadmehr, 2006).

From a neurobiological point of view, the persistence of LTP and its resistance against interfering stimuli could be the crucial mechanism allowing for proper skill consolidation (Cantarero et al., 2013). Intact BDNF release and function of its receptor TrkB are important for the persistence of LTP (Bekinschtein et al., 2008). Therefore, the exercise-induced elevation of neurotrophins like BDNF and catecholamines like norepinephrine (Segal et al., 2012; Skriver et al., 2014) might contribute to enhance offline learning and/or to minimize the effects of interfering stimuli in the consolidation time window.

As mentioned in the previous section, Roig et al. (2012) showed that acute high-intensity exercise immediately after skill acquisition facilitates long-term motor memory. When directly comparing the two intervention groups (exercised before [PRE] or after skill acquisition [POST]) it was shown that the group that exercised after practice outperformed the group that exercised before practice in the retention test 7 days after skill acquisition. Hence, this study provided first evidence that a single bout of exercise after practicing a motor skill can enhance off-line learning.

But does post-learning exercise also protect against task interference within the consolidation window? Rhee et al. (2015) asked undergraduate subjects to learn a motor sequence task. Three experimental groups practiced according to the classical memory stabilization paradigm: acquisition of the target sequence followed by practicing an alternative (interfering) sequence 2 h later and a retention test (consisting of three trials) of the target sequence 24 h after the first practice session. While one of these groups rested between acquisition of the target and alternative sequences (ALT), the experimental groups conducted an acute bout of exercise either immediately after the target sequence (IMM) or immediately before the alternative sequence (END). The authors found that exercise contributed to the emergence of an off-line performance gain in the retention test session despite of task interference. But this was only true for the first retention test trial in the END condition.

Long-Term Exercise

Regular exercise training conducted over months or even years leads to numerous epigenetic adaptations in different organ systems and tissues including skeletal and cardiac muscle cells and the brain (Hawley et al., 2014; Heinonen et al., 2014). Recently, the use of long-term endurance exercise to prime the molecular machinery for subsequent learning is increasingly recognized by scientists from basic research (Berchtold et al., 2005, 2010; Korol et al., 2013). In line with this, long-term exercise before learning is assumed to be a promising intervention strategy especially for motor rehabilitation (Mang et al., 2013; Petzinger et al., 2013; Stoykov and Madhavan, 2015) suggesting a general positive transfer effect of endurance exercise

on motor skill learning (Kleim and Jones, 2008) that has already been proved empirically (Quaney et al., 2009; Wang et al., 2015). However, there is a general lack of studies examining the effects of long-term exercise on motor learning and performance so that this area of research must be considered as largely underexplored to date (Stoykov and Madhavan, 2015).

A pilot study assessing the role of long-term physical activity on motor skill learning was conducted with 10 elderly subjects (age range 72–91 years) divided into two groups (Bakken et al., 2001). The exercise group passed through a physical activity program including calisthenics, stationary cycling and walking over 8 weeks (three training sessions/week), whereas controls rested. A finger-movement tracking task was tested before and after the 8 weeks. The exercise group showed a significant positive development in the accuracy index of a finger-movement tracking task from pre- to post-intervention compared with controls, whose performance worsened over time. However, the small sample size and the between-group differences especially regarding resting heart rate and blood pressure makes a generalization of the results difficult even for this age group.

In a more recent animal study, Buitrago et al. (2004) introduced the rotarod motor learning paradigm (balancing on an accelerated stick) and provided five rats daily access to a closed running wheel for a period of 7 days. The rats were kept in the wheel until they ran a predetermined distance of 100 m per day (except for day 1). Wheel-running was followed by 8 days of rotarod training. In the control condition, five rats exclusively practiced the rotarod task. Interestingly, the exercise group showed higher initial levels of rotarod performance and this advantage remained stable until the end of the rotarod training period. The authors interpreted this finding as a positive transfer effect of wheel-running movements to the rotarod task by means of an improved motor control through placement of steps to maintain balance and speed. However, one might counter the assumption that wheel running led to a specific transfer effect (for example, on balance ability) since running is considered to be a simple, well-practiced, automated and therefore hardly challenging movement skill for mice (Black et al., 1990). In line with this assumption, prior studies failed to observe synaptogenesis in response to wheel running (Black et al., 1990). The occurrence of a general positive transfer effect evoked by long-term exercise (Adkins et al., 2006; Kleim and Jones, 2008) should at least be considered as an alternative hypothesis to the assumption of a specific transfer of wheel running on locomotion-related abilities like balance.

The (sparse) existing evidence suggests that even comparably short periods of exercise are sufficient to prime the underlying neurobiological substrates for motor learning. Whether regular exercise over several months or years reveals additional benefits for motor learning is purely speculative to date. While a minimum amount of exercise is required to prime the molecular machinery for learning (Berchtold et al., 2005), the sustainability of exercise-induced adaptations is likely higher in the case of long-term compared with short-term exercise periods (Hötting and Röder, 2013).

HYPOTHETICAL MECHANISMS FOR EXERCISE-INDUCED IMPROVEMENTS IN MOTOR LEARNING

Our working hypothesis is that endurance exercise improves motor learning through facilitation of motor learning-related neuroplasticity (**Figure 1**). However, the causal link between exercise- and motor learning-related neuroplasticity has not yet been established (see Introduction). We previously reviewed behavioral and neurobiological evidence obtained in separate studies and we will now continue with the development of hypotheses concerning their mechanistic link.

At the molecular level, skeletal muscles can act as endocrine organs capable of secreting molecules relevant for neuroplasticity (Phillips et al., 2014; Lucas et al., 2015). Understanding the link between exercise-induced changes in peripheral biomarkers and the brain is of critical importance. Here, solid correlations between brain tissue and peripheral BDNF levels were found (Karege et al., 2002; Klein et al., 2011). A possible way by which exercise increases BDNF under physiological conditions could be the transport of peripheral-derived BDNF to the brain via the blood-brain-barrier (Pan et al., 1998; Di Lazzaro et al., 2007). However, Matthews et al. (2009) showed that BDNF mRNA and protein are increased in skeletal muscles after exercise, but the increased BDNF seems not be released into circulation. Analyses of blood samples from the radial artery and the internal jugular vein under resting and exercise conditions indicate that the brain itself may account for 70–80% of the BDNF levels circulating in peripheral blood vessels (Rasmussen et al., 2009). Therefore, changes in peripheral BDNF levels seem to be mainly caused by alterations in brain BDNF release into circulation.

A biomarker of potential interest for motor learning-induced neuroplasticity is lactate. Lactate is released from skeletal muscles during exercise and lactate in brain tissue modulates several brain functions (for overview see Barros, 2013) such as the survival of neurons (Fünfschilling et al., 2012; Lee et al., 2012) and axonal myelination (Rinholm et al., 2011). As we have outlined in the previous section, peripheral-derived lactate contributes significantly to brain metabolism under the conditions of physical exercise (van Hall et al., 2009; Boumezbeur et al., 2010). Also, lactate is assumed to play a major role in the exercise-induced elevation of neural growth factors. The link between lactate and growth factors is supported by studies that mimicked endurance exercise by sodium lactate injections. For example, Coco et al. (2013) treated cultures of astrocytes and SH-SY5Y (a cell line used as a model for neurons) *in vitro* for a period of 4 or 24 h with sodium lactate concentrations ranging from 5 to 25 $mmol^{*}l^{-1}$. The results show that BDNF mRNA in the treated cultures is markedly increased in comparison to control cultures. When lactate was applied for 4 h, the BDNF mRNA increase was positively related to the concentration of sodium lactate in both cultures. This applied also for astrocytes after the 24 h treatment but not for the SH-SY5Y cells, where BDNF mRNA levels after 24 h returned to baseline. However, the exact mechanisms by which lactate increases BDNF mRNA remain to be clarified (Bergersen, 2015). In another *in vivo* study, Lezi et al. (2013)

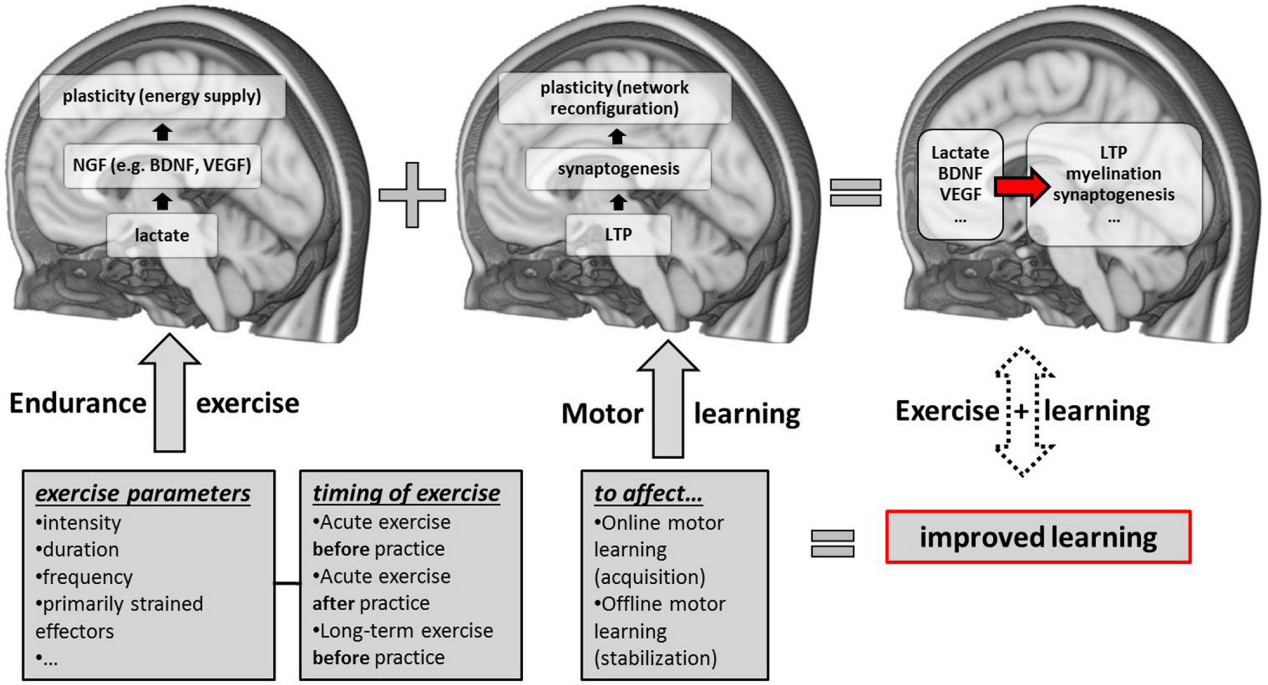

FIGURE 1 | Schematic overview of candidate neurobiological correlates and contributing factors (exercise parameters and the timing of exercise sessions with respect to motor practice) of exercise-induced improvements in motor learning. NGF, nerve-growth factors; LTP, long-term potentiation; BDNF, brain-derived neurotrophic factor; VEGF, vascular-endothelial growth factor.

reproduced certain endurance exercise-related effects by infusing sodium lactate in resting mice. One of the main findings of this study is a lactate-induced elevation of VEGF levels, another neuroplasticity-related growth factor in the brain. Importantly, Schiffer et al. (2011) recently showed that the peripheral infusion of sodium lactate enhanced levels of serum BDNF in humans in the resting-state. Since sodium lactate has a basic pH-value, it is likely that increasing lactate concentrations instead of acidosis are causally linked with the observed changes in BDNF. In line with this, pH buffering via bicarbonate infusion during high-intensity cycling does not abolish the BDNF response, providing additional evidence that the exercise-induced elevation in BDNF-levels is indeed due to increased lactatemia (Rojas Vega et al., 2012b). Furthermore, it was found that lactate stimulates the expression of genes required for long-term memory *in vitro* and *in vivo* (Yang et al., 2014). To sum up, at the molecular level, studies indicate a positive relationship between lactate levels and the concentration of neurotrophic factors, especially BDNF (Ferris et al., 2007), with strong evidence that this relationship may be causal in nature (Schiffer et al., 2011; Coco et al., 2013; Lezi et al., 2013). Despite of the absence of a correlation between exercise-induced elevations of lactate and BDNF levels after cessation of exercise, Skriver et al. (2014) showed that both biomarkers *per se* were highly associated with successful motor skill learning.

How can exercise regimens be improved to optimize neuroplasticity? The aforementioned studies indicate the importance of high exercise intensities for a high BDNF response (Knaepen et al., 2010; Huang et al., 2014), which may be mediated by an exercise-induced increase of lactate levels. Beyond that, high exercise intensities are proposed to increase cardiovascular health (Lucas et al., 2015) and showed beneficial effects on various cognitive functions (Angevaren et al., 2007; Ferris et al., 2007; Winter et al., 2007) and motor learning (Roig et al., 2012; Mang et al., 2014).

Exercise interventions that elevate peripheral BDNF levels include ramp or graded exercise tests to exhaustion (Rojas Vega et al., 2006, 2012a), continuous exercise of moderate to high intensities (Gold et al., 2003; Ferris et al., 2007; Schmidt-Kassow et al., 2012; Schmolesky et al., 2013) and high-intensity interval (HIIT) as well as sprint interval training (Winter et al., 2007; Mang et al., 2014; Skriver et al., 2014). In contrast to ramp exercise and continuous exercise, interval training consists of repeated bouts of exercise interspersed with recovery periods that comprise light exercise or rest (Billat, 2001) and is considered as an effective training method to improve endurance ability (Milanović, 2015). Moreover, as shown in animal research, 6 weeks of endurance training (six times weekly) with either HIIT (95–100% VO_2max) or continuous exercise (80% VO_2max) elevated BDNF and GDNF (glial cell line-derived neurotrophic factor) in rat brain tissue in comparison to a resting control group (Afzalpour et al., 2015). Moreover, the HIIT condition led to significantly higher BDNF and GDNF levels compared with the continuous condition (Afzalpour et al., 2015). The reason for the superiority of HIIT might be that HIIT training can be performed at velocities above the individual anaerobic threshold (IAT) (Billat, 2001), therefore allowing to

subsequently accumulate considerable levels of lactate (Buchheit and Laursen, 2013). On the contrary, continuous endurance exercise over longer durations have to be performed at intensities low enough *not* to induce lactate accumulation above the IAT to avoid fatigue (Rojas Vega et al., 2012b).

However, an important and unresolved issue to date is whether an exercise intervention should affect either the peak BDNF level at a fixed time point, for example after the cessation of exercise, or the total volume of circulating BDNF over time (Schmolesky et al., 2013). To make matters worse, the kinetics of exercise-induced BDNF changes during training are largely unknown to date, but existing data suggest that BDNF values reach their maximum level after approximately 10–20 min of moderate intensive continuous exercise and show a slight decrease thereafter (Schmidt-Kassow et al., 2012). Nonetheless, long-term exercise interventions aiming at priming the molecular machinery of motor skill acquisition and stabilization might be most effective when conducted with high intensities.

Of note, the exercise-effects on motor learning may also be dependent on the nature of the motor task (Wulf and Shea, 2002) because the brain networks involved in early and late practice depend on task complexity. Knowledge of the brain regions being involved in different stages of skill learning is critical to optimize exercise schedules that influence motor skill acquisition, consolidation and retention. Therefore, future studies are required that combine exercise and (subsequent) motor learning with observation of underlying brain changes. Disentangling the brain regions that correlate with the exercise-induced improvement in motor learning is critical to subsequently prove causality with, for example, focal brain stimulation (e.g., TMS).

Notwithstanding, recommendations regarding optimal exercise regimens are even more difficult to provide if motor skill learning should be affected by an acute bout of exercise. Even though some studies present evidence for a beneficial effect of HIIT on motor skill learning (Roig et al., 2012; Mang et al., 2014), this benefit might not apply for complex motor skill learning (McMorris et al., 2015). In the case of acute exercise prior to motor skill practice, reduced motor performance might be due to temporary peripheral and/or central fatigue effects (Taylor, 2012), especially relevant if the pre-strained effectors are at the same time critically involved in the performance of the motor skill. Besides increasing intracortical inhibition of pre-strained muscles (Sidhu et al., 2013), high exercise intensities are also known to enhance cortisol levels (Rojas Vega et al., 2006). Since low-to-moderate exercise intensities mainly revealed facilitating effects on various neuroplasticity indices and behavior, these intensities can be recommended for applied settings at the moment. However, high-intensity exercise might be useful if part-body movements of the upper limb should be facilitated by lower limb exercise (Roig et al., 2012; Mang et al., 2014) and maybe vice versa.

On the contrary, temporary fatigue effects theoretically should not be of disadvantage if exercise is conducted after practicing motor skills (Roig et al., 2012). However, further research is needed because the neuronal mechanisms that mediate motor memory consolidation in the time window after practice are not known well by now (Berghuis et al., 2015), let alone their potential interaction with a post-practice bout of exercise.

To conclude, considerable knowledge gaps remain regarding the optimal type, intensity, duration and, if applicable, frequency of exercise to promote motor learning related neuroplasticity (van Praag et al., 2014). However, especially the results obtained from basic research lay the foundation for more applied studies to be conducted in the future. In our view, properly scheduled endurance exercise protocols potentially reflect a promising intervention strategy to affect motor learning.

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frontiers in Human Neuroscience

REVIEW
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Online and Offline Performance Gains Following Motor Imagery Practice: A Comprehensive Review of Behavioral and Neuroimaging Studies

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There is now compelling evidence that motor imagery (MI) promotes motor learning. While MI has been shown to influence the early stages of the learning process, recent data revealed that sleep also contributes to the consolidation of the memory trace. How such "*online*" and "*offline*" processes take place and how they interact to impact the neural underpinnings of movements has received little attention. The aim of the present review is twofold: (i) providing an overview of recent applied and fundamental studies investigating the effects of MI practice (MIP) on motor learning; and (ii) detangling applied and fundamental findings in support of a sleep contribution to motor consolidation after MIP. We conclude with an integrative approach of online and offline learning resulting from intense MIP in healthy participants, and underline research avenues in the motor learning/clinical domains.

Keywords: movement imagery, dynamic imagery, motor consolidation, cerebral plasticity, mental processes, sleep, motor learning

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INTRODUCTION

Motor imagery (MI) is the mental representation of an action without engaging its actual execution. MI practice (MIP) refers to the repetitive use of MI to improve performance (Jackson et al., 2001). MIP research usually combines psychological and neurophysiological approaches, and represents a relevant research topic for integrative neuroscience. There is now compelling evidence that MIP positively affects motor learning, with pioneering reports dating from the first half of the 20th century (e.g., Sackett, 1934, 1935). MIP has now multiple applications in both sport sciences and rehabilitation (for an overview, see Guillot and Collet, 2008). Here, we will focus on the effects of MIP on performance in healthy individuals. Scanning the MEDLINE®/Pubmed® database (until June 2015) through the systematic crossover of the following terms: ["*Motor imagery*"/"*Movement imagery*"/"*Mental rehearsal*"/"*Mental imagery*"/"*Mental practice*"] by ["*Performance*"/"*Learning*"/"*Sport*"] yielded 188 studies (including 30, i.e., 16% of review articles). This was thought to provide a reliable corpus to convey both the development and current trends in the field. Only interventions targeting the acquisition/improvement of motor skills were included in the pool of

"motor learning" MIP articles. A related—yet distinct—research topic, since the pioneering contribution by Cornwall et al. (1991), is whether MIP can yield to force gains. Such studies primarily focused on isometric contractions, and therefore did not directly aim at improving movement kinematics. Additionally, results regarding the benefits of MIP on force remain contradictory (Guillot et al., 2010; Manochio et al., 2015).

Overall, MIP articles included both applied and fundamental motor learning studies (**Figure 1**). Applied MIP studies followed a pragmatic approach, and primary aimed at assessing MIP efficacy at the behavioral level. Interventions were delivered in the actual context of a specific sport/professional discipline (e.g., music, sports, surgery, etc.). Fundamental MIP studies additionally addressed research issues related to the psychophysiological underpinnings of the hypothesized effects on learning. Further, these studies frequently considered simple movements (typically single-joint actions) performed in standardized laboratory contexts. MIP studies published before the 1990s almost exclusively belong to the field of sport psychology. These have been elegantly summarized in seminal review articles and meta-analyses (Feltz and Landers, 1983; Driskell et al., 1994). MIP studies since 2000 include a larger proportion of fundamental studies, with an increase in functional brain imaging investigations intended to delineate the psychophysiological processes underlying MIP efficacy. Fundamental studies thus progressively outnumbered applied MIP studies (**Figure 1**). Fundamental findings on the psychophysiological underpinnings of MIP should ideally guide applied research (e.g., new domains of applications, optimal conditions of practice, etc.,). Yet, the field in fact progressively evolved from applied to more fundamental research. To convey how the field developed during the last decades, we chose to first discuss applied, and then fundamental findings, in the forthcoming sections.

Motor learning is classically defined as a change in motor behavior resulting from practice. Accordingly, motor learning is quantified in terms of performance improvements before and after a practice intervention in longitudinal research designs. When the practice intervention involves multiple sessions within a span of several days/weeks, the cumulated effects on performance are evaluated to attest motor learning. These can be summarized as *online* learning processes, since they occur as a *direct* consequence of practice. Several authors underlined in conceptual frameworks that motor learning cannot be considered a linear process of performance improvement (e.g., Yelle, 1979; Mayer-Kress et al., 2009). For instance, Doyon and Benali (2005) highlighted the involvement of functional interactions between cortico-striatal and cortico-cerebellar brain systems during the early stages of motor learning, i.e., corresponding to the rapid performance improvements consecutive to a single/a series of practice session(s). The automatization stage of motor learning, corresponding to slower performance improvements yielding to increased motor efficiency, involved to a greater extent the cortico-striatal system (Doyon and Ungerleider, 2002). While learning stages differ in terms of behavioral/neurophysiological correlates, they commonly result from online learning processes. Doyon and Benali (2005) also acknowledged the consolidation stage, characterized by delayed performance gains occurring after a latent period of approximately 6 h, in the absence of additional practice. These can be summarized as *offline* learning processes, since they *indirectly* result from practice. Performance improvements consecutive to a night of sleep is a well-established correlate of offline learning (Brashers-Krug et al., 1996; Karni et al., 1998). Delayed/spontaneous performance improvements are also sensitive to motor interferences (e.g., Korman et al., 2007). Practically, delayed performance gains and robustness to interference are two important behavioral correlates of offline learning processes (for a review see Krakauer and Shadmehr, 2006), and should thus be considered concurrently

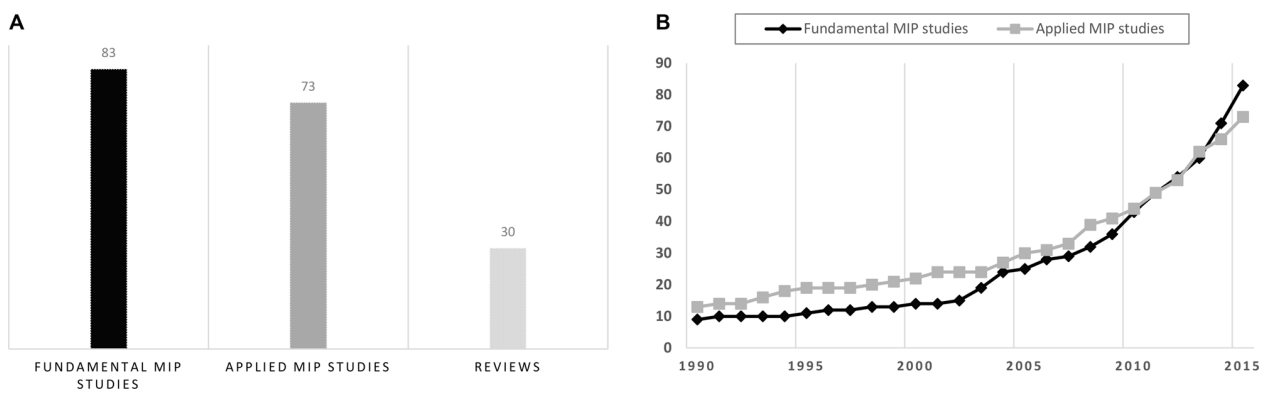

FIGURE 1 | Overview of the motor imagery practice (MIP) literature (1990–2015) based on the Pubmed/Medline® database. (A) Number of fundamental/applied MIP studies and reviews since 1990. **(B)** Cumulated number of fundamental and applied MIP studies from January 1990 to June 2015. The increase in number of functional brain imaging investigations paradigms carried out since 2000, which was due to the emergence of fundamental research topics addressing the neurophysiological underpinnings of MIP effects on motor performance, explains why fundamental studies progressively outnumbered applied MIP studies.

when investigating whether a period of sleep contributes to enhance motor performance. Former review articles considered performance improvements immediately resulting from MIP interventions (i.e., MIP effects on *online* learning processes). Surprisingly, they did not consider the potential *delayed* performance gains consecutive to MIP, in other words the MIP effects on offline learning. The present review was therefore designed to provide a comprehensive overview of motor learning after MIP in healthy participants in relation to both online and offline processes.

ONLINE LEARNING PROCESSES

Applied Studies

Effect on Quantitative and Qualitative Indexes of Performance

From a conceptual viewpoint, there has been a great deal of research on imagery processes for well over a century (Kosslyn et al., 2006), and there is now ample evidence that MIP can substantially contribute to promote motor learning. In the sport domain, MI is very popular among athletes and coaches, and has been described as a "*Centre pillar of applied sport psychology*" (Morris et al., 2005; Cumming and Williams, 2013). As mentioned previously, there has been an important number of relevant reviews and meta-analyses focusing on the benefits of MIP (Feltz and Landers, 1983; Driskell et al., 1994; Holmes and Collins, 2001; Guillot and Collet, 2008; Murphy et al., 2008; Weinberg, 2008; Schuster et al., 2011; Cumming and Williams, 2013; Rao et al., 2015). All focused on MIP findings attesting positive effects on online learning processes. This yielded multiple practical applications and theoretical models. Among them, Guillot and Collet (2008) distinguished four main imagery outcomes in their model (Motor Imagery Integrative Model in Sport), covering the main practical applications of MIP: *(i)* Motor learning and Performance; *(ii)* Motivation, Self-confidence and Anxiety; *(iii)* Strategies and Problem-solving; and *(iv)* Injury Rehabilitation. Overall, particular attention has been paid to the effect of cost-effective MIP interventions in enhancing online learning, MIP improving both quantitative and qualitative aspects of the motor performance (**Figure 2**).

MIP was first shown to enhance movement accuracy. For instance, Guillot et al. (2015) showed that embedded MIP blunted the decrease of subsequent tennis shot accuracy usually observed during high intensity interval training sessions, hence preserving the level of performance during intense practice. Afrouzeh et al. (2015) also reported greater pass accuracy in volley-ball players after MIP. A second set of applied studies provided strong evidence that MIP is likely to impact movement speed. Boschker et al. (2000) first reported that increasing or decreasing MI speed of a motor sequence might elicit comparable changes in actual movement speed. They investigated the effect of mentally or physically performing a sequence of 12 rhythmic basic steps at a slow/fast pace, and provided evidence that changing MI speed resulted in similar modifications of the actual speed during a subsequent retention test. Louis et al. (2008) confirmed that MI might affect the execution time of subsequent motor tasks, even in highly automated sport actions. Using sequential finger movement sequences, Debarnot et al. (2010) and Avanzino et al. (2009) reported that MIP, either performed in real time or at a faster pace, was likely to increase movement velocity, particularly for the most complex sequences (i.e., bimanual). Although such effects of MI on actual movement speed are not systematic (O and Munroe-Chandler, 2008), and even though decreasing MI speed to correct and adjust fine visual-motor tasks might be beneficial during the early stages of learning (O and Hall, 2009), this may be frequently detrimental to achieve expert performance—where accurate timing is seminal. Surprisingly, there is yet no experimental data examining the effect of MIP on actual movement speed which controlled, concomitantly, the possible alterations of the technical execution. Concluding about the effects of changing MI speed might thus be premature before ensuring that movement efficacy is not altered.

Finally, MIP was found to improve movement efficacy. This is reflected through both objective and subjective evaluations which addressed qualitative/quantitative aspects of the motor performance (e.g., scoring performance in a given discipline, technical realization). Overall, there is accumulated evidence that MIP contributes to achieve a greater level of sporting performance (Schuster et al., 2011; Guillot et al., 2013a; Williams et al., 2013), or through a subjective/qualitative appreciation of movement efficacy (Arora et al., 2011; Guillot et al., 2013b). Furthermore, MIP was shown not only to improve the overall performance, but also to impact specific movement kinematics. For instance, Battaglia et al. (2014) reported that both the flight time and the ground-contact time were significantly improved during performance of the Hopping and Drop Jump tests, after a mental training program in national rhythmic gymnasts. Likewise, Giron et al. (2012) provided evidence that MIP contributed to enhance pelvis and hip kinematics during dance movements, with visual and kinesthetic imagery leading to distinct peak external hip rotations. Olsson et al. (2008b) further reported that MIP might specifically improve some technical components of complex motor tasks (i.e., high-jump). The authors investigated the efficacy of an internal imagery intervention in active high jumpers by measuring four appropriate outcome measures of performance: jumping height, number of false jumps, take-off angle, and bar clearance (i.e., the virtual line-distance from the foot to the shoulder when the athlete is over the bar). Data revealed a significant improvement on bar clearance only, which is the most complex technical component of the motor sequence. Such findings confirm that researchers should not only pay attention to the final performance, but also consider technical outcome measures. This conceptual approach of performance analysis is of importance, as improving bar clearance might result in higher jumping height over time, even in the absence of immediate positive effects.

Practical Implications

Both the theoretical accounts of MI use and the experimental data designed to determine the best way to perform MI

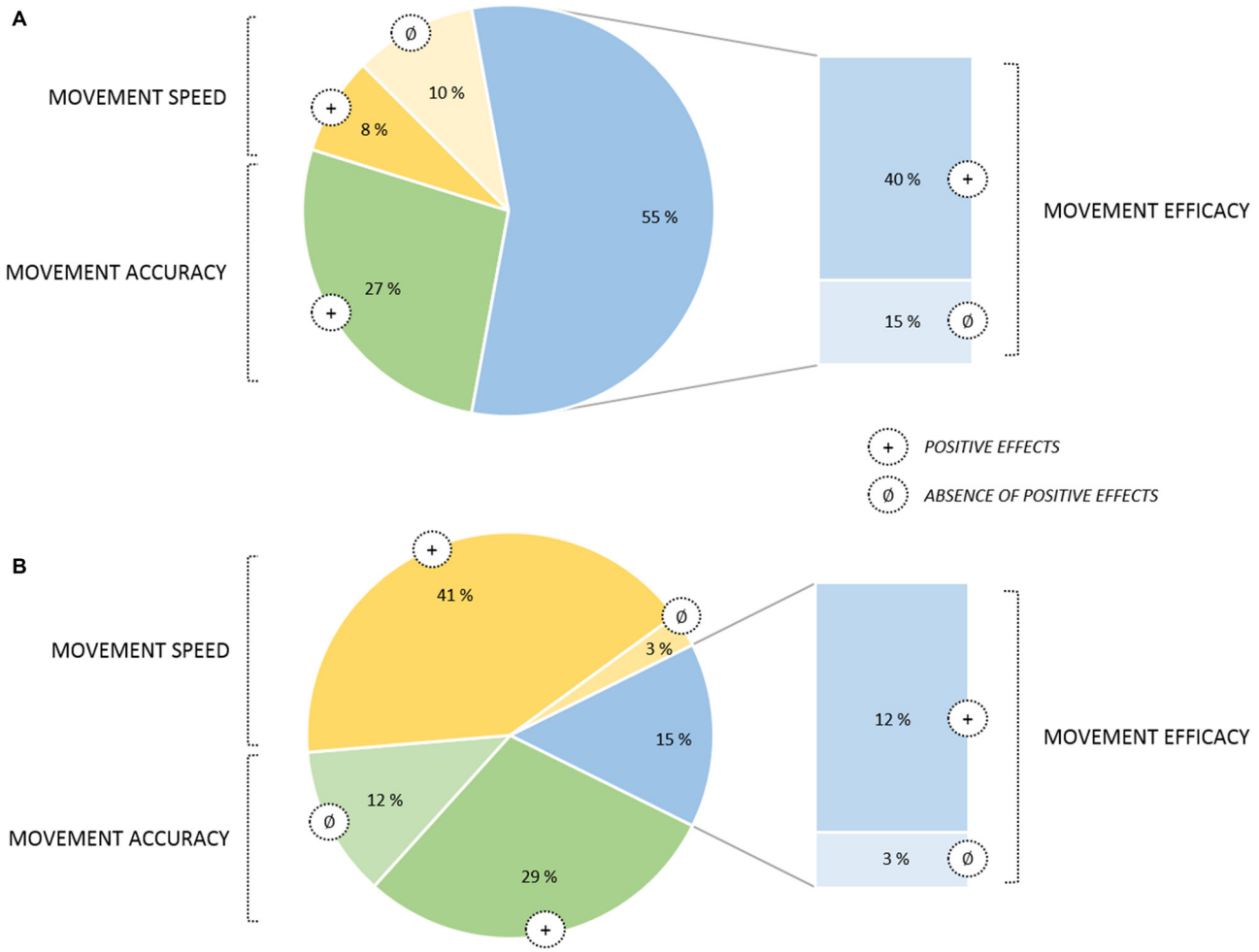

FIGURE 2 | Pie chart of movement parameters affected by online learning through MIP (based on a sample of 122 studies published from January 2000 to June 2015). (A) Graph for applied MIP studies ($n = 52$). **(B)** Graph for fundamental MIP studies ($n = 70$). Improvements quantified in terms of movement efficacy are displayed in a separate sector of the chart since this category involved a broader set of motor performance indexes. Movement efficacy encompassed both objective (e.g., movement coordination, success rate) and subjective (e.g., scale ratings on the technical execution) criterion. Noteworthy, no deleterious effects of MIP were found. Also, while for applied studies MIP efficacy on movement speed yielded contradictory results, with positive effects were almost systematically reported in fundamental studies. A reversed pattern of results emerged for movement accuracy, with positive effects being systematically reported in applied MIP studies but less consistently in fundamental studies.

adequately cover the main key-components that need to be carefully controlled to ensure the effectiveness of MI to achieve greater motor performance. Several theoretical models and MI frameworks have been designed to support efficient MI interventions (e.g., Holmes and Collins, 2001; Guillot and Collet, 2008), enabling researchers to infer optimal MIP guidelines across several disciplines requiring motor expertise (for a systematic review see Schuster et al., 2011). This approach, which is nicely and extensively illustrated in the imagery literature, will not be developed in the present review. Interestingly, there is a substantial overlap of active brain regions during MIP and physical practice of the corresponding movement (for exhaustive reviews see Munzert et al., 2009; Guillot et al., 2012a; Hétu et al., 2013). Efficient forms of MIP may strongly engage the motor systems to increase the connectivity between motor system regions. MIP should thus be more efficient if it involves the same processes than those engaged while preparing, programming and controlling actual movements (see "Theoretical Implications" section, for further development). While common brain networks are activated during both physical practice and MI of the same task, and as there is no actual feedback during MI, an important question remained to determine how adequately combining these two forms of practice and the optimal ratio of physical vs. MIP.

Courtine et al. (2004) demonstrated the superiority of alternating MI and physical practice compared to performing a single block of MI trials, as shown by a significant decrease in timing variability. A recent study by Rozand et al. (2015) further showed that performing a prolonged session of MI without any sensory feedback might be harmful, but including regular physical execution trials contributed to reduce the sensation of mental fatigue and prevented from the alteration of actual and

imagined movement durations. Interestingly, Allami et al. (2008) examined the selective efficacy of different ratios of physical to MIP. Overall, data revealed that performing MI at high rates (e.g., 50–75%) along with physical practice might result (at least) in comparable levels of performance compared to physical practice alone. A similar finding was reported by Sanders et al. (2004), who investigated the benefits of MIP in medical students learning basic surgical procedures. They concluded that MI might be as effective as PP once students have received adequate instructions and followed a monitored physical practice session beforehand.

When considering the place of MIP in mental training programs, another promising avenue is its combination with action observation (for an extensive review see Vogt et al., 2013). While the effects of action observation and MI have been extensively studied and documented in isolation from each other, Vogt et al. (2013) recently proposed an interesting spectrum ranging from congruent to conflicting action observation and MI coupling, in order to probe the two component processes. Results from recent neuroimaging and electrophysiological studies have confirmed that combining MI and action observation might result in enhanced cortical and subcortical activations relative to each form of practice alone, in regions of interest including the motor systems and the parietal areas (Macuga and Frey, 2012; Nedelko et al., 2012; Berends et al., 2013; Villiger et al., 2013; Taube et al., 2015). A substantial overlap is also observed when comparing combined action observation/MI with action execution, hence supporting the degree of functional equivalence and both the immediate facilitative and longer-term positive effects of coupling these techniques (Taube et al., 2014, 2015). Therefore, instead of contrasting the respective benefits of action observation and MIP on motor (re)learning, the best training effects might be expected by combined MI/action observation practice. Such mental training procedures might yield to a higher level of functional equivalence and potentiate the stimulation.

A debated point of consideration is the intrinsic nature of the MI work, and how it relates to physical practice. The static/dynamic distinction of imagery processes has been early considered by researchers. Paivio and Clark (1991) provided a comprehensive review of how one can imagine stationary objects, but also objects in motion or being rotated and transformed. This conceptualization refers to the perception of movement during MI of objects with a dynamic quality, or images of objects being transformed and manipulated. Since these studies, however, the dynamic properties of MI no longer characterize the symbolic representation of movements and transformations. A second and more practical consideration of static/dynamic imagery considered whether participants were moving or remained motionless during MI. According to Gould and Damarjian (1996), however, replicating the actual movements during MI, while holding a piece of equipment relevant to the sport/situation, might contribute to facilitate and increase the efficacy of MIP. We all have in mind pictures of athletes moving while imagining their subsequent performance during pre-performance routines, which challenges the traditional assumption that MI requires the athlete remaining motionless. The fact that athletes often move slightly while engaged in MI has therefore spawned interest in MI research. Experimental studies showed that such dynamic imagery might contribute to increase the vividness and temporal accuracy of MI (Callow et al., 2006; Guillot et al., 2013b; Fusco et al., 2014). As initially suggested by Gould and Damarjian (1996), who proposed that dynamic imagery promotes the recall of the sensations associated with the actual performance, we state that moving while imagining may prime and facilitate the MI experience based on the actual feedback, and therefore contribute to improve subsequent motor performance. This might also improve temporal congruence by emphasizing the degree of behavioral matching, and possibly enhance the functional equivalence between MI and motor performance (van der Meulen et al., 2014). Interestingly, Ferreira Dias Kanthack et al. (2016) investigated whether the benefits of dynamic over static MI remained effective under physical fatigue. They showed that the optimal use of static and dynamic MI may be linked to exhaustion/energy expenditure, as dynamic MI was superior to static MI to improve movement accuracy when athletes were not fatigued. In contrast, static MI remained more efficient to enhance performance under physical fatigue. They argued that the current physical state might affect the body representation, so that performing dynamic MI under fatigue may create interferences between actual and predicted body states (Demougeot and Papaxanthis, 2011). Dynamic MI might therefore be prioritized in the absence of fatigue, while static MI should be preferred under fatigue state. Based on these data, we state that dynamic imagery should incorporate slight congruent movements to enhance the process, but the amplitude of these movements should be carefully defined to avoid a misunderstanding between MI and motor performance. We therefore propose to define dynamic MI as:

"A type of MI where athletes adopt a congruent body position and embody spatial and/or temporal invariants of the movement without entirely performing it".

Conceptually, performing dynamic imagery is different from imagining while moving by engaging the full body in the action. The latter form has received less attention and is not common, even though athletes can punctually form mental representations during physical practice (Van Gyn et al., 1990; Hanrahan, 1995; Nordin and Cumming, 2007). For instance, Vergeer and Roberts (2006) investigated the efficacy of MIP during stretching on flexibility gains, imagery vividness, and perceived comfort. While there was no significant effect on performance, they reported a positive effect on the perceived comfort. More recently, Kanthack et al. (2016) examined the short-term effects of MIP during a stretching exercise, with a specific focus on its effects on muscle and autonomic nervous system responses. They reported reduced muscle activation allowing a more effective stretch of the connective tissues, hence eliciting significant stretching performance gains. Taken together, these data provide evidence of the benefits of using MI during movements, even though it challenges the common belief that MI occurs in the absence of sensory input. As outlined by MacIntyre and Moran

(2010), performing dynamic imagery and/or using MI during actual practice requires reconsidering our theoretical conceptual definitions of MI.

Fundamental Studies

Effects on Neural Plasticity

There is a general consensus that experience-dependent changes in motor behavior originate from structural and/or functional reorganizations in the connectivity of neurons, i.e., activity-dependent neuroplasticity (for reviews see Salmon and Butters, 1995; Sanes, 2003; Ioffe, 2004; May, 2011). Empirically, the assumption that MIP could induce activity-dependent neuroplasticity has been early considered (e.g., Warner and McNeill, 1988). This postulate was driven by: *(i)* motor learning experiments attesting MIP efficacy (behavioral changes being hypothetically grounded in parallel neurophysiological adaptations to those underlying the effects of physical training); and *(ii)* functional brain imaging findings supporting the functional equivalence principle. Accordingly, MI and physical practice of the corresponding action engage both overlapping neural networks and comparable patterns of connectivity between brain motor system regions (e.g., Lotze and Halsband, 2006; Munzert et al., 2009; Gao et al., 2011). Peripheral neurophysiological recordings of somatic and autonomic activities have further established a solid scientific background supporting that physical practice and MI belong to the same action-state continuum (for reviews see Stinear, 2010; Guillot et al., 2012a; Collet et al., 2013). This is in keeping with the early postulate by Stephan and Frackowiak (1996), who considered MI as an intermediate motor behavior between the cognitive motor processes and the physical performance of an action. MI would thus represent an efficient method to stimulate brain motor networks mediating skill acquisition (for recent insights, see Kraeutner et al., 2014).

While scientific evidence of activity-dependent neuroplasticity is accumulating in the field of brain computer interfaces and neurologic rehabilitation (Mokienko et al., 2013; Di Rienzo et al., 2014; Ahn and Jun, 2015), scientific reports of learning-dependent brain changes after MIP in healthy participants remain somehow limited. Pascual-Leone et al. (1995) provided a pioneering straightforward evidence of activity-dependent neuroplasticity consecutive to MIP. Using transcranial magnetic simulation, the authors observed an enlargement of the cortical representation of hand muscles controlling a piano sequence learned by MI (2 h of practice per day during 5 days). The cortical changes were identical in the MIP and physical training groups, although physical training outperformed MIP in terms of performance improvements. Interestingly, the adjunction of a single physical practice session in the MIP group enabled to reach a similar level of performance. The authors suggested that while MIP prompts activity-dependent neuroplasticity at the brain level, physical practice facilitates the actualization of the central changes at the behavioral level (stabilization of labile reorganizations). Accordingly, for simple motor tasks, MIP may replace up to 75% the physical training if a minimal ratio of physical practice is delivered to compensate the deficits in performance improvements (Allami et al., 2014). In reference to the principle of functional equivalence, and in the same vein of Pascual-Leone et al. (1995), Jackson et al. (2003) hypothesized that MIP would induce learning-dependent brain changes comparable to those observed after physical practice, and that such changes would be measured during both physical and mental performance. Based on a sequence of foot movements learnt over the course of 1 week (5 MIP sessions), functional brain imaging data with positron emission tomography confirmed the main hypotheses. Increased contralateral orbitofrontal cortex and reduced ipsilateral cerebellum activations were recorded in the MIP group, but not in the control group. These brain changes corresponded to those elicited after physical practice of the same task, as reported in an earlier study (Lafleur et al., 2002). Findings of: *(i)* reinforcement of brain activity within motor system regions (i.e., more intense and focused activations, sometimes with reduced recruitment of associative regions, **Figure 3**); and *(ii)* preservation of functional equivalence between MI and physical practice after motor learning (i.e., learning-dependent changes being reflected in brain activations during both physical and MI) were later replicated in several experiments (e.g., Lacourse et al., 2004; Nyberg et al., 2006; Olsson et al., 2008c; Zhang et al., 2011), in spite of the different nature of the motor tasks across protocols (e.g., sequential hand/foot movements, locomotor abilities).

The effects of MIP on activity-dependent neuroplasticity in longitudinal designs have not only been observed as participants physically performed the task learnt, but also as they imagined it before and after a MIP program. For instance, Sacco et al. (2006) administered a 5-day MIP intervention embedded within classical tango dance lessons, to emphasize the attentional control of locomotion in participants without any prior dance experience. During the post-test, the authors observed increased activation of the bilateral primary sensorimotor and left parietal cortices, with concomitant decrease of cerebellar activations during MI of walking. In a more fundamental approach, Sauvage et al. (2015) observed reduced fronto-parietal activations and increased cingulate/basal ganglia recruitment during MI of a sequence of foot movements learnt by MIP over a 1 week period (five sessions of 100 MI trials). Notably, transversal studies examining the neural networks controlling MI in novices and expert athletes/professionals emphasized long-term brain reorganizations mediating expertise. The most recent experiments reported differences in the resting state brain networks after MIP intervention. Particularly, these experiments emphasized increased connectivity between regions of the brain motor system, rather than differences in resting state levels of activation (Zhang et al., 2014; Ge et al., 2015). These data therefore suggest that MIP leads to large-scale functional reorganizations of the motor networks, which can be assessed from various brain states.

Recent findings keep extending the knowledge regarding the effects of MIP on online learning processes. For instance, in addition to classical brain activation contrasts, functional

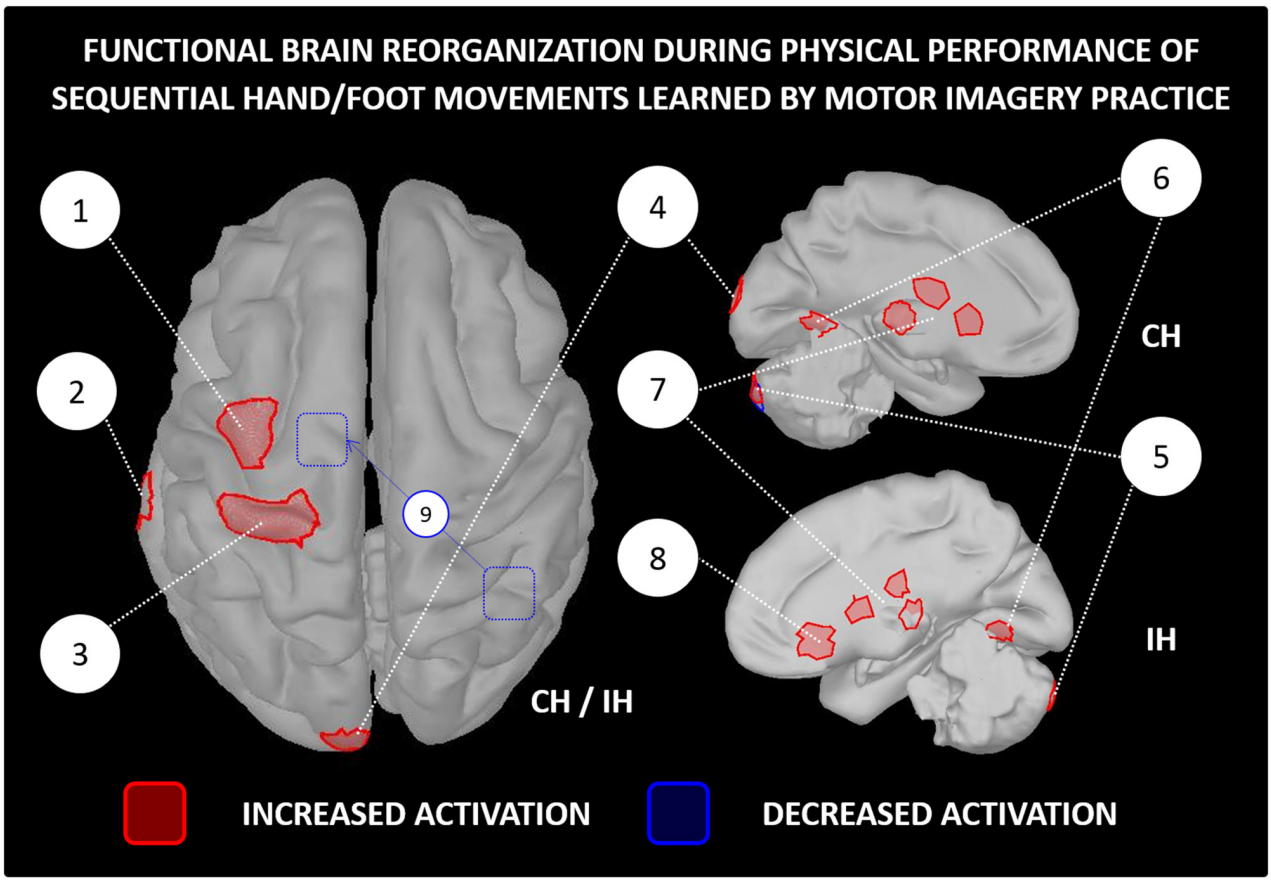

FIGURE 3 | Functional reorganization of the brain networks controlling the physical performance of a motor task learnt by MIP only. The figure is based on functional brain imaging experiments which performed source reconstruction analyses. Only paradigms involving sequential hand/foot movements met such inclusion criteria (e.g., Jackson et al., 2003; Lacourse et al., 2004; Nyberg et al., 2006; Zhang et al., 2011, 2012). Functional brain imaging experiments assessing neuroplasticity following MIP by examining brain activations *during MI* were not included (e.g., Sauvage et al., 2015). 1-Premotor cortex, 2-Middle temporal gyrus, 3-Primary motor cortex, 4-Occipital cortex, 5-Cerebellum, 6-Fusiform gyrus, 7-Thalamus and basal ganglia (caudate nucleus and putamen), 8-Orbitofrontal cortex, 9-Decreased functional connectivity between the right inferior parietal lobe and the supplementary motor area after MIP. MIP, Motor imagery practice; CH, Contralateral hemisphere; IH, Ipsilateral hemisphere.

connectivity measures brought further knowledge regarding how MIP affect the functional interplay between brain motor regions. Using graph theory analyses, Zhang et al. (2012) observed that learning effects during a finger tapping sequence in the MIP group (2 weeks of practice, 30 min of practice per day) reduced the connectivity of the ipsilateral posterior parietal cortex with cortical/subcortical regions of the motor network, notably the SMA, during both actual and imagined performance. Such changes were absent in the no-learning control group, and thus potentially reflected a more efficient allocation of mental resources to complete the task after MIP. Additionally, brain stimulation paradigms demonstrated their efficacy to facilitate or interfere with the effects of MIP on motor learning. For instance, Transcranial Magnetic Stimulation (TMS) applied over the inferior parietal lobe interfered with implicit learning of a sequential button-press task (Kraeutner et al., 2015). Conversely, applying transcranial direct current stimulation to the primary motor cortex during MI increased its beneficial effects on the online learning of a finger tapping sequence (Saimpont et al., 2015). Previously, Foerster et al. (2013) reported similar findings on writing skills using transcranial direct current stimulation of the primary motor cortex and dorsolateral prefrontal cortex. Yet, these paradigms did not include a physical training condition (with or without brain stimulation). Nonetheless, they adopted a radically different use of electromagnetic brain stimulations compared to the early neurophysiological MIP studies. Brain stimulation techniques were primarily used to assess brain changes after MIP (e.g., Pascual-Leone et al., 1995; see also Avanzino et al., 2015 for a recent TMS investigation of primary motor cortex neuroplasticity). Fundamental MIP experiments on healthy participants frequently put their findings in the perspective of clinical applications, albeit the guidelines for efficient MIP with clinical populations may vary to a great extent compared to those in healthy participants (Di Rienzo et al., 2014). In this vein, recent approaches attempted to evaluate *a priori* the clinical efficacy of MIP (and their neurophysiological basis) from data measured in healthy participants. Particularly, Volz et al. (2015)

studied whether a single session of MIP (20 min of finger-to-thumb oppositions) decreased the pain threshold evoked by thenar pressure (see Einsiedel et al., 2011; Frenkel et al., 2014 for a similar approach in a clinical model of joint immobilization). The authors measured reduced pain threshold in the MIP group, but not in control subjects. The changes in pain perception were correlated to decreased corticospinal excitability in the efferent pathways targeting the thenar during voluntary contractions, which may have implications for patients suffering from chronic pain.

The positive effects of MIP on neuroplasticity in motor performance paradigms may not be systematic. For instance, Bassolino et al. (2014) observed that, contrary to action observation, MIP of grasping exercises failed to prevent the corticomotor depression caused by 10 h of arm immobilization in healthy subjects (i.e., reduction of the corticomotor map of the first dorsal interosseus evoked by TMS). Unfortunately, the experimental paradigm did not involve any behavioral measures. While the authors concluded that MIP was inefficient to prevent corticospinal depression after immobilization (for an opposite pattern of results of MIP and action observation on corticospinal excitability, see Bianco et al., 2012), this lack of behavioral control is somehow problematic as the results contradict several experiments attesting at a behavioral and/or neurophysiological level the efficacy of MIP to limit the deleterious effects of immobilization on joint range of motion (Einsiedel et al., 2011; Frenkel et al., 2014). The number of experiments investigating activity-dependent brain changes in healthy participants after MIP increases on a regular basis since 2000, hence reflecting the consideration of neuroscientists for the method. Future research should highlight new factors which may influence the outcome of MIP interventions, thereby explaining divergent results. A recent work by Herholz et al. (2015) underlined the issue of individual profiles of responsiveness to MIP. In a piano-sequence learning paradigm, the authors detangled the neurophysiological correlates of the inter-individual predispositions to benefit from MIP. Before the intervention, participants who exhibited the highest activation intensities in the primary auditory cortex and hippocampus (while listening to the piano sequence), and in the premotor cortex and thalamic regions (while imagining the piano sequence), achieved the highest learning rates. Notably, reduced activations in frontal and occipital cortices (as well as in the precuneus) were also significant predictors of the learning rate. Future research on the neurophysiological correlates of individual predispositions towards MIP effects on activity-dependent neuroplasticity may enable to adjust MIP intervention frameworks to optimize their efficacy and potentially account for contradictory results related to the efficacy of some interventions.

Theoretical Implications

Until the end of the 20th century, the effects of MIP on online learning processes were attributed to psychological and/or cognitive factors (Kohl and Roenker, 1983). For instance, the "*Symbolic learning*" theory by Sackett (1934) proposed that mental rehearsal involved a specific focus on symbolic components such as the spatial and/or temporal invariants of the movement (due to the absence of actual motor output). This was assumed to facilitate cognitive processing during the forthcoming task performance. These theories of MIP were emphasized in early reviews that focused on MIP and online learning (Feltz and Landers, 1983) as an account of higher benefits of MIP on online learning of skills requiring a high cognitive demand (Driskell et al., 1994). Another classification of MIP use was based on the 2 × 2 conceptual framework by Paivio (1985). MI was assumed to impact both cognitive and motivational functions and to operate on general and specific levels. This resulted in four functions of MIP. Hall et al. (1998) extended this model by subdividing the motivational-general function into motivational general-arousal and motivational general-mastery sub-modalities. Overall, such classifications support a contribution of MIP to improve motor performance by driving focus on psychological factors such as strategies and routine, self-achievement, arousal/affect, self-confidence and mental toughness (for an extensive review see Cumming and Williams, 2013).

The seminal contribution of M. Jeannerod, referred to as the "*simulation theory*" (e.g., Jeannerod, 2001) conceptualized MI as an inhibited form of voluntary motor behavior (see also Jeannerod and Decety, 1995; Jeannerod, 1995). According to this framework, MI is a conscious access to the content of the motor preparation. The motor preparation would be emulated into a sensory experience due to its active inhibition during mental rehearsal: "*If motor preparation (. . .) could be prolonged, the intention to act would become progressively a MI of the same action (. . .). Actions which fail or which are cancelled at the last moment may be situations where a non-conscious program is transformed into a conscious image*" (Jeannerod, 1994, p. 7–8). Gandevia et al. (1997) argued, in the same vein, that MIP facilitates neural processing within the neural circuits controlling the action, due to subliminal activation of the somatic pathways. These theories of MIP share the postulate that MIP improves performance through the preliminary rehearsal of psychological/cognitive/neurophysiological components, which exerts a preparatory effect on the actual performance. MIP effects on performance would thus reflect "*priming effects*", namely: *"(. . .) A type of implicit learning wherein a stimulus prompts a change in behavior"* (Stoykov and Madhavan, 2015, p. 1).

These approaches are obviously sound and scientifically grounded. They may be extended at the scope of recent evidence that MI not only engages the psychophysiological processes involved during motor preparation but also those mediating the actual execution. Functional brain imaging demonstrated that MIP stimulates both premotor and primary sensorimotor brain structures (for recent insights, see Gemignani et al., 2004; Burianová et al., 2013; Kraeutner et al., 2014)[1].

[1]This result is unanimously supported by neurophysiological methods affording a high temporal resolution (e.g., magnetoencephalography, electroencephalography, transcranial magnetic stimulation), but less consistently reported in functional brain imaging experiments with a lower temporal resolution (e.g., functional magnetic resonance imaging, positron emission tomography).

Functional brain imaging evidence of activity-dependent brain reorganizations consecutive to MIP is accumulating (**Figure 3**). Assuming that both short- and long-term effects of MIP on motor performance are mediated by activity-dependent neural reorganizations (e.g., short-term changes in synaptic gain and/or long-term scaling of labile networks through stabilization of latent synapses), a neural plasticity approach of MIP effects would represent a unified framework to explain/interpret the positive results of MIP on online learning processes (for pioneering insights, see Decety and Ingvar, 1990). It is worth mentioning that this postulate derives from findings yielded by explicit online learning paradigms, where participants focused on a specific movement during MIP. Original findings by Kraeutner et al. (2016) revealed that MIP could also promote implicit learning of sequential movements (see Ingram et al., 2016 for recent insights regarding the nature of implicit learning through MIP, as revealed by transfer/interference conditions). TMS data further revealed that inhibiting parietal structures prevented implicit learning (Kraeutner et al., 2015). Detangling the neurophysiological correlates mediating implicit vs. explicit online learning through MIP thus represents a novel and exciting research issue. Finally, the postulate that MIP efficacy is grounded in activity-dependent neural reorganizations provides a neurophysiological rationale to the practical guidelines supporting efficient MIP. For instance, practicing MI in an environmental context and according to sensory modalities matching those encountered during physical practice contributes to reduce the "*subjective distance*" (Jeannerod, 1995) between overt and covert motor performance, which in turn enhances recruitment of brain motor areas (e.g., Fourkas et al., 2008; Lorey et al., 2009; Mizuguchi et al., 2013; Bisio et al., 2014; Wang et al., 2014).

OFFLINE LEARNING PROCESSES

Applied Studies

Despite some challenging results (Rickard et al., 2008; Nettersheim et al., 2015), sleep has been shown to play a critical role in the consolidation of motor performance after physical practice (Stickgold and Walker, 2005; Doyon et al., 2009; Albouy et al., 2013b), as well as action observation (Van Der Werf et al., 2009). Yet, looking for similar effects following MIP has received little attention but showed promising results. However, experimental studies looking at this issue only investigated whether a period of sleep contributed to delayed performance gains for simple movements performed in a standardized laboratory context. There is therefore no real applied studies exploring offline learning processes according to the theoretical definition of applied vs. fundamental studies adopted for the present review. Such line of research is of practical interest in the motor learning and clinical domains, but preliminarily requires fundamental studies providing strong evidence of the benefits of sleep after MIP, and determining the neural underpinnings of such offline learning effects.

Fundamental Studies

Based on the functional equivalence between MI and actual motor performance, offline performance gains following MIP might be expected during sleep, as it has been established for physical practice. First evidence of such effects comes from studies in which healthy participants performed either a motor adaptation task (requiring compensating the movement for environmental changes, Doyon and Benali, 2005; Hardwick et al., 2013), a motor sequential learning task, or a mental rotation task, before and after a night of sleep (Debarnot et al., 2009a,b, 2013). In all cases, data revealed the existence of substantial sleep-related gains following MIP. Interestingly, there was no correlation between the measure of underestimation of the time to imagine the motor sequence, which is likely to affect the MI quality (Louis et al., 2008; Guillot et al., 2012b), and actual speed gains after sleep. These results provided evidence that sleep contributes to motor memory consolidation after MIP, and further suggested that offline delayed gains are not related to the intrinsic characteristics (e.g., speed) of MI. As shown by Kuriyama et al. (2004) for actual practice, Debarnot et al. (2012a) later demonstrated that the most complex sequential finger movements to be imagined were the most effective in promoting sleep-related performance gains, with larger overnight improvement for movements involving bimanual coordination. These findings support that delayed performance gains for imagined movements partially depend on motor skill complexity. Analyses of the transitions between the elements of the motor task further revealed greatest speed enhancement for the most difficult transitions. In a more recent study, Debarnot et al. (2015) compared the effects of variable and constant MIP on the acquisition, consolidation, and transfer of visuomotor sequential learning. Data revealed significant delayed performance gains after variable MIP compared to both constant MI and the simple passage of daytime, hence providing new insight in the scheduling and content of MI sessions. Interestingly, not only a night of sleep, but also daytime naps were found to facilitate the motor memory consolidation of imagined movements, compared with spending a similar time interval in the awake state (Debarnot et al., 2011). Delayed performance gains were observed regardless of the nap duration, i.e., after short naps including 10 min of stage 2 sleep or long naps of 60–90 min period including slow-wave and rapid eye movement sleep. This result highlights the importance of non-rapid eye movement sleep including the stage 2 for efficient motor consolidation (Nishida and Walker, 2007; Morin et al., 2008; Albouy et al., 2013a).

Besides delayed gains in performance (Korman et al., 2007), the susceptibility to retrograde interference (disruptive effect of a later experience on the consolidation in memory of a prior training experience) should also be considered (Krakauer and Shadmehr, 2006). Yet, only Debarnot et al. (2010) examined the effect of a retroactive motor interference (administered 2 h after MIP) on motor consolidation after a night of sleep. As in Korman et al. (2007), they showed that performing a motor interference task prevented the expression of delayed gains at 24 h post-physical training,

while practicing the first motor learning through MIP followed by the physical interfering task did not alter the motor consolidation process (Debarnot et al., 2010). This result highlights the relevance of a period of sleep for motor consolidation after MIP, and further supports that MIP might result in a durable and flexible representation of task requirements (Wohldmann et al., 2008). Moreover, this finding suggests that MIP may occasionally be a better alternative to consolidate motor skills than physical practice, by strengthening an abstract representation that does not involve specific effectors. Interestingly, in contrast to such procedural motor interference, Debarnot et al. (2012b) later showed that a declarative interference task might affect the offline motor consolidation following MIP. Data revealed that declarative interference (i.e., word-list task) altered overnight and daytime consolidation of MIP learning, but with delayed gains in performance still occurring after a night of sleep compared to wakefulness. In other words, sleep compensated the detrimental effect of declarative interference, unlike wakefulness. Surprisingly, a last issue that has been neglected in the current literature is the potential (lack of) retrograde interference of a secondary MI task on the motor consolidation of a first motor task also learnt through MIP. Future studies will certainly consider this retrograde influence and contribute to better understand the effects of MIP on motor consolidation.

Spurred by the data mentioned above, and albeit this line of research is quite recent, combining sleep and MIP in motor learning protocols is a promising avenue. From a more theoretical viewpoint, determining the neural processes underpinning the need for sleep to consolidate motor memories after MIP, as well as the factors susceptible to limit benefits of sleep, are questions currently under consideration. Yet, whether brain plasticity observed during MI is later reactivated during the period of sleep following MIP, as shown for physical practice (e.g., Stickgold and Walker, 2007), needs to be addressed. Likewise, future research should better determine the stages of sleep that are critical for discrete steps in motor consolidation following MI. As for motor skill consolidation, there may be more than a single phase of sleep-dependent consolidation. In particular, as sleep-spindle activity is thought to play a critical role in motor consolidation by facilitating the neuronal plasticity (Barakat et al., 2011; Albouy et al., 2013b), further investigations including recording sleep-related polysomnographic data after MIP are required.

CONCLUSION

We reviewed the effects of MIP on both online and offline learning processes in healthy participants. Activity-dependent neuroplasticity resulting from MIP is a plausible origin to online learning effects assessed at a behavioral level (e.g., movement accuracy, movement speed and movement efficacy, **Figure 2**). Yet, the neurophysiological correlates of MIP on offline learning processes remain unexplored. Overall, MIP can facilitate access to motor expertise, which can be considered the long-term result of successive online and offline learning processes. Interestingly, motor expertise, in turn, yields to activity-dependent neural reorganizations of brain networks controlling both actual and imagined performance. The imagery literature provided ample evidence of such reorganizations across various disciplines (Olsson et al., 2008a; Sacco et al., 2009; Wei and Luo, 2010; Chang et al., 2011; Baeck et al., 2012; Bezzola et al., 2012; Olshansky et al., 2015; Wolf et al., 2015), hence attesting that brain activations during MI reflected life-long brain changes resulting from successive online and offline neural reorganizations elicited by intense amounts of practice.

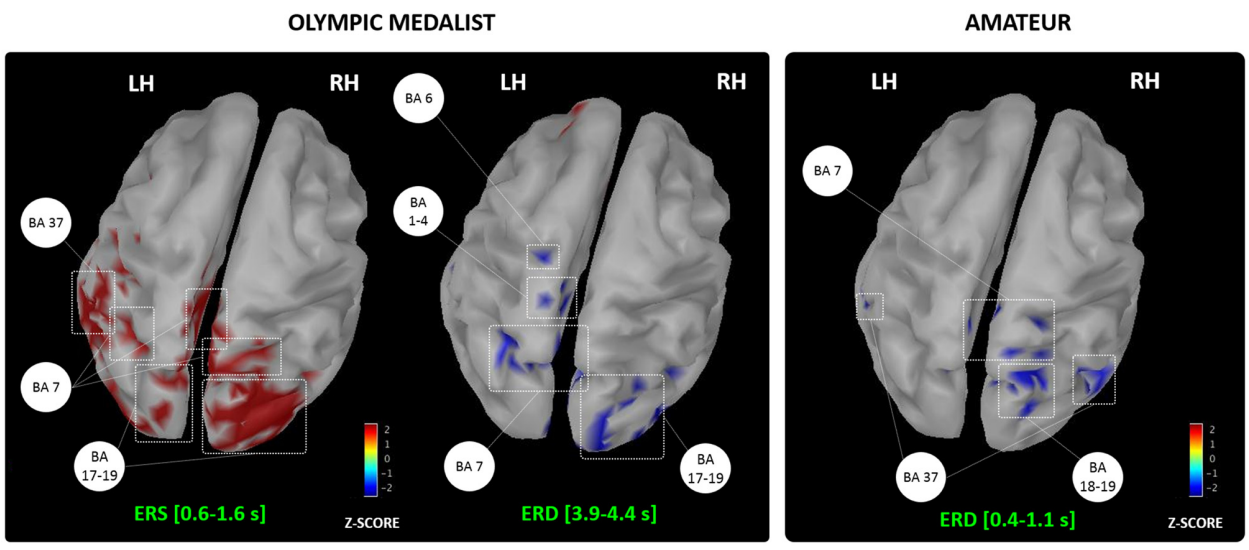

FIGURE 4 | Generators of the alpha event-related synchronization/desynchronization recorded in an Olympic and amateur athletes during MI of the snatch. BA, Brodmann areas; LH/RH, Left/Right hemispheres; ERS, Event-related synchronization; ERD, Event-Related desynchronization. Adapted with permission from Di Rienzo et al. (2016).

Brain activity during MI reflects the motor automatization taking place along the course of development (Cebolla et al., 2015), but also mirrors expertise-dependent changes in the brain networks of *athletes* (for a review see Debarnot et al., 2014). Nonetheless, past studies on expertise-dependent changes of MI networks rarely compared two *extreme* levels on the expertise continuum, namely an Olympic level champion vs. a novice athlete. The study by Di Rienzo et al. (2016) may be an original and informative illustration of such contrast to punctuate this review. Using magnetoencephalography (MEG), they gained access to the generators of mu desynchronization during the representation of MI of a snatch in an Olympic weightlifting athlete and a novice participant competing at a departmental level (**Figure 4**). They discussed the dynamic and interdependent nature of the relationship between MI and online/offline learning processes leading to motor expertise.

They first reported an event-related synchronization of alpha and beta frequencies during the first instants following the MI onset stimuli in the Olympic athlete, usually reflecting neural inhibition and resting brain areas (Pfurtscheller, 1992; Neuper et al., 2006). They argued that the Olympic participant engaged in a kind of "reset phase" involving the occipital and parietal associative cortices, which is congruent with his subjective reports of absence of visual focus and "*empty mind*". This phase appears very close from a meditative state of internal attentional focus (for a review see Aftanas and Golocheikine, 2001; Fell et al., 2010), and possibly allowed greater focus during forthcoming MI. Interestingly, the novice athlete did not report such use of contextualization strategies. Second, both participants exhibited an alpha desynchronization, but this comparable oscillatory pattern originated from the activation of very distinct neural networks. In the Olympic athlete, in addition to the bilateral precuneus activation emphasized for its role in the generation of motor images (Ogiso et al., 2000; Cavanna and Trimble, 2006), the desynchronization originated from premotor, primary sensorimotor and parietal activations. In the novice athlete, brain activations were more diffuse, and involved, in addition to associative parietal and occipital regions, the fusiform gyrus, which is emphasized for its role in online learning processes resulting from MIP interventions (Olsson et al., 2008c; Zhang et al., 2011). Overall, these data not only provide new insight about the time course of neural oscillations during MI, but also confirm that expertise is associated to a more focused recruitment of brain motor system regions during MI (for a review see Debarnot et al., 2014). By contrast, novices engage to a greater extent associative areas involved in the early phases of learning, and allocate a greater amount of mental resources to complete the MI task.

Historically, applied and fundamental MIP findings in healthy participants frequently provided a scientific rationale preceding clinical applications. Prompted by insights from Warner and McNeill (1988) (see also Decety, 1993), the number of clinical uses of MIP dramatically increased since the beginning of the 21th century (Di Rienzo et al., 2014). This attests an effective and positive transfer of MIP findings from sport sciences to clinical rehabilitation. Yet, this primarily concerns MIP findings related to online learning processes. Whether a greater understanding of MIP effects on offline learning processes (for instance at a fundamental level by determining the brain correlates of delayed performance gains) will contribute in the near future to the efficacy of clinical interventions represents a promising research issue. For instance, scheduling MIP sessions before/after periods of sleep could substantially boost the benefits and promote motor recovery. Likewise, whether current findings on online learning in healthy participants will also contribute to design effective MIP programs for clinical applications is a critical challenge. Considering the state-of-art in the field, extending our current understanding of: *(i)* the neurophysiological underpinnings of the individual predispositions to benefit from MIP; *(ii)* the relationship between MI ability and MIP effects on motor performance, assessed at behavioral and/or neurophysiological level; and *(iii)* the efficacy of combined MIP intervention (e.g., dynamic MI, action observation, etc., see "*Practical Implications*" Section) will have strong practical implications.

AUTHOR CONTRIBUTIONS

FDR, UD, SD, ES, CD, JD, CC and AG contributed to the conceptual background of the review and to the preparation of the manuscript (structure of the article, determination of the content of each section, search of information within the relevant literature). FDR, AG, UD, CC and JD wrote the article. FDR, UD, SD, ES, CD, JD, CC and AG all read and commented the article throughout the writing. They also reviewed the final article before submission.

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