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Original Article

Motor sequence learning in a goal-directed stepping task in persons with multiple sclerosis: a pilot study

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Motor sequence learning in persons with multiple sclerosis (pwMS) and healthy controls (HC) under implicit or explicit learning conditions has not yet been investigated in a stepping task. Given the prevalent cognitive and mobility impairments in pwMS, this is important in order to understand motor learning processes and optimize rehabilitation strategies. Nineteen pwMS (the Expanded Disability Status Scale = 3.4 ± 1.2) and 18 HC performed a modified serial reaction time task by stepping as fast as possible on a stepping tile when it lit up, either with (explicit) or without (implicit) knowledge of the presence of a sequence beforehand. Motor sequence learning was studied by examining response time changes and differences between sequence and random blocks during the learning session (acquisition), 24 h later (retention), and in three dual-task (DT) conditions at baseline and retention (automaticity) using subtracting sevens, verbal fluency, and vigilance as concurrent cognitive DTs. Response times improved and were lower for the sequenced compared with the random blocks at the post- and retention tests (*P's* < 0.001). Response times during DT conditions improved after learning, but DT cost improved only for the subtracting sevens DT condition. No differences in learning were observed between learning conditions or groups. This study showed motor sequence learning, by acquisition and retention, in a stepping task in pwMS with motor impairments, to a similar degree as HC and regardless of learning conditions. Whether automaticity increased remains unclear.

Keywords: multiple sclerosis; motor learning; stepping; dual tasking

Introduction

Multiple sclerosis (MS) is an inflammatory, neurodegenerative, and demyelinating disease of the central nervous system.[1] Around the world, 2.8 million people are thought to be living with MS, with the highest prevalence in Europe and the Americas.[2] MS manifests with a wide range of symptoms, among which are motor and cognitive impairments and fatigue.[1] More than 50% of persons with MS (pwMS) report gait[3] and balance[4] deficit and have been found to fall at least once within a 3-month period.[5,6] A vast majority of pwMS state that walking problems have a disruptive effect on their lives[4] and a quarter of the falls in pwMS occur during general mobility activities, such as walking and turning.[7] Furthermore, cognitive deficits can occur in various domains, such as information processing speed, learning, and memory.[8] Consequently, rehabilitation directed at mobility difficulties is an important component in the treatment of MS symptoms,[9,10] where learning or relearning movements is an essential part of most rehabilitation programs.[11,12] Given the cognitive and motor impairments in pwMS, understanding motor learning in MS is important to optimize rehabilitation.[13]

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In motor learning, a distinction is often made between explicit and implicit forms.[11] Explicit learning is thereby thought to be a more conscious form of learning and has been defined as "learning which generates verbal knowledge of movement performance, involves cognitive states within the learning process and is dependent on working memory involvement (p. 5)."[14] By contrast, implicit learning methods are thought to occur with little conscious awareness and thus do not depend as much on working memory (WM), attentional resources, and verbal knowledge compared with skills that are learned explicitly, but instead, rely more heavily on automatic processes.[14,15] Implicit forms of motor learning might, therefore, have potential advantages for rehabilitation in pwMS where cognitive deficits are prevalent.[13]

In pwMS, seven studies have been conducted on implicit and/or explicit motor learning, and all focused on sequential motor skill learning. Sequential movements are an essential component in daily life functioning,[16] think of typing, tying a knot, or riding a bike. Experimentally, these seven studies all used a serial reaction time (SRT) paradigm involving the upper limb, such as key pressing tasks,[12,17–19] touching the right finger with the thumb,[20,21] or an isometric visuomotor tracking task.[22] Within this paradigm, participants are required to react as fast as possible to certain stimuli, while repeated sequences of stimuli are hidden between random stimuli, either known (explicit) or unbeknownst (implicit) to the participant. Sequence learning has occurred when reaction times and/or errors are decreased in the repeated sequences as compared with random stimuli.[23,24] Studies comparing motor sequence learning in upper-limb tasks between pwMS and healthy adults indicated, in general, similar performance improvements during implicit conditions, but reduced improvements under explicit conditions,[12,13,17–19] although contrastingly Tacchino *et al.* reported no improvements for pwMS in the implicit condition, while healthy adults did improve.[21] Furthermore, only four studies examined both implicit and explicit learning conditions within pwMS, and contrasting results were reported on differences between those conditions in pwMS, with two studies reporting less learning in explicit conditions[17,18] and two in implicit conditions.[12,21]

However, in pwMS, motor impairments in walking and balance are prevalent, and it is unknown whether the findings on motor sequence learning from the abovementioned upper-limb tasks generalize to motor tasks involving dynamic balance.[25] Additionally, most of these studies examined motor performance only within one session, while retention of the learned motor skill after a period of not practicing the task is considered a hallmark of motor learning.[26] Therefore, it is of interest to examine whether implicit or explicit learning of sequences also occurs in pwMS during a stepping task that involves the whole body and whether retention of the learned motor skill is shown.

Another hallmark of motor learning is automaticity, a stage in which the performance can occur with relatively little attention.[27,28] Motor skills that are automatized are thought to be less easily disturbed when the cognitive resources of the performer are compromised because of psychological pressure and fatigue, or when they are less available because of a concurrent cognitive task, that is, a dual task (DT). The more automatized a motor skill is, the more robust performance on that task will be when performed under DT conditions.[29–31] In pwMS, a growing number of studies have shown that gait or balance performance is reduced while performing a concurrent cognitive task.[32–34] Previous research reported pwMS to perceive more problems with dual tasking in daily life than healthy adults[35,36] and difficulties with dual tasking during walking to be related to higher risks of falls and lower quality of life.[37,38] Considering additionally the relevance of DT performance for daily life,[39] where many activities require successful locomotion in an environment that puts demands on cognitive functioning as well, the importance of assessing DT performance in pwMS is evident. As implicit learning strategies are thought to rely more strongly on automatic processes, it could be hypothesized that implicit learning results in superior DT performance compared with explicit motor learning.[29,30]

This is, to our knowledge, the first study that investigates motor sequence learning in pwMS in a task involving whole-body movements, being more functionally related to walking, and that examines motor sequence learning as acquisition, retention, and automaticity of skill. Therefore, the SRT paradigm was adjusted to a goal-directed stepping task. A pilot study was conducted in pwMS and healthy controls (HC) to examine whether motor sequence learning of a goal-directed stepping task

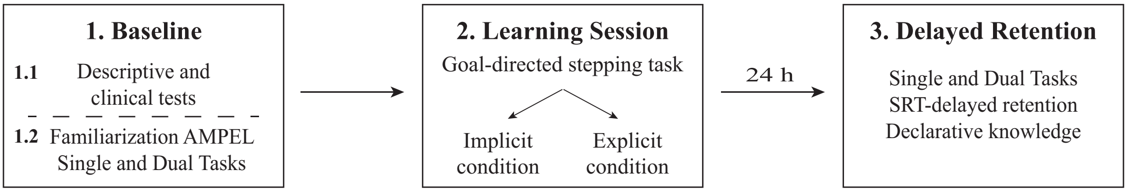

Figure 1. **Overview of the study design, with measurements divided over 3 or 4 days for healthy controls and persons with multiple sclerosis, respectively. After baseline measurements, participants were assigned to either the implicit or explicit learning condition, wherein, respectively, no knowledge or explicit knowledge of the to-be-learned sequence was given. Abbreviations: AMPEL, the Augmented Movement Platform for Embodied Learning; SRT, serial reaction time.**

involving dynamic balance occurs and whether it differs between implicit and explicit learning strategies by studying effects on, primarily, learning a sequence embedded within that task (acquisition), and secondarily, on the performance 24 h later (retention) and under transfer to DT conditions (automaticity). We hypothesize that motor sequence learning can occur in a stepping task, with similar learning for pwMS and HC in the implicit learning groups, but less learning for pwMS in the explicit learning group, and that DT performance improves, with greater automatization of the task for the implicit compared with the explicit learning strategy.

Methods

Participants

PwMS and HC were recruited between July 2020 and April 2021 via flyers and social media and via the National MS Center Melsbroek in Belgium.

Inclusion criteria were aged between 18 and 65 years, a minimal cognitive functioning as measured with the Montreal Cognitive Assessment test of ≥26, able to step upon the experimental device without using aid, and able to walk for 6 min consecutively, according to the participants' own estimate. Additional inclusion criteria for pwMS were diagnosis of MS according to the McDonald criteria, no relapse within the past 30 days, and the Expanded Disability Status Scale (EDSS) ≥2.0 and ≤5.5. Participants were excluded when there were other medical conditions interfering with mobility, other neurological diagnoses, or when participants were unable to understand instructions or had major hearing or visual problems. The study was approved by the Ethical Committee of Hasselt University, Belgium, and all participants received written information and signed informed consent (ClinicalTrials identifier NCT04538872). No *a priori* sample size calculation was conducted owing to the lack of existing data on this particular task performance.

Study design

The study was a randomized controlled pilot trial with pwMS and HC assigned to one of the two learning groups, with the implicit or explicit learning conditions explained below. Randomization stratified by sex (female and male), age (18–35, 36–50, and 51–65), and EDSS (≤3.5 and > 3.5) was done on the basis of a blocked list generated online via Sealed Envelope Ltd. 2020.[40] The study consisted of three test moments (see Fig. 1), namely (1) baseline testing, (2) the learning session using the SRT paradigm with the goal-directed stepping task, and (3) a delayed retention session 24 h after the learning paradigm. The interval between the baseline testing and learning session was on average 4.2 ± 1.3 in the implicit and 4.6 ± 2.7 in the explicit group for pwMS, and for HC, 12.0 ± 10.7 in the implicit and 22.3 ± 18.6 in the explicit group. For pwMS, baseline testing was divided over 2 days (Fig. 1; 1.1 and 1.2, with an average interval of 3.1 ± 7.6 days).

Baseline

At baseline, various descriptive and clinical measures were taken, which are described in more detail below. Furthermore, participants were familiarized with the device used for the goal-directed stepping task and with the task itself. Thereafter, single tasks (STs) and DTs of the goal-directed stepping task and walking were performed.

Descriptive and clinical measures

Demographic measures included age, sex, height, weight, and level and years of education. For pwMS, date of diagnosis, type of MS, EDSS,[41] and MS medication were noted. Cognitive functioning was

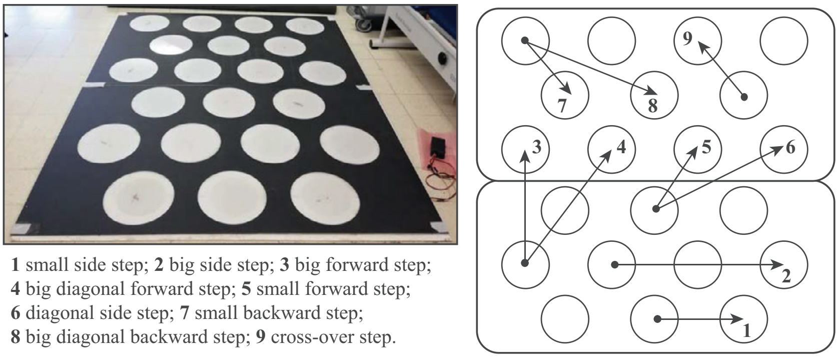

Figure 2. **The Augmented Movement Platform for Embodied Learning (AMPEL) with the steps used throughout the study. The dot is the left foot and the arrow points to the tile the right foot has to go. The same type of steps were used for the left foot, only in the left (mirrored) instead of the right direction.**

assessed using multiple tests. The Brief Visuospatial Memory Test (BVMT) was used to assess visual learning and the 10/36 Spatial Recall Test (SPART) to assess visuospatial learning.[42,43] The Symbol Digit Modalities Test (SDMT) and the Paced Auditory Serial Addition Test (PASAT)−3 seconds were conducted to assess information processing speed and concentration.[42,43] Last, the auditory digit span backward and the visually digit span forward (Corsi) tests were conducted to assess auditory and visual WM, respectively.

Motor functioning was assessed using the 6-min walking test (6MWT),[44] the Timed-Up and Go (TUG),[45] the Timed 25-foot walk (T25FW),[46] the Four Square Stepping Task (FSST),[47] the Berg Balance Scale (BBS),[48,49] the Timed Tandem Walk 3 meters (TTW-3),[50] and the Motricity Index.

Patient-reported outcomes were the Movement Specific Reinvestment Scale (MSRS) to assess someone's disposition for conscious control of movement,[51] the dual-task questionnaire (DTQ),[52] the Multiple Sclerosis Walking Scale-12 (MSWS-12),[53] the modified Fatigue Impact Scale (MFIS),[54] the MS neuropsychological screening questionnaire (MSNQ),[55] and the activities-specific balance confidence scale (ABC).[48,49]

Goal-directed stepping task and familiarization

The experimental task was a goal-directed stepping task for which the device AMPEL (Augmented Movement Platform for Embodied Learning)[56] was used (see Fig. 2). AMPEL is a platform consisting of 21 interactive stepping tiles equipped with LEDs in a color of choice and able to recognize impact (e.g., whether someone is on the tile or not). The software controls the light of each tile separately. The experimental task was a reaction time task in which participants started in front of AMPEL and were asked, *"to step as fast as possible on the tile that lights up."* When the participant stepped on the target tile with, for example, the right foot, that tile turned off, and the next one lit up, on which they subsequently stepped with the left foot. The response–stimulus interval was set at 100 milliseconds. Participants were asked to start with their right foot, continue with alternated right-left stepping, and keep the same orientation on the board (face forward).

Figure 2 shows the type of steps that were used throughout the study for the right foot (i.e., the tiles that could have lit up after placement of the left foot on a tile). The same steps were used for the left foot but mirrored. At baseline, before testing on AMPEL, participants were familiarized with stepping on AMPEL and with the task. First, they were asked to just walk over the platform; second, participants performed every type of step once with both feet, and, last, the goal-directed stepping task was practiced twice with 28 steps consisting of an order of steps not used in the rest of the experiment. This practice order was repeated once again before starting the learning session on the second test moment.

Dual-task paradigms

Multiple cognitive and motor tasks were performed as STs and DTs at baseline and delayed retention. Two types of motor tasks and three types of cognitive tasks were performed as STs and combined into

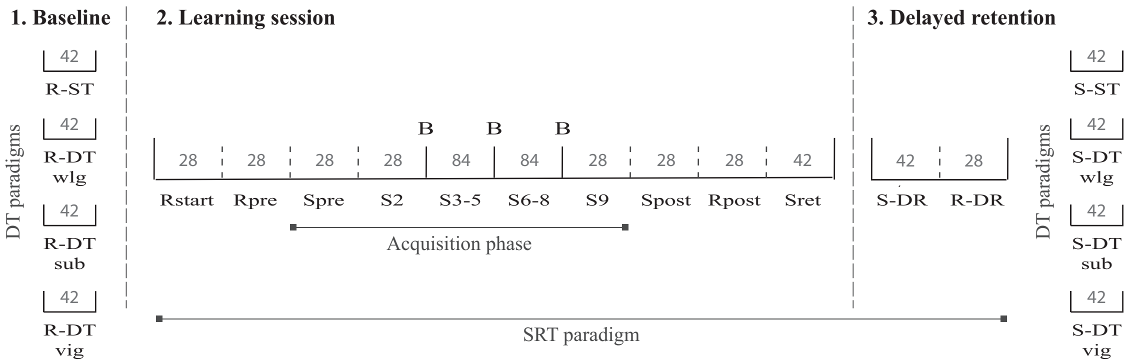

Figure 3. **Designs of the goal-directed stepping tasks performed on AMPEL at baseline, during the learning session, and at delayed retention. The figure presents the different goal-directed stepping tasks performed on AMPEL, with the ST and DT stepping tasks illustrated vertically on baseline (left) and delayed retention (right), and the design of the SRT paradigm with the goal-directed stepping task during the learning session and at delayed retention illustrated horizontally (middle). The number of steps is given per task or block. Abbreviations: B, break; DR, delayed retention; DT, dual-task; R, pseudorandom block; ret, retention; S, sequence block; ST, single-task; SUB, subtracting sevens; VIG, vigilance task; WLG, phonemic word list generation task.**

six DTs. For the DTs, participants were instructed to perform both tasks as well as possible. The order of the blocks ("ST cognitive," "ST motor," and "DT"), as well as the order of the cognitive tasks within the ST cognitive and DT blocks, were alternated between participants.

The motor tasks used were (1) the goal-directed stepping task on AMPEL ("step") and (2) walking at self-selected speed ("walk"). (1) In the stepping tasks, the goal-directed stepping task on AMPEL was used as described previously, with participants stepping as fast as possible on the target tiles. Every stepping task consisted of 42 steps, and the duration was thus dependent on the participants' speed (Fig. 3, vertically). (2) During walking tasks, participants walked for 30 s at a self-selected speed on a 30-m, free-of-obstacles, quiet walkway marked with cones at the ends. The examiner walked closely behind the participant and measured the distance walked within 30 seconds. The stepping tasks were always performed before the walking tasks within the ST motor and DT blocks.

The three cognitive tasks were (1) the serial subtraction sevens (SUB) tasks, (2) the word list generation task (WLG), and (3) the vigilance (VIG) task. These three were chosen on the basis of previous research showing the greatest interference for the subtraction task,[35] specific interference for pwMS compared with HC for the WLG,[32] and the VIG task as an externally interfering task in contrast with the previous two being internally interfering tasks.[57] (1) In the subtraction task, participants were asked to continuously subtract seven starting from a given number (152, 167, 174, 186, or 198). (2) In the phonemic WLG, participants were asked to come up with as many words as possible, starting with a certain letter. Personal, geographical, and brand names or numbers were not allowed. The letters D, G, O and K, T, M were used and randomized between participants across test moments (i.e., baseline and delayed retention) and type of tasks (i.e., ST and DT steps and DT walk). (3) In the VIG task, participants listened to a string of letters and were asked to respond with "yes" as quickly as possible when they heard one of the two given target letters ("L" and "R") and not respond to other letters. The letters were presented at the rate of one letter per 2.5 seconds. The cognitive tasks were performed for 30 s during the cognitive STs and when combined with the walking task. As the duration of the stepping task was dependent on the participants' speed to complete the 42 steps, the cognitive tasks were performed over the full duration of the DT stepping task.

Learning session

The SRT paradigm with the goal-directed stepping task. During the learning session, the goal-directed stepping task on AMPEL was performed using an SRT paradigm. During the goal-directed stepping task, the target tiles could either light up in a predetermined sequence or a pseudorandom order. The determined sequence used in the paradigm consisted of 14 steps. Per sequence block (S-block), this sequence was repeated twice, resulting in 28 steps. The pseudorandom blocks (R-blocks) also consisted of 28 steps, of which the difficulty had to be matched to the S-blocks, meaning that the steps could not be completely at random and were, therefore, also predefined. The names *S-* and *R-blocks* are used to make the distinction between the blocks consisting of the to-be-learned and repeated sequence order and the blocks consisting of the pseudorandom orders (see Supplementary Data 1 for more information on the stepping patterns during these tasks (online only)).

See Figure 3 for an illustration of the SRT paradigm. The first two blocks were R-blocks (Rstart and Rpre) to establish initial task performance. Thereafter, there was an acquisition phase consisting of nine S-blocks, with the first (Spre) giving an initial performance on the sequence and the one after these nine S-blocks (Spost) giving performance after practicing the sequence. Subsequently, a random block (Rpost) and again an S-block (Sret, consisting of three times the sequence) were performed to establish whether sequence learning had occurred. To minimize fatigue, three breaks were provided, between S2 and S3, S5 and S6, and S8 and S9, resulting in 112, 84, 84, and 126 steps per time. Physical and mental fatigue were assessed during every break and at the end of the learning session in order to monitor changes in the level of subjective fatigue during the developed stepping task and to check whether there were differences between groups during the learning session. This was done by taking a visual analogue scale (VAS) score asking, "How tired do you feel physically and mentally, respectively."

Learning groups. There were two learning groups to which the participants could be assigned: a group with either the explicit or implicit learning condition. In both groups, participants were instructed to step on the target tile as fast as possible. In the explicit group, participants were additionally given the information that, after some time, the tiles would light up in a certain repeating sequence, and the sequence was shown to the participants on the device. In the implicit group, participants were not given extra knowledge and were thus unaware at the start that the order of steps would be repeated. Participants were asked not to talk about the learning part when they knew other participants.

Delayed retention

Here, the same stepping task as used in the learning session was performed on AMPEL to examine retention of the practice-related improvements on the goal-directed stepping task and the learning of the sequence embedded in this task. This task of delayed retention consisted of three times the sequence in an S-block (S-DR) and one R-block (R-DR) (Fig. 3).

In addition, the same STs and DTs of the DT paradigms used during the baseline session were performed at delayed retention. The difference between the single and dual stepping tasks at baseline and delayed retention was the order of the steps. At baseline, the tiles lit up in a pseudorandom order to examine baseline ST and DT performance on the stepping task. At delayed retention, the tiles lit up in the predetermined sequence that participants practiced in either the implicit or explicit condition the day before at the learning session (Fig. 3). Therefore, at delayed retention, all stepping tasks also consisted of 42 steps, similar to baseline, but now these steps were three times the 14-item sequence that was repeated in the SRT paradigm of the goal-directed stepping task (see Supplementary Data 1, online only).

Last, at the end of the study, a test of declarative knowledge was conducted to check participants' awareness and knowledge of the sequence. Participants were first asked, *"What was the aim of this study?"* Participants in the implicit learning group were thereafter asked, *"Did you notice anything particular?"* and *"Did the tiles light up in a random order or was there a certain pattern?"* The last question could be answered with "at random," "pattern in a part of the task," or "constant repeating pattern in the complete task." Finally, all participants were first asked to step the sequence on the board without the tiles lighting up (autonomous stepping) and were thereafter shown four sequences from which they had to guess which one they thought they had executed. The percentage of tiles that were stepped on in the correct order during autonomous stepping was noted (percentage declarative knowledge (DK%)).

Outcome measures

Primary outcome measure. The outcome measure for the goal-directed stepping task on AMPEL was response time in milliseconds (ms), which was the time it took somebody to react to the stimulus by stepping on the target tile. Response time is, therefore, an accumulation of reaction and movement time. To calculate this response time, the time a target tile turned on (stimulus) and the time it turned off by the participant stepping on it (response) were logged for each step. Response time was calculated per step, after which an average of the response times per block was taken as an outcome measure for the SRT paradigm during the learning session and at delayed retention.

Secondary outcome measures. For the DT paradigms, the outcome measure for the ST and DT stepping tasks on AMPEL was also response time, averaged per task. The outcome measure for the ST and DT walking tasks was the walked distance (meters). Performance on the subtraction and WLG cognitive tasks in the DT paradigms was determined as "number of correct answers" in 30 s, while for the VIG task, reaction time was measured using a smartphone app developed for this study ("vigilance test"). To be able to compare cognitive task performance across tasks, only the first 30 s of the score on the cognitive task during DT step conditions was used in the analyses (although the cognitive task was performed over the full duration of the DT stepping task). DTCs were calculated for each outcome measure of the DT paradigms (i.e., response time, meters, number of correct answers, and reaction time) using the formulas below. For the outcome measures response and reaction time for the stepping and VIG tasks, respectively, the sign was flipped, such that for all outcomes, a positive DTC reflected reduced DT versus ST performance (see the equations).

$$\text{DTC}_{\text{motor}}\,(\%) = \frac{(ST\ motor\ score) - (DT\ motor\ score)}{ST\ motor\ score} * (-)100$$

$$\text{DTC}_{\text{cognitive}}\,(\%) = \frac{(ST\ cognitive\ score) - (DT\ cognitive\ score)}{ST\ cognitive\ score} * (-)\,100$$

As the ST and DT walking conditions at delayed retention were not of primary interest, it was decided to only include the baseline measurements as descriptive measures in the analysis.

Statistics

All analyses were conducted with JMP Pro 14 (SAS Institute Inc., Cary, NC). Two-sided *P* values were set at α level 0.05, and missing data were handled by listwise deletion. Normality was checked with Shapiro–Wilk tests or on the basis of conditional residual plots when linear mixed model analyses were done.

First, separately for pwMS and HC, continuous baseline measures of descriptive and clinical measures were compared between the implicit and explicit learning groups using independent *t*-tests or Mann–Whitney tests for normally and nonnormally distributed data, respectively. For categorical measures, frequency distributions were compared between learning groups using the Chi-square test. Furthermore, the same analyses were done to compare descriptive baseline measures between pwMS and HC.

Sequence learning (acquisition and retention) on the goal-directed stepping task. First, initial performance on the goal-directed stepping task in the SRT paradigm (i.e., response time on Rstart) was compared between the four groups with a two-way ANOVA, including main and interaction effects of *group* (two: MS and HC) and *learning group* (two: the implicit and explicit learning groups).

Second, to examine whether sequence learning embedded in the goal-directed stepping task occurred and whether it differed between pwMS and HC and between learning groups, a 2 × 2 × 7 mixed model was conducted with response time on the stepping task as the dependent variable. Between-subjects factors were *group* (two: MS and HC) and *learning group* (two: implicit and explicit learning groups), and the within-subjects factor was *block*, with the seven following blocks of the stepping task: Rpre, Spre, Spost, Rpost, Sret, S-DR, and R-DR. Post-hoc testing was done using Tukey's HSD.

Third, correlational analyses were conducted between the percentage declarative knowledge (DK%) and the sequence learning effect (Rpost – Spost) using Spearman correlations, as the latter outcome was nonnormally distributed for pwMS.

Fourth, physical and mental VAS scores were compared between the first break (B1) and at the end of the learning session for pwMS and HC separately, in a mixed model with learning group, break, and the interaction. VAS scores were compared between pwMS and HC using Mann–Whitney tests, as the scores were nonnormally distributed for the HC.

Performance during the DT paradigms (automaticity). Additionally, the effects of the learning session on the performance in the cognitive–motor DT step paradigms were studied by examining ST motor performance and the DTCs. First, ST motor performance of the stepping task (i.e., by response time) on baseline and delayed retention was compared for the four groups to check whether the possible motor learning in the goal-directed stepping task transferred to the ST step of the DT paradigms. To this end, a 2 × 2 × 2 mixed model was conducted with main and interaction effects of within-subjects factor *time* (two: baseline and delayed retention) and between-subjects factors *group* (two: MS and HC) and *learning group* (two: implicit and explicit).

Second, the DTC_{motor} and $DTC_{cognitive}$ for the step conditions were investigated for changes from baseline to delayed retention. Similar to the ST response time, time, group, learning group, and their interactions were included in the mixed models. Additionally, the factor cognitive DT (SUB, WLG, and VIG) and interactions with the other factors were included. The models were simplified by removing four- and three-way interactions, one-by-one, when they were not significant, as well as two-way interactions not of primary interest, leaving models only including the main effects and two-way interactions with time, where possible. Post-hoc testing was done using Tukey's HSD. In all mixed models, participants were included as random factors. In total, 0.8% of data of the SRT learning paradigm blocks and 0.6% of the DT outcomes were missing at random because of technical errors.

Results

Descriptive characteristics

Of the 23 pwMS recruited for the study, four did not comply with the inclusion criteria and could not participate. In total, 19 pwMS (EDSS 3.4 ± 1.2) and 18 HC participated in the study. Table 1 shows descriptive characteristics separately for pwMS and HC per learning group. Between pwMS and HC, in general, no significant differences were found on demographic and cognitive outcomes or the DTC_{motor} and $DTC_{cognitive}$ during the walking DT paradigms at baseline. PwMS did perform worse on all mobility- and balance-related and patient-reported outcomes.

For pwMS, no significant differences were found between the implicit and explicit learning groups on any of the descriptive outcomes. For HC, a significant difference between learning groups was only found for the TTW-3 and the DTC_{motor} of the subtraction walking condition, with lower performances for the implicit compared with the explicit learning HC group.

Sequence learning (acquisition and retention) on the goal-directed stepping task

First, initial performance on the SRT paradigm with the goal-directed stepping task (Rstart) was analyzed. Two-way ANOVA of the response time at Rstart showed no main ($P = 0.201$) or interaction ($P = 0.197$) effects for learning group, and only a main effect of group ($P = 0.002$), indicating similar initial performance between the implicit and explicit learning groups for pwMS and HC, and higher response times for pwMS compared with HC.

Second, the occurrence of learning within the goal-directed stepping task was analyzed. For each group, Figure 4 depicts the average response times on the goal-directed stepping task for all blocks of the SRT learning paradigm during the learning session and delayed retention, while Table 2 (part A) only shows the average response times for the seven blocks included in the mixed model analysis. There was no main effect for learning group ($P = 0.151$), nor were any of its interactions significant (Learning group*Block: $P = 0.195$, Learning group*Group: $P = 0.159$, and Learning group*Group*Block: $P = 0.967$), indicating no differences between the implicit and explicit learning groups for pwMS and HC. A significant interaction between group and block was found ($P < 0.001$).

Post-hoc tests showed no difference between Rpre and Spre for both pwMS and HC, indicating that the random and sequence blocks were of similar difficulty (*P's* = 1.00). Furthermore, pwMS showed faster response times in all other blocks as compared with Rpre and Spre (*P's* ≤ 0.010),

Table 1. Descriptive measures (mean ± SD) for the implicit and explicit learning groups of persons with MS and HC separately

Background characteristics	MS-IM ($n = 9$)	MS-EX ($n = 10$)	P	HC-IM ($n = 10$)	HC-EX ($n = 8$)	P	MS-HC P
Age (years)	43.1 ± 12.5	40.0 ± 11.9	ns	46.9 ± 12.9	43.3 ± 14.3	ns	ns
Gender F/M (n)	8, 1	7, 3	ns	7, 3	6, 2	ns	ns
Education (years)	15.7 ± 3.6	15.2 ± 2.7	ns	15.4 ± 2.1	17.0 ± 2.0	ns	ns
Education level 1,2,3,4,5,6[a] (n)	0,4,1,1,0,3	0,2,2,2,1,3	ns	0,1,1,6,0,2	0,0,1,2,1,4	ns	ns
EDSS (score, (min – max))	3.5 ± 1.3, (2 – 5.5)	3.4 ± 1.2, (2 – 5.5)	ns	na	na		
Disease duration (years)	8.5 ± 7.5	9.5 ± 6.1	ns	na	na		
MS type RR, SP, and PP (n)	5, 1, 3	9, 1, 0	ns	na	na		
Cognitive functioning							
SDMT (0−110)	53.4 ± 13.4	65.1 ± 19.5[b]	ns	59.8 ± 10.5	61.9 ± 5.6	ns	ns
CORSI (0−144)	50.4 ± 17.5	56.0 ± 14.7	ns	56.3 ± 24.4	50.0 ± 10.4	ns	ns
SPART (0−30)	18.6 ± 4.2	21.6 ± 5.2	0.059	23.2 ± 4.8	22.5 ± 3.5	ns	ns
BVMT (0−36)	21.2 ± 6.9	24.8 ± 7.5	ns	27.2 ± 4.7	24.0 ± 5.5	ns	ns
PASAT (0−60)	46.4 ± 8.5	48.4 ± 13.2	ns	47.4 ± 13.6	50.5 ± 12.8	ns	ns
Digit span backward (3−9)	4.0 ± 1.2	4.8 ± 1.3	0.098	4.4 ± 0.8	4.6 ± 1.4	ns	ns
Motor functioning							
6MWT (m)	435.8 ± 112.1	509.4 ± 139.8	ns	622.5 ± 104.8	628.6 ± 56.0	ns	<0.001*
TUG (s)	8.4 ± 2.0	7.2 ± 2.1	ns	6.2 ± 1.8	5.4 ± 0.7	ns	0.002*
T25FW (s)	5.8 ± 1.4	4.9 ± 1.2	ns	4.2 ± 1.2	3.6 ± 0.5	ns	0.001*
FSST (s)	11.4 ± 4.8	8.9 ± 2.1	ns	8.4 ± 2.3	6.8 ± 1.3	0.089	0.020*
TTW-3 (s)	24.3 ± 32.2	13.7 ± 8.6	ns	9.9 ± 3.6	6.0 ± 0.9	0.009*	<0.001*
BBS (0−56)	53.6 ± 4.0	54.2 ± 2.4	ns	56.0 ± 0.0	56.0 ± 0.0	ns	0.006*
MI R/L (0−100)	84.9 ± 16.7/84.9 ± 15.1	84.1 ± 19.8/93.6 ± 9.9	ns/0.088	99.1 ± 2.8/99.1 ± 2.8	100.0 ± 0.0/98.9 ± 3.2	ns/ns	<0.001*
Patient-reported outcomes							
MSRS (10−60)	44.0 ± 11.7	37.5 ± 8.1	ns	23.9 ± 7.3	27.8 ± 10.5	ns	<0.001*
MSWS-12 (0−100)	51.6 ± 21.6	41.7 ± 35.2	ns	0.0 ± 0.0	0.0 ± 0.0	ns	<0.001*
MSNQ-P (0−60)	27.6 ± 11.7	22.6 ± 15.4	ns	20.4 ± 7.7	14.5 ± 7.9	ns	0.060
DTQ (0−40)	18.6 ± 8.8	16.2 ± 12.3	ns	9.0 ± 5.0	6.8 ± 6.0	ns	0.002*
ABC (0−100)	62.5 ± 16.1	74.1 ± 22.0	ns	97.2 ± 6.1	93.8 ± 9.7	ns	<0.001*
MFIS (0−84)	47.2 ± 12.5	39.9 ± 20.8	ns	18.6 ± 16.9	9.3 ± 9.5	ns	<0.001*
Dual walking tasks							
SUB-WALK ($DTC_{motor/cognitive}$)	11.2 ± 12.8/10.1 ± 30.2	14.4 ± 15.7/2.4 ± 33.2	ns/ns	21.5 ± 11.1/11.3 ± 13.7	11.6 ± 8.5/9.0 ± 22.0	0.046*/ns	ns/ns
WLG-WALK ($DTC_{motor/cognitive}$)	14.8 ± 19.5/−8.1 ± 39.9	16.5 ± 16.9/8.0 ± 16.3	ns/ns	17.1 ± 10.7/−21.3 ± 30.2	10.8 ± 8.5/−12.2 ± 35.5	ns/ns	ns/0.093
VIG-WALK ($DTC_{motor/cognitive}$)	−0.2 ± 11.1/0.9 ± 10.5	−0.0 ± 5.1/−0.2 ± 8.3	ns/ns	2.2 ± 7.1/−3.3 ± 8.9	4.0 ± 6.2/−1.5 ± 6.0	ns/ns	ns/ns

*P value < 0.05.
[a] Educational levels: 1 (elementary school), 2 (high school), 3 (technical/vocational education), 4 (college), 5 (university Bachelor's), 6 (university Master's).
[b] One person with the maximum score of 110.
Abbreviations: ABC, activities-specific balance confidence scale; BBS, Berg balance scale; BVMT, brief visuospatial memory test; CORSI, CORSI block test; $DTC_{motor/cognitive}$, dual-task cost for the motor and cognitive domain; DTQ, dual-task questionnaire; EDSS, Expanded Disability Status Scale; EX, explicit group; F, female; FSST, four square stepping test; HC, healthy controls; IM, implicit group; L, left; M, male; MFIS, modified fatigue impact scale; MI, motricity index; MSNQ-P, MS neuropsychological screening questionnaire; MS, multiple sclerosis; MSRS, movement-specific reinvestment scale; MSWS-12, MS walking scale 12; na, not applicable; ns, not significant; PASAT, paced auditory serial addition test 3; PP, primary progressive; R, right; RR, relapsing remitting; SDMT, symbol digit modalities test; SP, secondary progressive; SPART, 10/36 spatial recall test; SUB, subtracting sevens; 6MWT, 6-min walking test; TUG, timed-up and go; T25FW, timed 25-foot walk; TTW-3, timed tandem walk 3 meters; VIG, vigilance; WLG, word list generation.

indicating a generally increased task performance, plus faster response times in Spost, Sret, and S-DR as compared with Rpost and R-DR (P's < 0.001), indicating sequence learning immediately and 24 h after the learning session (see also Fig. 4). No differences were found among the latter sequence blocks (Spost, Sret, and S-DR: P's ≥ 0.939) or the random blocks (Rpost and R-DR: $P = 1.00$), indicating consolidation of the task and learning performance. Post-hoc testing for HC showed similar results, with faster response times on Spost, Sret, and S-DR compared with Rpre, Spre, Rpost, and R-DR (P's < 0.001). However, no differences between Rpre and the latter random blocks, Rpost and R-DR, were found (P's $= 1.00$). The post-hoc tests on differences between groups (pwMS and HC) per block showed significantly faster response times for HC compared with pwMS on Rpre ($P = 0.001$) and Spre ($P = 0.002$), but not on Spost, Rpost, Sret, S-DR, and R-DR (P's ≥ 0.102).

Table 2 (part B) shows the VAS scores at the first break and at the end of the learning session per group. For the mental VAS scores, no effect of learning group, break, or interaction was found for

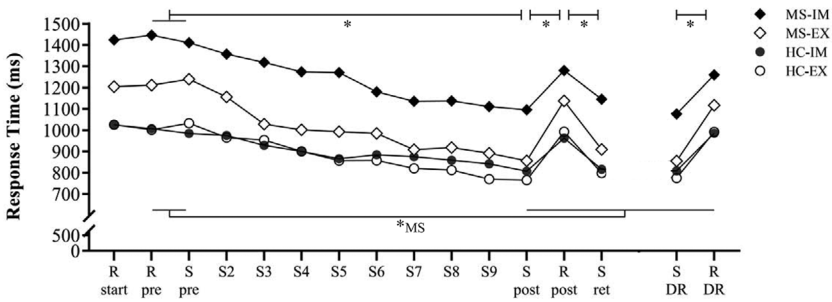

Figure 4. Response times (ms) on the goal-directed stepping task on AMPEL in the SRT learning paradigm. Response times are shown for the pwMS (diamonds) and HC (circles) in the implicit (black) and explicit (white) learning groups. * *P* value < 0.05 for all groups, *MS only for the group of MS. Differences between pwMS and HC are not shown. Abbreviations: DR, delayed retention; EX, explicit; HC, healthy controls; IM, implicit; MS, multiple sclerosis; R, pseudorandom blocks; ret, retention; S, sequence blocks.

pwMS (P's $\geq$ 0.384) and HC (P's $\geq$ 0.156), nor a difference between the groups at B1 ($P = 0.075$) or after the learning session ($P = 0.097$). For the physical VAS scores for pwMS, a main effect of break ($P = 0.003$) was shown but not of the learning group ($P = 0.060$) or of the interaction ($P = 0.060$). No significant effects were found for HC (P's $\geq$ 0.112). PwMS and HC differed significantly in physical VAS scores on both moments (P's $\leq$ 0.001).

Performance on DT step paradigm (automaticity)

Table 2 (part C) presents the absolute ST and DT motor and cognitive performances of the DT paradigms for the stepping tasks at baseline and delayed retention for the four groups. Table 3 depicts the results of the mixed model analysis for the ST motor performance and DTCs.

The ST motor performance. Mixed model analysis showed a significant main effect of time for response time on the step ST ($P < 0.001$), with no significant interactions of time with group and/or learning group (P's $\geq$ 0.064), indicating overall improved motor performance, by a reduced response time, from baseline to delayed retention, regardless of the group and learning condition. The main effect of group showed higher response time ($P = 0.001$) for pwMS compared with HC. Furthermore, the main effect of the learning group was reported for the ST step with overall higher response times in the implicit versus explicit groups ($P = 0.035$).

DTCs during the stepping task. Table S1 (online only) shows the results of the full factorial mixed model analyses of all main and interaction effects between time, group, learning group, and cognitive DT, while Table 3 presents the P values of the main effects and interactions of interest (i.e., with time), after simplifying the models. Figure 5 and Supplementary Table S2 (online only) show the DTCs for the implicit and explicit learning groups for the pwMS and HC separately.

For both the DTC_{motor} and $DTC_{cognitive}$ in the stepping task, none of the three- or four-way interactions with time were significant (P's $\geq$ 0.144). Furthermore, no main or interaction effects were found for group and learning group (P's $\geq$ 0.086), indicating no differential effect of group or learning condition over time, irrespective of the cognitive DT used. A significant interaction effect is reported for Time*Cognitive DT for both DTC_{motor} ($P = 0.018$) and $DTC_{cognitive}$ ($P = 0.030$).

Post-hoc tests show reduced DTC_{motor} and $DTC_{cognitive}$ from baseline to delayed retention for the SUB stepping task ($P < 0.001$ and $P = 0.018$, respectively), and not for the WLG or VIG stepping tasks (P's $\geq$ 0.976). Furthermore, for both baseline and delayed retention test moments, the DTC_{motor} was greatest in the SUB stepping tasks and lowest in the VIG stepping tasks (P's $\leq$ 0.026), and the $DTC_{cognitive}$ was greater in the SUB compared with the WLG ($P \leq 0.002$) and VIG ($P \leq 0.031$) stepping tasks.

Declarative knowledge

After asking whether something particular was noticed during the learning session, 2 out of 9 pwMS and 6 out of 10 HC in the implicit learning groups commented that they noticed a repetition

Table 2. **Performances on the goal-directed stepping task on AMPEL for the SRT paradigm and the DT "step" paradigms for the four groups**

(A) SRT paradigms (ms)		MS-IM ($n = 9$)		MS-EX ($n = 10$)		HC-IM ($n = 10$)		HC-EX ($n = 8$)	
Rpre		1446.6 ± 355.3		1211.2 ± 326.9		1007.4 ± 133.3		1001.2 ± 102.8	
Spre		1410.6 ± 297.9		1238.8 ± 348.9		985.0 ± 89.1		1032.7 ± 172.7	
Spost		1095.8 ± 306.0		856.0 ± 281.2		807.1 ± 98.1		764.9 ± 116.7	
Rpost		1280.2 ± 311.4		1137.8 ± 283.5		962.2 ± 78.8		992.9 ± 101.45	
Sret		1145.2 ± 302.4		909.5 ± 293.8		815.5 ± 109.8		798.2 ± 97.6	
S_DR		1076.5 ± 229.6		855.2 ± 270.7		808.6 ± 102.2		775.0 ± 118.2	
R_DR		1259.5 ± 250.9		1117.6 ± 217.2		985.7 ± 101.3		992.0 ± 108.0	
(B) VAS scores		B1	End	B1	End	B1	End	B1	End
VAS physical		4.3 ± 2.1	5.4 ± 2.1	3.6 ± 2.3	3.9 ± 2.3	2.0 ± 1.4	2.6 ± 1.6	1.1 ± 1.6	1.3 ± 1.2
VAS mental		3.6 ± 2.6	3.7 ± 2.7	2.6 ± 2.8	2.5 ± 2.4	2.0 ± 1.6	2.0 ± 1.9	0.8 ± 0.9	1.3 ± 1.4
(C) DT paradigms		Baseline	DR	Baseline	DR	Baseline	DR	Baseline	DR
STEP (ms)	ST	1467.7 ± 284.6	1143.9 ± 251.9	1209.8 ± 283.5	884.4 ± 268.9	1080.3 ± 156.2	839.2 ± 74.4	1047.6 ± 140.4	794.4 ± 106.8
	+SUB	2492.7 ± 633.8	1567.7 ± 397.8	2030.6 ± 834.8	1253.5 ± 412.7	1996.8 ± 423.1	1458.6 ± 383.6	1705.7 ± 499.6	1160.5 ± 209.5
	+WLG	2122.7 ± 756.6	1566.8 ± 407.0	1748.7 ± 548.3	1152.7 ± 363.2	1406.8 ± 260.8	1167.3 ± 228.1	1413.5 ± 259.2	1029.8 ± 149.5
	+VIG	1519.9 ± 339.0	1187.3 ± 243.4	1372.5 ± 544.2	912.1 ± 317.4	1130.6 ± 152.2	877.9 ± 131.2	1149.4 ± 128.8	906.9 ± 121.2
SUB (#*n* correct)	ST	8.6 ± 5.2	9.1 ± 4.7	9.0 ± 3.8	11.0 ± 4.6	12.0 ± 3.9	14.3 ± 3.8	13.5 ± 9.3	13.6 ± 8.7
	+STEP	4.2 ± 3.2	7.1 ± 4.0	5.6 ± 3.7	8.0 ± 3.5	6.5 ± 2.5	10.1 ± 1.7	7.1 ± 5.4	7.7 ± 3.8
WLG (#*n* correct)	ST	8.6 ± 3.0	9.3 ± 2.3	10.3 ± 3.0	10.1 ± 5.0	9.0 ± 3.2	10.1 ± 2.6	10.1 ± 2.5	10.4 ± 1.9
	+STEP	8.1 ± 2.2	9.0 ± 2.1	8.3 ± 3.9	8.4 ± 3.8	9.2 ± 4.6	9.8 ± 3.3	8.9 ± 4.1	10.5 ± 2.9
VIG (reaction time, ms)	ST	996.7 ± 144.0	958.0 ± 76.1	974.9 ± 120.6	906.0 ± 88.8	986.0 ± 130.5	966.3 ± 119.2	918.7 ± 49.8	891.7 ± 86.8
	+STEP	1087.9 ± 97.9	1088.1 ± 230.3	1000.6 ± 120.8	1011.8 ± 129.5	991.7 ± 91.8	978.1 ± 92.4	1062.6 ± 174.3	1009.2 ± 117.7

(A) Response times on the seven blocks included in the analysis of the SRT paradigm. (B) VAS scores during the first break and at the end of the learning session. (C) Response times and cognitive performances on the single and dual "step" and cognitive tasks. Abbreviations: AMPEL, augmented movement platform for embodied learning; B1, first break; DR, delayed retention; DT, dual-task; EX, explicit group; HC, healthy controls; IM, implicit group; MS, multiple sclerosis; #*n*, number of correct answers; ret, retention; SRT, serial reaction time; ST, single-task; SUB, subtracting sevens task; VAS, visual analogue scale; VIG, vigilance task; WLG, word list generation task.

of steps. When specifically asked whether there was a regularity in the steps, responses were "at random" by two pwMS and one HC, "regularity in a part of the task" by three pwMS and four HC, and "constant repeating regularity in the complete task" by four pwMS and five HC.

The choice of which sequence was conducted out of four was correctly chosen by 10/10 of pwMS and 7/8 of HC in the explicit group and by 7/9 of pwMS and 6/10 of HC in the implicit group. Last, when having to step the sequence spontaneously without tiles lighting up, the means and standard deviations of the percentage correct steps were 46.3 ± 35.9% for pwMS and 50.0 ± 29.3% for HC in the explicit groups, and 32.5 ± 23.3% for pwMS and 47.4 ± 25.8% for HC in the implicit groups. Correlational analysis showed a significant correlation between DK% and the learning effect for HC ($\rho s = 0.73$, $P < 0.001$) and borderline for pwMS ($\rho s = 0.44$, $P = 0.08$).

Discussion

This study showed that persons with mild to moderate MS could learn a sequence in a stepping task to a similar degree as HC, regardless of knowledge of the sequence beforehand. Furthermore, the learned sequence and increased motor performance on the goal-directed stepping task were retained over time and during the simultaneous performance of a cognitive task in pwMS, despite the presence of significant motor impairments compared with HC.

Persons with MS show motor sequence learning by acquisition and retention

Doyon and colleagues described motor adaptation and motor sequence learning as two types of motor learning.[27] Previous studies in pwMS demonstrated intact motor learning and retention through motor adaptation in various functional mobility tasks as a precision walking task with altered visuomotor mapping,[58] perturbations during walking,[59] or postural control tasks on a moving platform.[60–62] The present study shows that also motor sequence learning in a mobility task is possible in persons with mild to moderate MS, and to a similar extent as in HC, even in the presence of significant mobility deficits. Moreover, the learning was retained over a short period of time.

Similar to common practice in the traditional key pressing SRT tasks, a single outcome of response time was used in the present study to capture performance on the stepping task. The pattern

Table 3. Mixed model analyses for ST motor performance and DTC_{motor} and $DTC_{cognitive}$ for the DT "step" paradigms

	Time	Group	Learning	Time*Group	Time* Learning	Group* Learning	Time*Group* Learning
ST Step (ms)	<0.001*	0.001*	0.035*	0.064	0.867	0.114	0.897
	Time	Group	Learning	Cogn. DT	Time*Group	Time*Learning	Time*Cogn. DT
DTC_{motor} STEP (%)	0.003*	0.550	0.608	<0.001*	0.086	0.673	0.018*
$DTC_{cognitive}$ STEP (%)	0.052	0.960	0.283	<0.001*	0.845	0.654	0.030*

*$P \leq 0.05$. Abbreviations: Cogn. DT, factor cognitive dual task (subtracting sevens, word list generation, and vigilance); DT, dual task; DTC, dual-task cost; group, factor group (persons with multiple sclerosis and healthy controls); learning, factor-learning group (implicit and explicit learning groups); ST, single-task; time, factor time (baseline and delayed retention).

of response time changes reported in our SRT stepping task is similar to the patterns in the classic SRT studies, with improvements over sequence blocks, an increase during a random block, and a decrease again when the sequence was reintroduced. This provides evidence for sequence-specific motor learning in a complex, whole-body movement stepping task in pwMS. In pwMS, this paradigm was thus far only performed with upper limb tasks,[12,17,18] but the findings are in accordance with a previous study in older adults that showed sequence-specific postural motor learning during a postural control weight-shifting task.[63] Some studies that applied the SRT paradigm in reaching or weight-shifting tasks made a distinction between response initiation (i.e., a measure of anticipation or plan-based control) and movement time (i.e., a measure of movement optimization and online control), reflecting different motor and cognitive processes.[63,64] In the stepping task used in the current study, the movement time plays a relatively greater role in the total response time compared with its role in key-pressing tasks, where the fingers often are already on the buttons; but still, the classic pattern of change was seen. Yet, future studies could focus on those distinct measures to gain more detailed insight into which processes improved during training.[63–65]

No difference between the implicit and explicit learning conditions

Where previous studies on motor learning in functional mobility tasks in pwMS were thought to rely on implicit learning processes,[59,60] in studies concerning motor sequence learning of upper limb tasks, distinctions have been made between implicit and explicit learning. Those studies indicated similar motor sequence learning between pwMS and HC in implicit learning conditions but impaired motor sequence learning in explicit learning conditions.[13] It has even been reported that explicit knowledge disrupted motor sequence learning in pwMS.[18] Contrastingly, our present study showed motor sequence learning in the stepping task, regardless of explicit knowledge of the sequence beforehand. Most participants recognized the correct sequence pattern when given a choice out of four, although few were able to reproduce it correctly. Moisello and colleagues described that declarative knowledge of 40% is usually taken as significant knowledge in SRT studies.[64] Here, only the implicit MS group scored on average below 40%, but the percentages of correct known steps varied over the whole spectrum from zero to all correct. For HC, the percentage of declarative knowledge did show significant, positive correlations with the learning effect (i.e., the difference between Rpost and Spost), indicating that the more declarative knowledge, the greater the learning effect. For pwMS, this effect was borderline, although for them, the correlation seemed to be driven by two highly performing subjects in the explicit group. Despite the correlation, also participants who could not autonomously perform any part of the sequence (DK 0–40%) showed a learning effect. Therefore, the question is posed to what extent the groups indeed learned the task implicitly or explicitly and whether knowledge of the present sequence was used during learning. Future research might provide more insights by making the experimental difference between learning conditions greater and making a distinction between reaction time and movement time.

Furthermore, sample characteristics might moderate findings on sequence learning. Previous

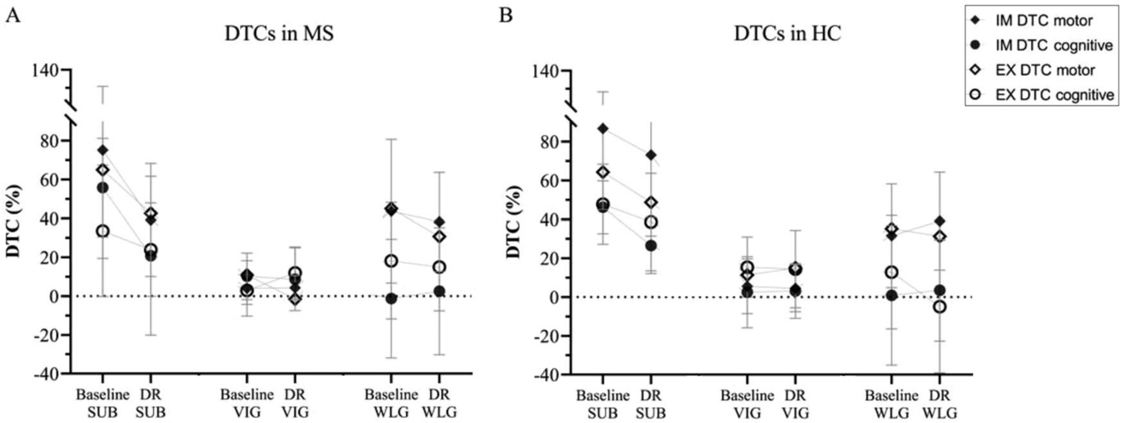

Figure 5. **Dual-task costs (DTCs) for (A) pwMS and (B) HC at baseline and delayed retention for the stepping tasks on AMPEL. Motor DTCs are depicted by diamonds and cognitive DTCs by circles for the implicit (black) and explicit (white) learning groups. Abbreviations: DR, delayed retention; DTC, dual-task cost; HC, healthy controls; MS, multiple sclerosis; SUB, subtracting sevens task; VIG, vigilance task; WLG, word list generation task.**

studies on sequence learning in pwMS included persons with low (quantified by EDSS ≤ 2)[18,20,21] or moderate to severe[12,17,19,22] disability, with the latter being most comparable to the sample in the present study in which on average moderately impaired pwMS (EDSS 3.4 ± 1.2) participated. Nevertheless, the literature reports contrasting findings regarding the learning rate of explicit compared with implicit learning in these studies, therefore, not providing explanations in light of disability. Furthermore, also cognitive functioning might affect findings regarding sequence learning. The literature in other populations suggests, for example, a moderating role for WM performance[66,67] and attention capacity.[68] However, relations between cognitive functioning and motor sequence learning have rarely been investigated in pwMS. Only Deroost and colleagues reported neuropsychological measures but showed no correlations with sequence learning for the pwMS.[12] Still, future research is warranted considering the prevalent impairments in information processing speed and acquisition of new information in pwMS[8,69] and the possible moderating role of cognitive functioning in sequence learning. Future studies could increase the comprehension of learning processes and the discrepancies between studies by reporting on cognitive functioning of their sample and specifically investigating implicit and explicit learning within the subgroups of pwMS with low and high cognitive functioning.

Transfer to DT conditions and automatization

This study was, to our knowledge, the first study in pwMS to examine automatization after a learning session by studying the transfer of a learned motor sequence task to a DT situation. All groups retained improvements under cognitive DT conditions, as was shown by better ST performances accompanied with similar or reduced DTCs, even though the DT still posed a cost on both the motor and cognitive performances. If improved performance was only shown during the ST, and not retained during DT situations, one would have expected the DTCs to increase. Notably, the motor and cognitive DTCs for the subtraction stepping task were lower at delayed retention compared with baseline. The findings indicate that participants performed the stepping task not only faster but also more automatically after practicing, reducing the cost in both domains. However, the costs during the step conditions were in general higher than during the walk conditions, indicating still more interference in the step conditions. Furthermore, this significant difference in DTCs was specific for the subtraction stepping task as they were not shown in the WLG or VIG stepping tasks. Although absolute cognitive performance on the subtraction task itself increased, shown as a learning effect in a previous study,[70] the possible learning effect in the cognitive task could not completely explain the reduced DTCs. Furthermore, the subtraction task resulted in the greatest cost for all DT paradigms, with very high costs at baseline. Even after a significant decrease in delayed retention, the cost was still substantial. Apparently, a mental tracking task interfered more with goal-directed stepping than a verbal fluency or externally interfering VIG task, similar to previously reported in DT literature.[57] Another explanation for the reduction in DTCs might, therefore, be that the participants quickly learned a certain strategy on how to perform the subtraction and stepping task concurrently, thereby showing a practice effect specifically for this task. An extra DT paradigm at delayed retention on AMPEL with a random instead of the learned sequence pattern could have given more insights in these distinctions. Furthermore, as the acquisition phase was short with a long sequence (only 18 repetitions of a 14-item sequence) in order to avoid fatigue, it would be interesting to see effects after a longer training period.

Methodological considerations

A couple of methodological considerations and limitations should be taken into account when interpreting the results of the present study. First, in the explicit learning group, the sequence was only shown at the start of the learning session, and no emphasis was put on its presence, nor did we ask the participants to explicitly discover or learn the sequence in order to avoid a higher cognitive load due to a discovery task. Quotes from participants in this group were *"My legs knew where to go before my head did"* and *"I did not even think about the sequence at all, I forgot about it,"* indicating that at least not all participants consciously used the information given. In the implicit learning group, on the other hand, participants noticed the repetition in the steps during the acquisition phase. In previous studies, a cognitive task has been added to avoid that participants in the implicit condition would discover the sequence;[63] however, in this pilot study in which we also examined the effects on DT performance as a measure of automatization, we wanted to avoid providing a DT training in which integration of a task might be practiced.[71]

Second, as previously mentioned, comparing DT conditions before and after the learning session, once with the learned sequence pattern and once with a random pattern, could have provided more insights. Although it was shown that the improvement in motor performance, as quantified by faster response times, was retained during DT conditions, it cannot be deduced to what extent this was due to learning of the sequence, generally improved motor performance on the task, or an acquired strategy to cope with the DT condition. Also, DT performance on baseline and delayed retention was compared, while different stepping sequences were used for the goal-directed stepping task (e.g., pseudorandom orders and the sequence order, respectively), which might have influenced performance. However, these sequences were set up so that the type of bodily movements (sideward, forward, backward, …) were generally evenly presented across sequences. Importantly, these sequences were piloted beforehand on similarity in difficulty. Moreover, the findings in the present study also indicated this similarity as no significant differences were found between the pseudorandom and first sequence blocks (i.e., Rpre and Spre) at the beginning of the learning session.

Additionally, owing to our rather small sample sizes, interpreting our findings should be performed with caution. Although the learning effect was robust, a small sample size might challenge the ability to find group differences. Similarly, no differences between the implicit and explicit groups were found on descriptive and clinical averages, but subtle contrasts and differences could still have influenced the learning processes. The aim was to match groups on age, gender, and the EDSS score. However, although statistically not different, participants were not entirely matched because of practical issues. Last, the pwMS participating in our study had mobility and balance deficits but were not cognitively impaired compared with the HC. Therefore, results cannot be generalized to cognitively impaired or more severely disabled pwMS. Future research might specifically focus on cognitively impaired pwMS since this study has provided proof-of-concept for motor sequence learning in a stepping task in pwMS.

Conclusion

This pilot study gave proof-of-concept of a modified SRT paradigm within a goal-directed stepping task and provided evidence for a robust sequence learning effect in persons with mild to moderate MS, regardless of explicit knowledge of the sequence beforehand. Sequence and motor learning were shown by retention over time and during simultaneous performance of cognitive DTs.

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Author contributions

R.V.: conception and design, acquisition of data, analysis and interpretation of data, and drafting and revising the manuscript. L.M.: interpretation of data and revising the manuscript. K. van D.: analysis and interpretation of data and revising the manuscript. J.S.: acquisition of data software and revising the manuscript. A.V.: acquisition of data and revising the manuscript. D.K.: revising the manuscript. P.F.: conception and design, interpretation of data, and revising the manuscript.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1. *P* values of the full factorial mixed model analyses for the DTC_{motor} and $DTC_{cognitive}$ of the step conditions.

Table S2. DTCs for all DT-paradigms with the stepping task, per group at baseline and delayed retention

Supplementary Data 1. Sequence and random orders for the stepping task.

Competing interests

The authors declare no competing interests.

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