

# The serial reaction time task revisited: a study on motor sequence learning with an arm-reaching task

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**Abstract** With a series of novel arm-reaching tasks, we have shown that visuomotor sequence learning encompasses the acquisition of the order of sequence elements, and the ability to combine them in a single, skilled behavior. The first component, which is mostly declarative, is reflected by changes in movement onset time (OT); the second, which occurs without subject's awareness, is measured by changes in kinematic variables, including movement time (MT). Key-press-based serial reaction time

tasks (SRTT) have been used to investigate sequence learning and results interpreted as indicative of the implicit acquisition of the sequence order. One limitation to SRT studies, however, is that only one measure is used, the response time, the sum of OT and MT: this makes interpretation of which component is learnt difficult and disambiguation of implicit and explicit processes problematic. Here, we used an arm-reaching version of SRTT to propose a novel interpretation of such results. The pattern of response time changes we obtained was similar to the key-press-based tasks. However, there were significant differences between OT and MT, suggesting that both partial learning of the sequence order and skill improvement took place. Further analyses indicated that the learning of the sequence order might not occur without subjects' awareness.

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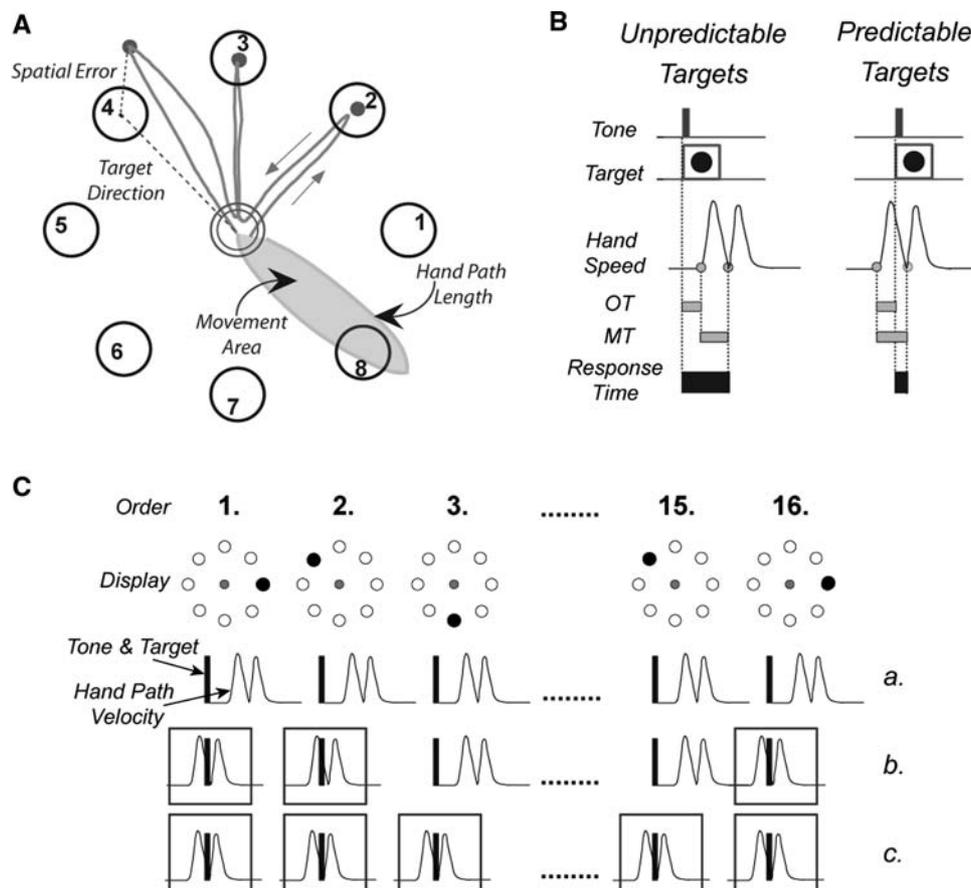
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## Introduction

The acquisition of motor sequences is an essential part of our life, as we learn to play a sport, to drive our car, or even simply to dial the phone number of a new friend. The sequence learning process encompasses two distinct components: the acquisition of the order of the elements in the sequence, and the ability to “perform” the sequence, thus combining the elements in a single, skilled behavior.

In these last years, we have developed a sequence learning task that allows us to identify and measure these two components in an intentional learning paradigm (Ghilardi et al. 2003, 2007, 2008) (see Fig. 1). In this task, subjects move a cursor on a digitizing tablet and reach for targets



in negative onset times (OT) and shorter or negative response time.

**c** Schematic illustration of the development of anticipatory movements during sequence learning. Tones and targets are presented at a constant time interval of 1 s, so that the temporal occurrence, but not the spatial location, is always predictable. At the beginning (a.), movements must be initiated by responding in reaction time to the target appearance. In the course of learning (b.), movements start before target (boxed hand paths). Finally, when the sequence is entirely known (c.), all target appearances are anticipated

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**c** Schematic illustration of the development of anticipatory movements during sequence learning. Tones and targets are presented at a constant time interval of 1 s, so that the temporal occurrence, but not the spatial location, is always predictable. At the beginning (a.), movements must be initiated by responding in reaction time to the target appearance. In the course of learning (b.), movements start before target (boxed hand paths). Finally, when the sequence is entirely known (c.), all target appearances are anticipated

appearing on different locations at a constant temporal frequency. Subjects are informed that a fixed sequence of targets is going to be presented, although they are not told the specific order of such sequence in advance. However, they are explicitly instructed to anticipate target appearance when they know which one is going to be presented; otherwise, they have to wait for the target to appear and move to it afterwards. Therefore, the acquisition of the sequence order, one of the sequence learning components, can be assessed through the progressive increase of the number of the correct anticipatory movements, a discrete variable that is defined based on changes in the movement onset times. As it is highly correlated with the declarative score collected at the end of each trial block (Ghilardi et al. 2003), the number of correct anticipatory is indeed an index of the declarative knowledge of the sequence order. The second learning

component, that is the ability to perform the sequence, is measured by comparing the kinematic characteristics of the anticipatory and non-anticipatory movements. In fact, we have found that, as movements become anticipatory, their duration increases while peak velocity and acceleration decrease, and spatial accuracy increase (Ghilardi et al. 2008). Thus, as declarative knowledge of the sequence order evolves, the movement can be better specified in advance (i.e., increased trajectory accuracy) with a significant saving in energy (i.e., lower peak velocity and acceleration) (Ghilardi et al. 2008). This type of learning, which can be equated to an optimization process, is a fundamental part of skill acquisition and is complete only after the entire sequence order has been acquired (Ghilardi et al., *in press*).

The idea of such a switch in kinematic strategy—from short to longer movement durations—that accompanies the

learning of a motor sequence may seem rather odd and counterintuitive. However, it is important to remark that, in normal life, movement velocity and duration can be modulated and optimized depending upon the situations and the task requirements. For instance, when they know “where and when” to go, subjects usually start moving in advance, take longer time and use less energy. On the other hand, when responses have to be made as fast as possible to unpredictable stimuli, subjects are able to shorten movement duration, producing high velocities and accelerations. We have captured these two situations with two motor tasks where the targets are presented at a constant time interval (Ghilardi et al. 2003, 2008). In one, targets are presented in a predictable order (counterclockwise, CCW): subjects start their movements before the targets’ appearance. In the other one, targets’ order is unpredictable (random order): subjects need to wait for their appearance and reach them as soon as possible. In the first task, spatial accuracy is greater and movement duration is longer than in the second, as trajectories are specified better when information about the upcoming target is available in advance. Thus, the changes in movement duration, peak velocities and accuracy occurring in our sequence learning task, represent the optimization process that accompanies a progressive transition from the unknown, or unpredictable, to the known, or predictable.

Importantly, with practice, all types of responses can be further perfected with increased spatial accuracy and asymptotic changes in movement time, peak acceleration and velocities, depending upon the task requirements.

Most of the sequence learning studies have been done with reaction time paradigms involving key presses in response to specific stimuli. The most popular of them is the serial reaction time (SRT) task (Nissen and Bullemer 1987; Willingham et al. 1989; Doyon et al. 1997; Robertson 2007), in which subjects press one of four buttons as response to a target appearing on a specific location of the monitor. Targets are presented either in a repeating order (sequence blocks) or in random order (random blocks). The peculiarity of this task is that subjects are never informed about the presence of repeating sequence, but only instructed to react to the stimulus as fast as possible, as in a normal reaction time paradigm. Thus, the learning occurring in this type of tasks can be considered incidental. Most studies have shown that response times decrease across successive sequence blocks and increase when a catch random block is introduced after several sequence blocks (Willingham et al. 1989; Goedert and Willingham 2002; Wilkinson and Shanks 2004). In general, these changes are interpreted as evidence of “sequence learning”; this incidental learning is usually considered “implicit” as subjects perform poorly in recalling the sequence structure (Willingham et al. 1989; Curran and

Keele 1993; Destrebecqz and Cleeremans 2001; Wilkinson and Shanks 2004). In many instances, the level of declarative knowledge of the sequence order has been assessed at the end of the entire session with a series of “generate tasks” (Willingham et al. 1989; Curran and Keele 1993; Destrebecqz and Cleeremans 2001; Wilkinson and Shanks 2004). This methodology has, however, intrinsic limitations. Namely, the retention of the order of a newly learnt sequence, especially of a long, complex one, is subject to decay in time and to interference from many sources, including the interposition of random blocks, as it happens in many SRT experiments.

All things considered, it is difficult to establish from the analysis of average response time changes alone what people actually learn in this task, whether the order of the sequence elements, or to optimize motor performance, or both. Indeed, as noted by Pascual-Leone et al. (1993), response time encompasses both the time between stimulus appearance and response initiation (reaction or onset time, OT) and the time for the execution of the response (movement time, MT). The simple breakdown of response time into OT and MT, two components that reflect different cognitive and motor processes, could provide some new insights on how and which part of the motor sequence is learned. Decreases in OT would mostly reflect anticipation and thus, acquisition of the sequence order. MT changes (decreases or increases) would reflect different optimization processes that define the nature of the movement itself. In other words, as we have discussed in the previous paragraphs, shortening of MTs would suggest that optimization has occurred when target appearance cannot be predicted; prolongation of MTs, instead would indicate that movements have been optimized in a context of target predictability.

So far, SRT studies have never attempted to separate and analyze OT and MT, as it is not easy in key-pressing tasks. Thus, we used our arm-reaching tasks in a typical SRT experimental design and measured changes in OT and MT. In addition, as our task allows for a complete description of the kinematic characteristics of each movement, we also measured changes in peak velocity, acceleration and spatial accuracy. We first performed a series of control experiments to measure such changes in two simple motor tasks, where movements were to either all predictable or all unpredictable targets and no sequence learning was taking place, and during the intentional learning of a motor sequence. The results of these control experiments provided sound bases for the interpretation of the results of the main experiment, where we analyzed the changes occurring during the incidental (SRT-like) learning of a motor sequence. In this way, we ascertained, first, whether an SRT arm-reaching task produced similar response time changes as in the classical key-pressing

tasks and then, whether such changes were similarly reflected in OT, MT and spatial accuracy. Our results show that, indeed, in our incidental learning task, response time has a pattern similar to the key-pressing tasks. Interestingly, the response time changes are not equally reflected in OT and MT. The characteristics of the OT and MT changes suggest that, during our incidental learning task, there is the development of a declarative, although fragmentary, knowledge of the sequence order. The results reported in this paper provide novel insights into the understanding and interpretation of the processes underlying incidental learning in SRT tasks.

## Methods

Forty right-handed subjects (15 males, 25 females, age: mean 29.2 years, SD 8.8) participated in the experiment and were assigned to one of four groups. (Six more subjects participated in an experiment reported in the supplemental material). Written informed consent was obtained from all participants and the experiments were conducted with the approval of our Institutional Review Board. All the subjects were naïve to the purpose of the experiment.

General features of the motor tasks are reported in details in previous studies (Ghilardi et al. 2000, 2003). Briefly, subjects moved a cursor on a digitizing tablet with their right hand (sampling rate 200 Hz). They made out and back movements from a central starting point to one of eight targets (distance 5.8 cm) displayed as circles (1 cm radius) on a computer screen (Fig. 1). Instructions were to move as fast and accurately as possible, without corrections and to reverse sharply within the target circle. The target always appeared in synchrony with a tone, at 1 s intervals. Targets were presented in separate trial blocks (64 or 128 s each), either in a pseudo-random, non-repeating order (random blocks, R), or in a predictable counterclockwise order (CCW blocks), or as a repeating sequence of 16 elements (8-2-7-5-3-1-4-6-1-5-7-2-4-8-6-3, see target number in Fig. 1a), in which each target appeared twice (sequence blocks, S). Cursor and targets were always visible during movements.

We first performed a series of control experiments to determine the effects of target predictability and timing instructions on the characteristics of movements performed either during RAN, CCW or intentional learning of motor sequences (see also Supplemental material). Then, in the main experiment, we analyzed the characteristics of movements during the incidental (SRT-like) learning of a motor sequence. Subjects were assigned to one of four groups, each performing a specific experimental paradigm, defined as follows:

## Control experiments

- “RAN” ( $n = 8$ ): subjects performed eight consecutive R blocks of 64 movements; subjects were asked to move to the target as soon as it appeared, minimizing reaction time but avoiding anticipation;
- “CCW” ( $n = 8$ ): subjects performed eight consecutive CCW blocks of 64 movements. Subjects were told that targets would appear in counterclockwise order and they were instructed to reach and reverse in the target in synchrony with the tone, thus anticipating its appearance;
- “Intentional” sequence learning ( $n = 8$ ): it consisted of alternating random and sequence blocks (R-S-R-S-R-S-R-S-R-S-R-S-R-S-R-S-R) of 64 movements (that is, 4 complete cycles or repetitions, for a total of 512 movements in S blocks). For S blocks, subjects were informed of the presence of the repeating sequence of 16 elements, instructed to learn it and to anticipate target appearance when they knew which target was going to appear next, thus reaching the target in synchrony with the tone. At the end of each block, they reported the sequence order and a verbal score was computed (see below). Subjects were informed when R blocks were presented, and instructions were as per “RAN”.

## Main experiment

- “Incidental” sequence learning ( $n = 16$ ): subjects performed nine blocks of 128 movements (8 complete cycles or repetitions, for a total of 640 movements in S blocks), in the following order: R-S-S-S-S-R-R-S-R. In all blocks, subjects were asked to move to the target as soon as it appeared and the presence of a sequence was never mentioned;

## Data analysis

As in previous publications (Ghilardi et al. 2000, 2003), for each movement we measured several spatial and temporal parameters (see also Fig. 1). In this context, we were particularly interested in:

- response time: the time from target presentation to end point, the sum of OT and MT;
- OT: the time from target presentation to movement onset. In R blocks OT always corresponds to reaction time (i.e., the values are always positive). In S blocks, negative values indicate movements starting before the presentation of the target. For each subject we also computed the *floor reaction time* in R blocks, i.e., the minimum OT value across all R blocks (Ghilardi et al. 2003, 2008).
- MT: the time from onset to movement reversal;

- the amplitude of peak velocity and peak acceleration;
- directional error: the difference between the target direction and the movement direction at peak velocity; this measure was used to identify movements to the correct target ( $<22^\circ$ ) (Ghilardi et al. 2000);
- spatial error: the linear distance from the movement end point and the center of the target;
- normalized hand path area: the area enclosed by the hand path divided by the squared movement length, an index of hand path shape and strokes' overlap (Huber et al. 2006; Moisello et al. 2008a).

For each S block, we also computed the number of correct anticipatory movements, defined as those movements directed to the correct target and with OT below the floor reaction time. We also computed a verbal or declarative score from 0 (unawareness of a repeating sequence) to 16 (or 100%, complete knowledge of the sequence), as described in previous papers (Ghilardi et al. 2003, 2008). In the control experiments about “Intentional” learning, such declarative scores were collected after each S block, while in the main experiment on “incidental” learning, scores were collected only at the end of the experiment.

For each subject, the mean of each parameter in each cycle of 16 movements was determined and their average in a block was computed.

To compare the learning in our task to that of the classic SRT, in the “incidental” group, we computed the following learning scores for response time and OT:  $\Delta 1_{R-S}$  corresponds to the difference  $R6 - S5$ ;  $\Delta 2_{R-S}$  is the difference between the final S block (S8), and the average of the R7 and R9. In the “Intentional” group we computed the difference between each S block (8 in total) and the average of the two adjacent R blocks, thus obtaining eight learning scores (R–S delta).

Unless otherwise specified, we performed mixed model ANOVAs ( $\alpha = 0.05$ ). Factors are described in the pertaining sections of “Results”. Significant main effects and interactions were analyzed further using Bonferroni corrected post hoc tests.

#### Methodological considerations on the selection of the study design

##### *Selection of different types of instructions and target presentation conditions in the control and main experiments*

Instructions and modalities of target presentation conditions are the main determinants of strategy selection (either conscious or unconscious) that results in distinctive kinematic characteristics. In the first set of experiments, a series of control tasks, that is CCW, RAN and intentional learning, instructions and target presentation have been varied

and specific kinematic characteristic were expected. With CCW, we tested the effects of target predictability and anticipation: this might occur when a sequence has been fully acquired. With RAN, we verified the effects of target unpredictability and time constraints, so that anticipation and advanced movement preparation are not allowed. This situation occurs when the order of target presentation is always unknown. Finally, with intentional sequence learning, we determined the course of the kinematic changes occurring during the development of awareness of the sequence order, thus, the transition from a reaction time mode to an anticipatory mode of response initiation. These three control conditions are necessary to understand the kinematic changes in the incidental learning task, where no instructions were given to subjects and targets were presented in a repeating order.

##### *Selection of different structure for intentional and incidental learning*

The reason has to do directly with the two major aims of the study, that is, to ascertain whether: (1) the changes in response time pattern seen in SRT key-press tasks are reproducible with arm-reaching movements; (2) OT and MT have similar pattern of changes in such incidental learning task—The comparison between incidental and intentional sequence learning time courses, or to establish the separate roles of amount of training, and training structure, are beyond the scope of this paper—Thus, to pursue these two aims, in the main experiment, we used for our incidental learning task the same structure as in the classical SRT key-press tasks, RSSSSRSR (Willingham et al. 1989). Instead, for intentional learning, one of the control conditions, we used an alternate-block structure. The main outcome measure of the classical SRT task is the delta between the response time of the last S and R blocks. With the alternate-block structure, we were able to obtain many and intermediate “delta” scores for the intentional learning paradigm by comparing adjacent RS values, similarly to the SRT task (Willingham et al. 1989). These delta scores were then correlated with the corresponding number of correct anticipatory movements and with the verbal score collected at the end of each block. These correlations allowed us to address another aim of the study, that is, to ascertain whether the delta scores of the incidental learning reflect a partial learning of sequence order. A RSSSSRSR structure would not had provided these advantages.

##### *Selection of different block length in intentional and incidental learning*

Although the numbers of total movements in the two learning conditions were similar (see above), the intentional

learning paradigm blocks were shorter. This was done in order to collect many intermediate learning scores for this condition, where the learning of sequence order is occurring at a faster pace than in incidental learning blocks.

### Supplemental experiments

To rule out the contribution of the “instruction of anticipation” on the kinematic characteristics we observed in the intentional sequence learning task, six naïve subjects were instructed to learn a repeated sequence without any instructions about timing. For this supplemental study, we used a RSSSSRSR design (see Supplemental material).

## Results

### Control experiments

#### *Movements to predictable and unpredictable targets are different*

We first describe the characteristics of movements directed to targets presented either in predictable (CCW) and unpredictable (R) order. Neither of these tasks required the learning of a sequence order. As expected, mean OT was negative in CCW blocks, as target anticipation was required, and it was positive in R blocks, as movements started after target appearance (Fig. 2a). Interestingly, while there was no difference in path length, we found longer MT in CCW compared with R blocks (Fig. 2b; ANOVA effect of Task:  $F(1, 14) = 8.83$ ,  $P = 0.01$ ), with a trend for lower peak velocity and acceleration in CCW (Fig. 2e, f). In addition, spatial error and normalized hand path area were lower in CCW, indicating that movements were more precise, straight, with overlapping strokes when

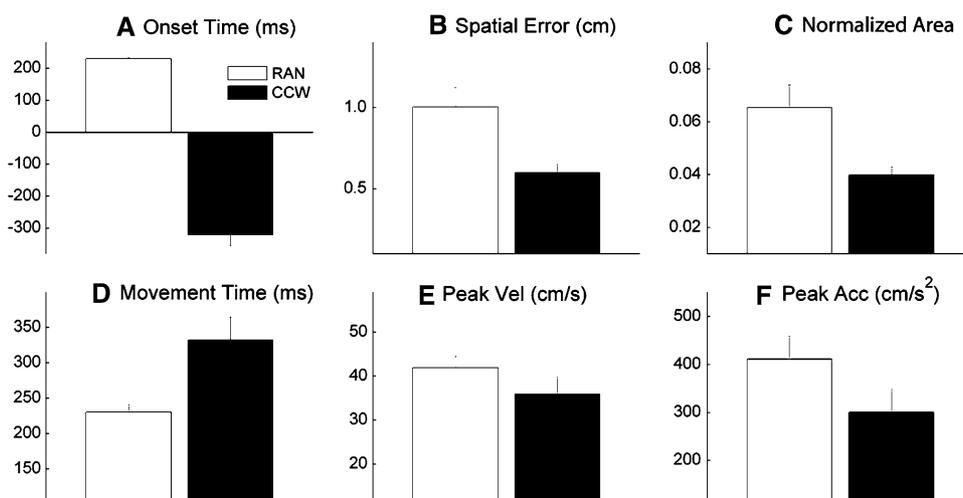
subjects knew in advance where to go (Fig. 2c, d; ANOVA effect of Task: spatial error  $F(1, 14) = 9.84$ ,  $P = 0.007$ ; Abs Norm Area  $F(1, 14) = 7.81$ ,  $P = 0.01$ ). These results confirm our previous findings (Ghilardi et al. 2008) and show that, when information about target appearance is available in advance, the movement can be specified better and time is managed in a more efficient way.

We then ascertained the effect of task repetition by analyzing the time course of changes across the eight blocks. Average MT progressively decreased across R blocks by 24.4 ms (SE: 9.82 ms,  $F(7, 49) = 7.25$ ,  $P = 0.00001$ ), whereas it was rather stable across CCW blocks ( $F(7, 49) = 0.51$ ,  $P = 0.83$ ). As for trajectory accuracy, spatial error slightly decreased across blocks in both tasks (CCW: ANOVA, effect of Block:  $F(7, 49) = 2.84$ ,  $P = 0.01$ ; R: ANOVA, effect of Block:  $F(7, 49) = 2.92$ ,  $P = 0.01$ ). Normalized area, instead, decreased across the eight CCW blocks (ANOVA, effect of Block:  $F(7, 49) = 4.65$ ,  $P < 0.001$ ), but was stable across R blocks ( $P = 0.47$ ). These results show that, even when the tasks are rather simple, with practice, spatial accuracy keeps improving in all instances. This improvement, which represents a type of learning, is accompanied by either an increase or a decrease of MT, depending upon the experimental conditions.

### *Intentional sequence learning*

In this experiment R and S blocks were alternated. Before each S block, subjects were informed of the presence of a sequence and instructed to anticipate target appearance (see “Methods”). Movements were straight and accurate, with sharp reversals. Movements in the wrong direction were more numerous in S ( $5.18\% \pm 1.13$ ) than in R blocks ( $1.32\% \pm 0.40$ ; ANOVA effect of Task:  $F(1, 14) = 5.41$ ,  $P = 0.04$ ), likely reflecting learning-related attempts to anticipate upcoming targets.

**Fig. 2** Movements to predictable and unpredictable targets: for each subject we computed the mean across the eight R or CCW blocks. Empty bars represent the means (+SE) for the “RAN” group, filled bar for the “CCW” group



We first analyzed S and R blocks separately. Figure 3a–c show that OT and MT, and thus their sum, the response time, remained constant across R blocks (ANOVA, effect of Block: OT:  $F(7, 49) = 1.11, P = 0.37$ ; MT:  $F(7, 49) = 0.40, P = 0.90$ ). Across the eight S blocks, where response time decreased by an average of  $211.9 \pm 53.1$  ms, there was a progressive and significant decrease in OT ( $255.8 \pm 62.8$  ms; ANOVA, effect of Block:  $F(7, 49) = 12.0, P < 0.000001$ ) accompanied by a significant increase in MT and a decrease of both peak velocity and acceleration (ANOVA effect of Block: MT:  $F(7, 49) = 3.98, P = 0.001$ ; PV:  $F(7, 49) = 1.70, P = 0.13$ ; PA:  $F(7, 49) = 2.54, P = 0.03$ ). The SRT task usually measures sequence learning by computing the difference between response time of consecutive S and R blocks. Thus, for each subject, we computed such ‘delta scores’ for OT and MT (see “Methods”). We found that OT delta progressively increased across blocks and, by block 4, it was significantly different from the first block (ANOVA effect of Block:  $F(7, 49) = 11.9, P < 0.000001$ ; post hoc tests: S4, S5, S6, S7, S8 vs. S1  $P < 0.02$ ). Interestingly, as MT increased across S blocks, also the MT delta increased significantly (ANOVA, effect of Block:  $F(7, 49) = 4.49, P = 0.0006$ ).

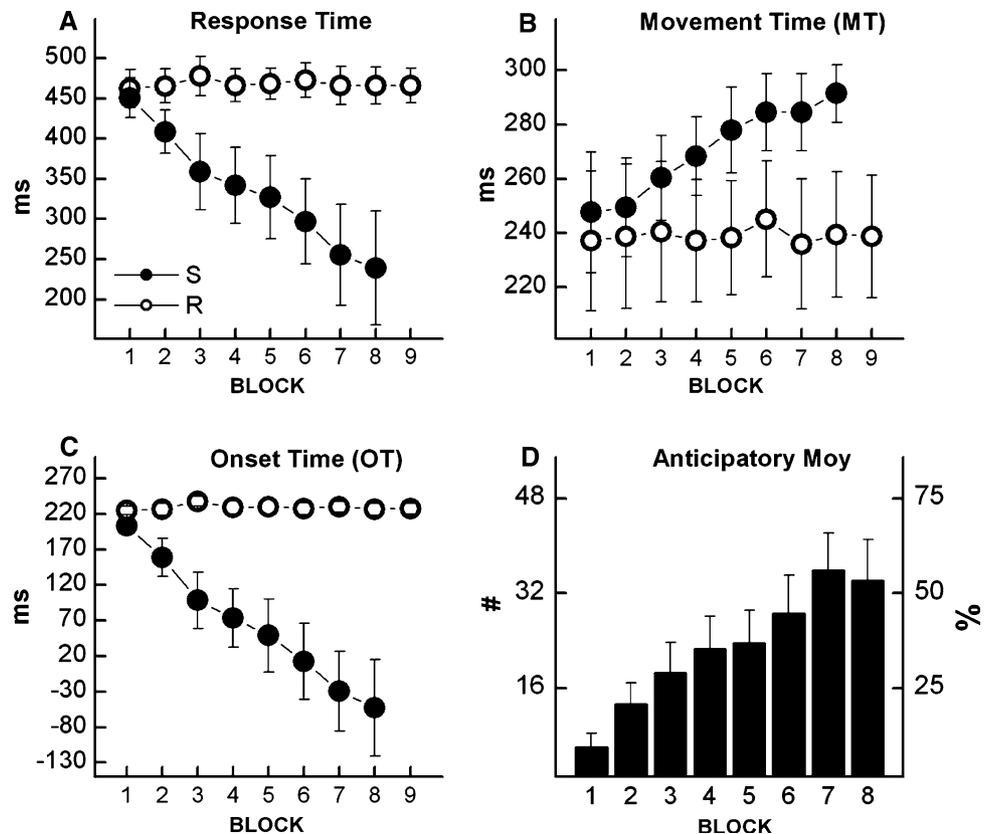
We then analyzed correct anticipatory movements. These movements, which are initiated below the floor random OT (see “Methods”), are strongly related to the

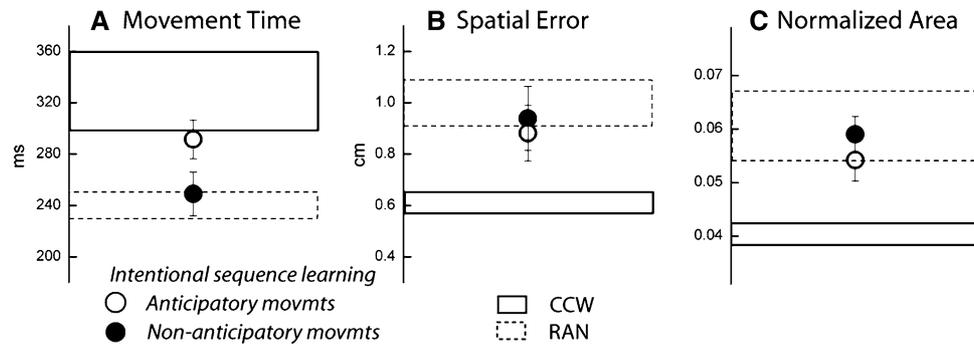
declarative knowledge of the sequence (Ghilardi et al. 2003). Anticipatory movements were already present in S1 and progressively increased across subsequent S blocks ( $F(7, 49) = 14.35, P < 0.000001$ ), reaching a maximum of  $56.1 \pm 6.3\%$  in S7 (Fig. 3d).

An unequivocally declarative index of the learning of sequence order is the verbal score collected after each sequence block. Verbal scores of sequence knowledge increased across S blocks, reaching 70.3% ( $11.3 \pm 1.6$  elements out of 16) at the end of session. As expected from previous results, we found a significant correlation between verbal scores and the number of correct anticipatory movements ( $r = 0.87, P < 0.0001$ ) and with OT delta ( $r = 0.88, P < 0.0001$ ). Since verbal scores reflect declarative learning of the sequence order, these correlations suggest that the number of anticipatory movements and OT delta are likely measures of declarative knowledge of the sequence order.

As described in the first paragraphs, movements to predictable targets have different characteristics than those produced in random reaction time paradigms. We thus expected that correct anticipatory movements in S blocks could be distinguished from the non-anticipatory ones based on their kinematic characteristics. Interestingly, anticipatory movements had longer duration than non-anticipatory ones (Fig. 4a; ANOVA effect of Anticipation:

**Fig. 3** Intentional learning results: average of mean response time (a), movement time (b), onset time (c) for R blocks 1–9 (empty circles) and S blocks 1–8 (filled circles); d average number (and %) of correct anticipatory movements for each trial block of 64 movements





**Fig. 4** The characteristics of anticipatory (*empty circles*, mean  $\pm$  SE) and non-anticipatory (*filled circles*, mean  $\pm$  SE) movements during intentional sequence learning are compared with those of tasks where

targets were always either predictable (CCW, *solid line boxes* representing the mean values  $\pm$  SE) or unpredictable (RAN, *dotted line boxes* representing the mean values  $\pm$  SE)

$F(3, 28) = 4.88$ ,  $P = 0.007$ ), and were more accurate (Fig. 4b, c; ANOVA effect of Anticipation: spatial error,  $F(3, 28) = 2.91$ ,  $P = 0.05$ ; hand path area  $F(3, 28) = 4.27$ ,  $P = 0.01$ ). This implies that in parallel with the learning of the sequence order subjects learn how to perform the movement to the targets that can be anticipated in a more efficient way. Thus, we can consider this change of MT and spatial accuracy as markers of skill acquisition and optimization.

#### Main experiment: incidental sequence learning

During this experiment, subjects were not informed of the presence of a repeating sequence, thus, they might have noticed the sequence order and learned it incidentally. Movements to targets were straight and accurate, with sharp reversals and overlapping out-and-back strokes. There were few movements to the wrong targets, without differences between S and R blocks ( $2.66\% \pm 0.70$  in random and  $2.45\% \pm 0.76$  in sequence blocks,  $P = 0.9$ ).

At the end of the experiments, subjects were asked whether they had noticed a repeating pattern in the order of target appearance. A declarative score for each subject was then computed as described in the methods. The mean declarative score was  $3.08 \pm 0.67$  (range 0–6), corresponding to an average of  $19.3\% \pm 4.17\%$  (range 0–37.5%). Therefore, none of the subjects achieved a significant declarative knowledge (more than 40%) of the sequence order, as per the criteria used in the majority of classic key-press implicit learning SRT studies (Willingham et al. 1989; Curran and Keele 1993).

The pattern of changes in the mean response time across the nine testing blocks (Fig. 5a) was overall consistent with the classical SRT task (Goedert and Willingham 2002). ANOVA showed a significant main effect of Block ( $F(8, 120) = 5.33$ ,  $P = 0.00001$ ): response time steadily decreased from R1 to S5 by an average of  $29.2 \pm 1.0$  ms (R1 vs. S5:  $P < 0.0001$ , see also Fig. 5a); it then increased

by  $18.4 \pm 0.76$  ms in R6 (R6 vs. S5:  $P = 0.008$ ) and, finally, decreased by  $10.3 \pm 1.75$  ms in S8 compared with the average of R7 and R9 ( $P = 0.02$ ).

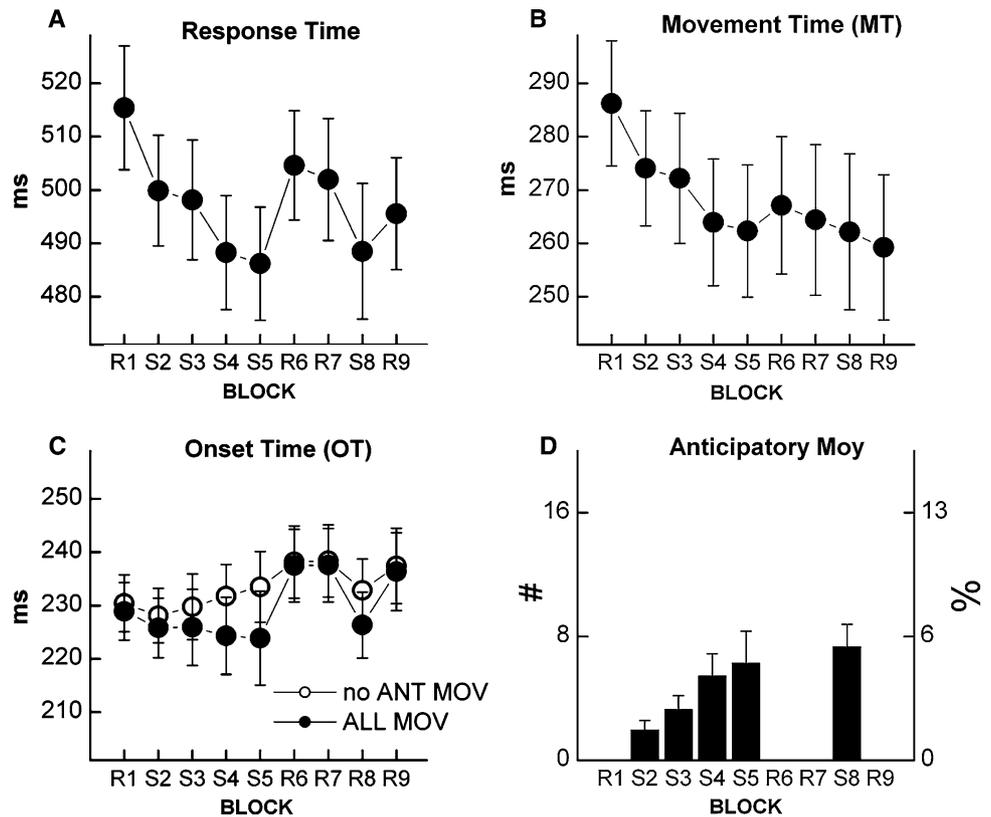
Unlike response time, OT was constant in the first five blocks (R1 vs. S5:  $5.0 \pm 3.4$  ms,  $P = 0.9$ ; Fig. 5c), suggesting that the early reduction in response time was not due to changes in OT. However, like response time, it increased in block R6 compared with S5 ( $13.6 \pm 2.0$  ms,  $P = 0.056$ ) and then decreased in S8 relative to the average of R7 and R9 ( $10.60 \pm 0.90$  ms,  $P = 0.27$ ).

To understand whether OT changes were uniform across all targets or were confined to specific elements of the sequence, we analyzed OT for each sequence element across all blocks. Indeed, we found that OT R–S deltas had different magnitudes for the different elements ( $F(15, 240) = 2.01$ ,  $P = 0.02$ ) and they were significant in only three of the 16 elements (element 5:  $P = 0.02$ , 12:  $P = 0.007$ , 15:  $P = 0.005$ , see Fig. 6). This result is against the hypothesis of a general optimization of attentional processes during learning and suggests that a partial order of the sequence might have been identified.

MT, on the other hand, continuously decreased from R1 to R9 ( $27.0 \pm 1.9$  ms,  $F(8, 120) = 6.4$ ,  $P < 0.00001$ , Fig. 5b), without differences between consecutive S and R blocks, but with significant difference between the first block (R1) and all the blocks starting from S4 ( $P < 0.001$ ). Altogether, these findings demonstrate that the reduction in response time over the first five blocks (Fig. 5a) is almost exclusively due to a reduction in MT, while the sudden increase in response time in blocks R6–7 is caused by an increase in OT.

The number of anticipatory movements (Fig. 5d) increased across S blocks, reaching 5.7% in S8 (~8 out of 128 movements). Interestingly, when such movements were excluded from the analysis, R–S differences in OT almost vanished (Fig. 5c). In addition, the number of anticipatory movements per subject was positively correlated with the corresponding R–S delta scores for both OT and

**Fig. 5** Incidental learning results: average of mean response time (a), movement time (b), onset time (c) for blocks 1–9, bars represent standard errors; d average number (and %) of correct anticipatory movements for each trial block of 128 movements. In (c) the empty circles represent the mean onset time following exclusion of corrected anticipatory movements ( $OT < \text{floor reaction time}$ )



response time (Table 1), further supporting the notion that R–S delta are the result of partial learning of the sequence order. We also computed the mean MT for the anticipatory movements in the S blocks. As in the previous experiment, we found they had longer MTs ( $290.3 \pm 11.6$  ms) compared with the non-anticipatory movements ( $266.2 \pm 12.0$  ms;  $P = 0.0001$ ), with lower values of peak velocity and acceleration (PV:  $42.3 \pm 1.8$  vs.  $45.1 \pm 1.8$  cm/s,  $P = 0.0001$ ; PA:  $364.2 \pm 21.2$  vs.  $409.8 \pm 20.9$  cm/s<sup>2</sup>,  $P = 0.0002$ ). Interestingly, we found no difference in spatial accuracy between the anticipatory (total: less than 5%) and non-anticipatory movements (spatial error:  $P = 0.66$ ; normalized area:  $P = 0.48$ ). This is in accordance with our recent results showing that improvement in trajectory accuracy occurs only when a consistent part of the sequence order has been learned (Ghilardi et al. Submitted). In fact, we have previously found that spatial accuracy reached asymptotic levels to any given target only when the whole target sequence was known. In other words, accuracy did not improve in a single step from RAN to CCW levels for each target as its position in the sequence was identified; instead, most of the sequence had to be known before spatial performance to any given target could be optimized to CCW levels.

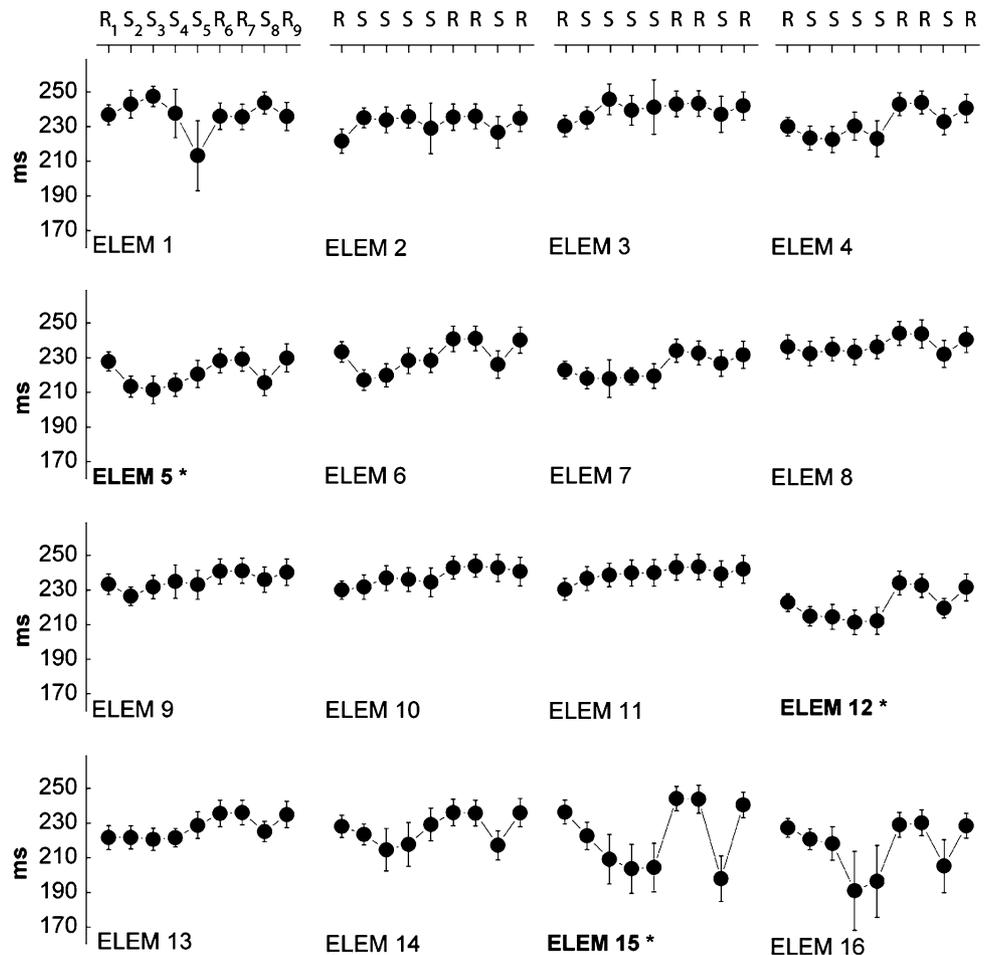
In summary, our response time data are consistent with those of traditional key press-based tasks; in addition, our results suggest that MT and related kinematic changes

represent motor optimization, while OT changes and anticipatory movements the learning of the order of a small subset of elements.

## Discussion

This is the first study on incidental motor sequence learning that combines an arm-reaching task with the SRT paradigm and decomposes response time into OT and MT. The main results are that the changes in response time are similar to the ones seen in classical key-pressing tasks, and are not equally reflected in OT and MT. Response time decreases across S blocks and increases in the catch R blocks, while MT steadily decreases across all successive blocks. The response time increases from S to catch R blocks are related to changes in OT that are largely due to a few (less than 5%) “anticipatory movements” directed to a small subset of targets. The anticipatory movements in this incidental learning task have increased MT and kinematic characteristics similar to those of the anticipatory movements in the intentional learning paradigms (both with and without instructions of anticipation, see Supplemental material), thus suggesting that they share a common nature. MT of the non-anticipatory movements, which are the majority during incidental learning, decreases over the course of both R and S

**Fig. 6** Incidental learning: mean OT for movements directed to each one of the 16 sequence elements in S blocks (8 movements per block), and for movements directed in the correspondent target direction for R blocks (16 movements per block since each direction is repeated twice in the 16-element sequence). Asterisks indicate the elements with statistically significant R–S delta



blocks. Thus, in SRT tasks, two parallel processes of optimization occur with opposite signs for anticipatory and non-anticipatory movements.

An arm-reaching task produces response time changes similar to the classic SRT task

The pattern of response time changes in our SRT arm-reaching task was qualitatively similar to that reported in the majority of studies performed with key-pressing tasks. However, the differences between S and R blocks never exceeded 30 ms, while in the classic SRT studies, they are usually around 100 ms (see (Willingham et al. 1989): 94 ms, (Curran and Keele 1993): 118 ms in the ‘less aware group’, (Wilkinson and Shanks 2004): 97 ms). Several reasons may account for this discrepancy. First, in our task a single effector (one hand) was used, as opposed to multiple effectors (four fingers, both hands, etc.) in the key-pressing task. Second, inter-stimulus interval in our task was shorter thus, allowing less time for information process than in classical SRT studies. Finally, in our task, sequences were longer and more

difficult (8 choices, 16 elements, vs. 4 choices, 10–12 elements).

That said, it is important to remark that reaching movements are substantially different from the finger presses used in the classic SRT task; thus, direct evidence and further experiments are needed to confirm that the results reported here, as well as their interpretation, apply to the key-press-based SRT task.

Changes in OT represent the acquisition of the sequence order

During incidental learning, OT and MT showed different time courses: OT increased from S to catch R blocks, while MT steadily decreased across successive blocks. Indeed, OT and MT reflect distinct processes (Schmidt and Lee 1998) and can be differentially affected by task demands and disease (Ghilardi et al. 2003, 2007, 2008).

Different processes contribute to OT, including stimulus processing, decision making and movement programming (Schmidt and Lee 1998). Therefore, reductions in OT might reflect, on one side, an improvement of stimulus-response

**Table 1** Correlations (expressed as *r* and *p* levels) between delta scores for OT and response time (RespT) and the number of correct anticipatory movements in block S5, S8, in the first four consecutive S blocks (S2–S5) and in all S blocks together

	S2–S5	S5	S8	ALL S
	<i>r</i> ; <i>p</i> level			
Δ1 OT	<b>0.77; 0.0005</b>	<b>0.91; 0.000001</b>	<b>0.63; 0.008</b>	<b>0.83; 0.00006</b>
Δ2 OT	0.31; 0.24	0.48; 0.06	<b>0.91; 0.0001</b>	<b>0.54, 0.03</b>
Δ1 RespT	<b>0.63; 0.009</b>	<b>0.81; 0.0001</b>	<b>0.54; 0.03</b>	<b>0.69; 0.003</b>
Δ2 RespT	0.21; 0.43	0.31; 0.24	<b>0.71; 0.002</b>	0.39; 0.12

Significant correlations are shown in bold

processing and movement planning (which can be considered implicit): in this case, OT reductions should have been seen equally in S and R blocks. On the other side, OT reductions might reflect the awareness of the upcoming target (a declarative component that influences decision making and results in the production of anticipatory movements). This is more likely to occur in blocks where the target appearance is predictable, as it was the case of both incidental and intentional learning. In addition, as shown in the experiments in the supplemental material, significant OT decreases occurred spontaneously, even without timing instructions, when the learning of the sequence order had occurred. Altogether, these considerations suggest that anticipation reflects the acquisition of the sequence order rather than temporal instructions.

Moreover, in the incidental learning task, the changes of OT were not uniformly distributed across targets but were confined to very few elements, as shown in Fig. 6. When movements to such elements were excluded from the analysis, R–S differences in OT virtually disappeared (Fig. 5c). Therefore, we conclude that the OT changes we saw in the incidental paradigm likely correspond to a partial learning of the sequence order. Further evidence comes from the fact that the number of anticipatory movements per subject was positively correlated with the corresponding R–S delta OT scores in both incidental and intentional learning paradigms and with the declarative scores in the intentional learning blocks (see Table 1).

#### MT changes reflect task-related movement optimization processes

In the incidental learning experiment, we found a mean, progressive decrease of MT across all S and R blocks. Interestingly, this decrease was confined to “non-anticipatory” movements: MTs of the few anticipatory movements in the S blocks, instead, increased. A different picture emerged during intentional learning, independently of timing instructions: on average, mean MT constantly increased across S blocks and this increase was more evident when

the anticipatory movements were analyzed separately. In addition, as also shown in previous work with simpler sequence of eight elements (Ghilardi et al. 2008), while anticipatory movements and movement duration progressively increased, amplitudes of peak accelerations and velocities decreased and spatial accuracy increased. As they happen both without explicit requests and subject’s awareness, all these changes should be considered implicit aspects of sequence learning. They, indeed, are part of a process of movement optimization that is task-dependent. That is, with practice, when a sequence or part of a motor sequence is learnt, optimization is reflected by increases in both movement duration and accuracy. Conversely, when subjects respond in a reaction time mode (and thus, movements cannot be prepared in advance), optimization process encompasses reaching higher speeds while maintaining or improving the spatial accuracy.

As mentioned in the introduction, that MT should increase (and peak velocity and acceleration decrease) in the course of sequence learning is perhaps counterintuitive. Indeed, in the context of reaction time tasks, where subjects are compelled to minimize response time and targets are not predictable, together with an increased spatial accuracy, movement optimization must entail a decrement of MT, as we saw in the non-anticipatory movements of the incidental learning paradigm. On the other hand, when target appearance is spatially and temporally predictable and anticipation is allowed as in either CCW or during S, subjects can initiate their movements before the stimulus appears or is processed in its entirety (Ghilardi et al. 2003). This allows them to move slower, use less force, and less net energy, (Moisello et al. 2008b) and to obtain better spatial accuracy (Ghilardi et al. 2003, 2008). In other words, when possible, the motor system seems to shift from a time-saving, but energy-costly strategy as in unpredictable reaction time tasks, to a preferable energy-saving strategy, as in predictable timed-response tasks (Todorov 2004). Thus, the increases in MT of anticipatory movements represent the natural, ecological, default selection of minimum energy consumption and are the result of an optimization process, akin to skill learning, in situations where advanced movement preparation is allowed.

Finally, two considerations should be made for the increase of MT in anticipatory movements. The first regards the contribution of on-line movement correction processes to spatial accuracy: when visual feedback is available during the movement, on-line corrections result in longer movement duration and higher spatial accuracy. Although well-learned movements depend less on such corrections, it is possible that the dependence on visual feedback may increase with the amount of practice, as proposed by one study (Proteau et al. 1992). Thus, well-learned movements may be associated with task-dependent optimal

on-line movement control processes that may produce an increased MT. The second consideration is that the MT increase of anticipatory movements might reflect an intermediate stage of learning where the priority is placed on spatial accuracy; it is possible that, later, with increasing practice and optimization of motor performance, MT would decrease. Further experiments are needed to verify these two possibilities.

Incidental sequence learning: what do you actually learn?

Many studies have attempted to assess the nature of the sequence learning in SRT tasks, trying to determine whether subjects developed awareness of a fragment of the sequence order during the execution of S blocks, thus expecting the appearance of specific elements. However, with the approaches used so far in the “implicit” versions of the SRT tasks (Willingham et al. 1989; Curran and Keele 1993; Destrebecqz and Cleeremans 2001; Wilkinson and Shanks 2004), it is possible to establish only whether “retention” of fragments or all sequence order has occurred after the task and not whether “learning” is taking place during the task itself. In fact, in ‘implicit’ or incidental learning paradigms, the presence of interference processes resulting from both the execution of R blocks and the temporal decay make any “a posteriori” assessment rather difficult to interpret. With the *intentional* learning paradigms of the control experiments, we bypassed all of these confounding factors, as subjects were a priori informed whether an R or S block was presented, explicitly instructed to learn the sequence order, and debriefed at the end of each block. In this way, we ascertained that, indeed, there is a high and positive relationship between changes in OT, the number of anticipatory movements and the declarative knowledge of sequence order. Interestingly, the data collected in the intentional sequence learning experiment without timing instructions (see supplemental material) suggest that the decreases in OT usually follow (and do not precede) the development of the declarative knowledge of the sequence order. In fact, at the end of the first S block, declarative scores were 100%, while the number of anticipatory movements reached 30% and the mean OT decrement was about 100 ms. Thus, it is likely that also in our incidental learning paradigm where no timing instructions were given, OT changes underestimate the level of the declarative learning.

In summary, the changes in OT in both incidental and intentional learning paradigms might reflect a declarative, on-going process leading to partial knowledge of the sequence order. The progressive acquisition of such order is indicated by the parallel increase of the R–S delta, of the anticipatory movements and of the declarative scores. Interestingly, the R–S differences in the first blocks of the “Intentional” learning experiment were higher than those of

the “Incidental” and were in the range of the values reported for the classical SRT tasks. Thus, these results provide compelling evidence that, first, awareness that a sequence is presented expedites the development of the order learning, possibly by focusing attention and by reducing or eliminating the need for stimulus-response transformation. In addition, they suggest that the R–S delta in the classical SRT task might be the expression of the initial development of a declarative, although still fragmentary, knowledge of the sequence order. This conclusion is also supported by the results of neuroimaging studies (Schendan et al. 2003) showing that, during an implicit learning SRT paradigm, dorsolateral prefrontal cortex and medio-temporal regions are significantly activated. These cortical areas are essential parts of the declarative memory system (Squire and Zola 1996) and are mostly active when learning occurs consciously (Grafton et al. 1992; McIntosh et al. 1999).

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