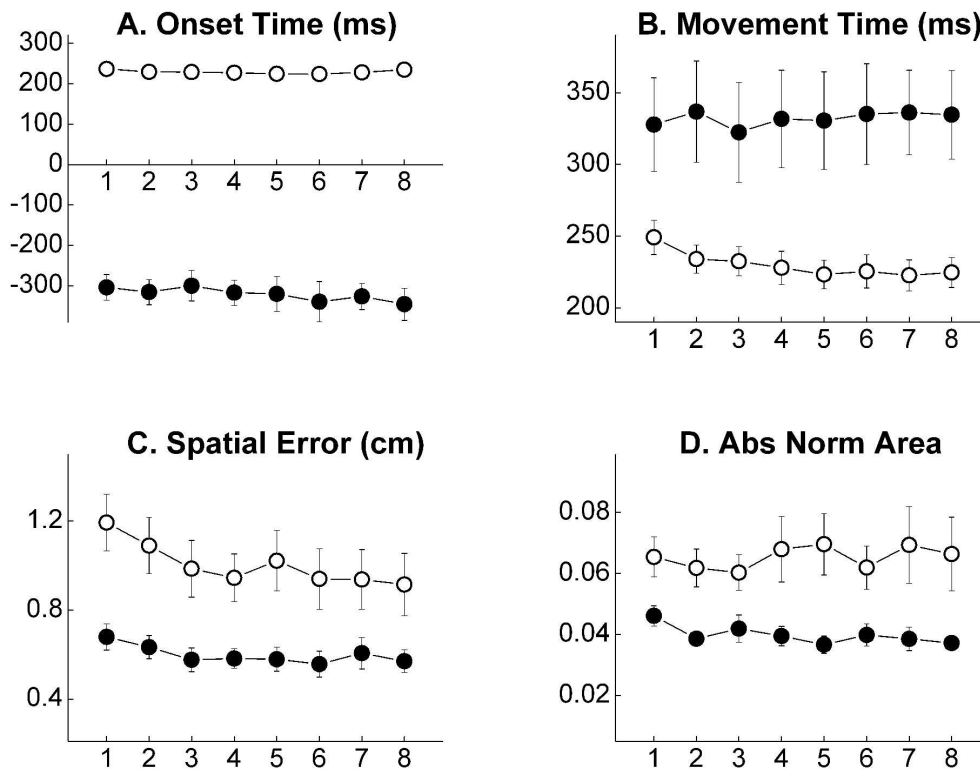


Supplemental Material

Figure 1S. Time course of kinematics and accuracy changes in RAN and CCW

control experiments



Mean Onset Time (A), Movement Time (B), Spatial Error (C), Normalized Area (D) for

Blocks 1-8 in RAN (white dots) and CCW (black dots), bars represent standard errors.

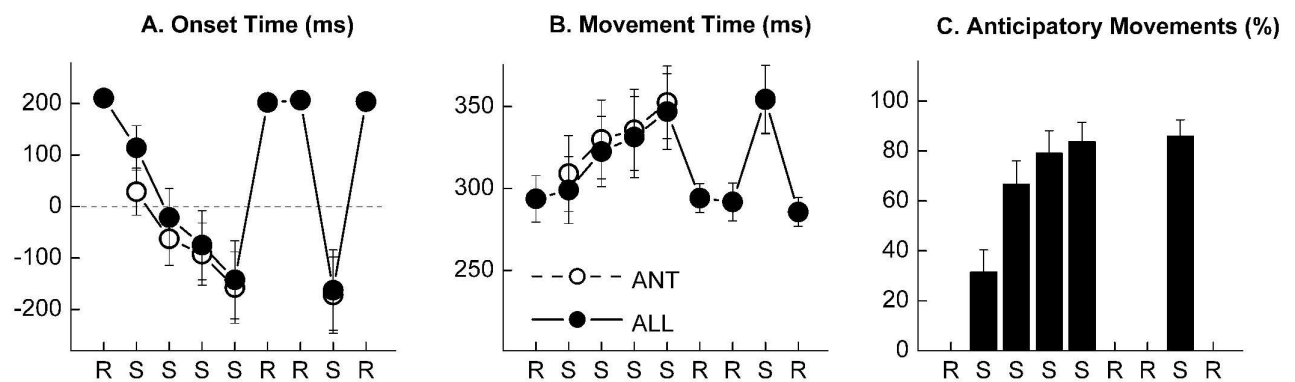
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3 **Control Experiment: the effect of the “anticipation instruction” on intentional**
4 **learning.**
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10 **Methods.** To rule out a possible contribution of the “instruction of anticipation” on the
11 kinematic characteristics we observed in the intentional sequence learning task, in a separate
12 experiment we tested 6 naïve subjects (age 25.5 ± 7.1 , 3 men, 3 women). They performed a
13 series of block with an RSSSSRSR design. For S blocks, subjects were informed of the
14 presence of the repeating sequence of eight elements and they were asked to learn it without
15 specific instructions about timing. At the end of each S block, they reported the sequence
16 order, and a verbal score was computed as described in the main text. Subjects were informed
17 when R blocks were presented, and instructions were as per “RAN” (see main text).
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29 **Results.** As in the intentional sequence learning task with “anticipation
30 instruction”, mean onset times in S blocks fell toward negative values (Figure 2S-A) and
31 were significantly lower than those in R blocks (ANOVA, Effect of Block:
32 $F(8,40)=15.458$, $p < 0.00001$). In parallel, the number of correct anticipatory movements
33 increased across S blocks, reaching 85.9 ± 6.6 % in the last S blocks (Figure 2S-C).
34 Most interestingly, movement time of the correct anticipatory movements increased
35 similarly to those in the intentional learning experiments (Figure 2S-B). Thus, movement
36 times of S blocks was significantly higher than those of R blocks (ANOVA, Effect of
37 Block: $F(8, 40)=6.8962$, $p = 0.00001$). These results suggest that the changes we
38 observed in the anticipatory movements during sequence learning tasks reflect the
39 acquisition of the order of the sequence and are independent from the temporal
40 instructions. Thus, “instruction of anticipation” per se does not determine the decrease
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in onset time or the increase in movement time and spatial accuracy. Interestingly, at the end of the first S block, declarative scores were already 100%, while the number of anticipatory movements reached 30% and the mean onset time decrement was about 100 ms. This suggests that, without explicit timing instructions, decreases in OT usually follow (and do not precede) the development of the declarative knowledge of the sequence order.

Figure 2S. Intentional Learning without instructions



Average of mean OT (A) and MT (B) for Blocks 1-9 (RSSSSRRSR), bars represent standard errors; C. Average % of Correct Anticipatory Movements for each trial block (64 movements). In A and B, the empty circles represent the mean OT and MT of corrected anticipatory movements alone (OT < floor reaction time). Note that, as the number of anticipatory movements approaches the total number of movements in a block (as in S8), this mean corresponds with the mean of the entire block.

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3 Dear Reza

4 Attached please find the manuscript: “The Serial Reaction Time Task revisited: a study
5 on motor sequence learning with an arm-reaching task” by Moisello et al. to be
6 considered for publication in this journal.

7 We would like to thank again both referees for their comments.

8 We have modified the manuscript as outlined below in response to the referees’
9 comments. Also please notice that the title has been slightly modified (in the current
10 version we have spelled out SRTT). We hope that you will find the paper suitable for
11 publication in EBR.

12 Thank you again.

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16 Lice Ghilardi
17 Clara Moisello

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21 Reviewer: 1

22
23 *Comments to the Author*

24 *The paper has been much improved by the authors based on previous reviews. The*
25 *experiments are interesting and the results are novel. Many changes were made*
26 *throughout the manuscript to clarify the principal aims of the study as well as to present*
27 *the main findings more clearly. In particular, authors added several details in the*
28 *methods as well as reorganized the presentation of experimental tasks so that there are*
29 *three control experiments and one main experiment. This helps the presentation and*
30 *interpretation of the results. Also, they added a new section “Methodological*
31 *considerations on the selection of the study design” in which they addressed several*
32 *methodological points raised in the previous reviews (selection of instructions and target*
33 *presentation, selection of different structure for the intentional and incidental learning,*
34 *selection of different block length in intentional and incidental learning) as well as added*
35 *supplemental experiments to test the contribution of the “instruction of anticipation” on*
36 *movement kinematics in the intentional sequence learning task. Finally, several new*
37 *considerations were presented in the discussion.*

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42 *I believe that the authors have addressed in detail the criticisms of the prior review and*
43 *that the changes that were made significantly improved the paper. I do not have anything*
44 *to add.*

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48 We thank the reviewer for the positive evaluation of our work and we are grateful for
49 his/her previous comments.

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51
52 Reviewer: 2

53 *Comments to the Author*

54 *The manuscript is much clearer in terms of motivation and theoretical context. I think*
55 *the paper is cleaner more convincing now that the focus is on the incidental condition*
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3 (now the “main” experiment). The central finding, that incidental sequence learning is
4 largely driven by changes in OT, is an important finding. Most of the points from the
5 previous letter are sufficiently addressed.
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8 ..., the additional experiment does alleviate my major concern (instructions and block
9 structure differences between incidental and intentional experiments). Moreover, I
10 appreciate that the differences between experiments are now explicit. Finally the
11 incidental experiment (the “main” experiment) in itself is quite interesting and these
12 results are important for our understanding of sequence learning.
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15 In summary, the central objectives of the manuscript (whether sequence learning occurs
16 in an arm-reaching task and whether OT and MT differentially evolve over the course of
17 learning) are interesting and nicely addressed by the data. The “control” studies are
18 actually not that well controlled, and, I think, not necessary for the central argument of
19 the paper. But so long as readers are made aware of the limitations, I think it is fair to
20 present the data along with the authors’ interpretation, and allow readers to interpret as
21 they choose. I am satisfied that readers are sufficiently informed in the current ms.
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26 We thank to the reviewer for his/her positive comments.
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29 **Response to additional queries**

30 **Q: Other concerns**

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34 *Declarative learning was queried in the supplementary experiment, but this data is not*
35 *presented in the results.*

36 **A:** We are grateful to the referee for pointing this out. We had previously reported the
37 results on the declarative scores of the supplemental material’s experiments only in the
38 discussion at the end of page 24. We are now reporting them in the supplemental material
39 itself:
40
41

42 “Interestingly, at the end of the first S block, declarative scores were already 100%, while
43 the number of anticipatory movements reached 30% and the mean onset time decrement
44 was about 100 ms. This suggests that, without explicit timing instructions, decreases in
45 OT usually follow (and do not precede) the development of the declarative knowledge of
46 the sequence order.”
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50 **Q:** *In the previous letter, I asked how we can be sure that declarative awareness alone*
51 *accounts for anticipatory movements. The authors respond that the correlation between*
52 *anticipatory movements and verbal sequence report is quite high (0.87), and this is likely*
53 *an underestimate given that verbal report may not capture the full extent of subjects’*
54 *knowledge.*
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Previous studies indicate that implicit learning can lead to anticipatory responses, and implicit and declarative learning may be expected to be correlated (see below). Even so, anticipation is probably a reasonable proxy for declarative sequence learning in this setting, and I don't argue with how the construct is used here in general. The issue is only critical if we debate the declarative nature of learning... see below.

Why does final summary paragraph (p 25) focus on the role of declarative learning? The initial ms reasonably included a section on this, but in the current version we're given the 'take-home' message that declarative learning may explain all sequence learning effects...? The hypothesis is interesting, but it wasn't the objective of the study and can't be tested with the current data, without the necessary control conditions.

A: First, we want to point out that we had not changed the final summary paragraph from the original version and we had not removed any section from the discussion.

We agree with the referee that crystal-clear conclusions 'on the declarative nature of learning' are not possible at this stage. For this reason, we were/are very cautious in the interpretation of our results, and thus, in our conclusions we say that:

"the changes in OT [...] **might** reflect a declarative, on-going process leading to **partial knowledge of the sequence order**"

and

"these results [...] 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**"

In other words, our work did not intend to provide any firm, conclusive statement about the topic, but rather to propose a new perspective to interpret the results of the classical SRT task, based on the results of arm-reaching tasks that allow for the separation of onset time from movement time. The referee must concede that the conclusions of the majority (if not all) of SRTT studies have been based on measurements of a single variable, response time, that combines (and mixes) both onset and movement times. Great focus has then been directed to understand the nature of response time changes correlating it with the results of several sorts of 'a posteriori' analyses (verbal reports, generate tasks, process dissociation procedure etc). None of the classical SRT studies to our knowledge has ever tried to categorize single movements as anticipatory or non-anticipatory based on specific criteria derived from each subject's performance. [Quite the reverse, in those cases where a generic threshold for anticipation was considered, this was only done for the purpose of excluding those movements from the analysis (see for example: Willingham et al. 1989)].

Our approach, instead, was to focus on the motor performance itself, rather than on subsequent tests of awareness, and for this purpose we used reaching movements, designing a task in which learning of the sequence order and performance optimization can be separated and reflected by different variables.

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3 This is clearly stated throughout the paper, so we think it is not correct to summarize our
4 findings saying that '*declarative learning may explain all sequence learning effects*'.
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8 **Q:** [As I mentioned in the previous review, the evidence isn't that compelling, and the
9 argument ignores quite a bit of the literature on this topic over the last 15+ years.]
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11 **A:** We are aware of the literature the referee mentions. However, we do not think that
12 such a discussion (which is of high interest to us) does not belong to this paper (which is
13 already rather long and complex), but rather to a review article.
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16 **Q:** *Minor concerns*

17 *The ms could use quite a bit of clean-up:*
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19
20 *Pg 3 line 29: "fix sequence of targets" à "fixed sequence of targets"*
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22 *pg 4, line 32: "subjects are capable to" à "subjects are able to"*
23

24 *Pg 6 line 20: Drop author from citation in parentheses for Pascual-Leone cite*
25

26
27 *Pg 11, line 39: "CONTROL" is lower case in all other instances*
28

29 *Pg 11, line 41: "Instructions" shouldn't be capitalized*
30

31
32 *Pg 11, line 43: "effects of targets predictability" -> "effects of target predictability" or*
33 *"effects of the targets' predictability"*
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35

36 **A:** We have now corrected all the above and cleaned up the manuscript.
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40 **Q:** *Does it make sense to divide studies into "control" and "main" experiments? (What*
41 *was controlled for in the "control" experiments?)*
42

43 **A:** The distinction of control vs main experiment was made in response to the criticisms
44 of both referees, who rightly pointed out that it was not clear which was the main
45 experiment. The new structure allowed us to clarify the main findings in the manuscript,
46 and received very positive feedback from referee #1 ("*In particular, authors added*
47 *several details in the methods as well as reorganized the presentation of experimental*
48 *tasks so that there are three control experiments and one main experiment. This helps the*
49 *presentation and interpretation of the results*").
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51 In both the previous and the current versions, we have justified the need for this
52 distinction in the introduction and in the methods (see: Methodological Considerations).
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