RESEARCH ARTICLE

Disentangling perceptual from motor implicit sequence learning with a serial color-matching task

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Abstract This paper contributes to the domain of implicit sequence learning by presenting a new version of the serial reaction time (SRT) task that allows unambiguously separating perceptual from motor learning. Participants matched the colors of three small squares with the color of a subsequently presented large target square. An identical sequential structure was tied to the colors of the target square (perceptual version, Experiment 1) or to the manual responses (motor version, Experiment 2). Short blocks of sequenced and randomized trials alternated and hence provided a continuous monitoring of the learning process. Reaction time measurements demonstrated clear evidence of independently learning perceptual and motor serial information, though revealed different time courses between both learning processes. No explicit awareness of the serial structure was needed for either of the two types of learning to occur. The paradigm introduced in this paper evidenced that perceptual learning can occur with SRT measurements and opens important perspectives for future imaging studies to answer the ongoing question, which brain areas are involved in the implicit learning of modality specific (motor vs. perceptual) or general serial order.

Keywords Implicit processes · Perceptual learning · Motor learning · Sequence structure · Serial reaction time task

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Introduction

Throughout live, we constantly learn skills that are based on a specific sequence of perceptual and/or motor events, e.g., learning to write, to tie your shoelaces, to ride a bike or to play a new instrument. Remarkably, such combinations of motor and sensory sequence learning can take place without intentional control (Stadler and Frensch 1998).

The implicit learning of motor sequences has been extensively explored using the serial reaction time (SRT) task. In this task, originally developed by Nissen and Bullemer (1987), participants are asked to press a key that corresponds with the location of the target on the computer screen. Unbeknown to the participants, the stimuli are not presented in a random order but appear according to a fixed sequence. Sequence learning is tested by presenting the participants with episodes in which stimulus presentation (and thus response generation) violates the order of the fixed sequence. The occurrence of sequence learning is inferred when reaction times (RTs) increase during such episodes. If afterwards, participants cannot verbalize, recall or recognize the sequence, sequence knowledge is believed to be implicit. Neuroimaging studies report a wide distributed network underlying SRT learning. Using this paradigm, motor sequence learning has been shown to involve motor, medial

temporal, parietal and prefrontal cortex, basal ganglia and cerebellum (Destrebecqz et al. 2005; Peigneux et al. 2000; Rauch et al. 1997; Schendan et al. 2003; Seidler et al. 2005).

On a perceptual level, implicit learning of ordered structure has been studied using classification paradigms such as artificial grammar learning (AGL) tasks or visual statistical learning (VSL) tasks. In a learning phase, subjects are exposed to several chains of stimuli in which the succession of stimuli obeys a complex rule. In a subsequent test phase, participants are informed about the presence (but not the identity) of this rule and asked to judge whether new chains of stimuli conform the regularities or not. Above chance discrimination, and thus perceptual learning, has been demonstrated, even without the ability to consciously describe the rule structure that has been acquired (Knowlton and Squire 1996; Turk-Browne et al. 2008). Although the neural basis of classification forms of implicit learning has been studied less than SRT learning, a prime role for the left superior occipital gyrus and left angular gyrus (Skosnik et al. 2002), caudate nucleus (Lieberman et al. 2004) and left inferior frontal cortex (Forkstam et al. 2006) has been described. With these paradigms, fMRI data are typically collected during the classification judgments of the test phase. Consequently, neural activations reflect the explicit retrieval of the rule representation (in contrasting grammatical vs. nongrammatical items) or the cognitive application of the rule knowledge (in contrasting correct grammatical judgments vs. incorrect judgments), but not the neural systems that are responsible for the actual encoding of the sequential regularities.

The comparison of imaging studies, using the SRT and classification tasks, reveals that shared but also distinct brain structures are involved in motor and perceptual sequence learning. However, the specific contribution of the different neural structures in motor and perceptual sequence learning remains far from clear, for three reasons.

First, the paradigms used to study the neural substrates of motor and perceptual sequence learning differ considerably and therefore cannot directly be compared. To reduce the influence of the task-specific context, it is imperative to use a paradigm that allows studying perceptual and motor sequence learning in the same task context. While it is possible to achieve such situation with SRT tasks, it is not clear how perceptual and motor learning could be distinguished with classification tasks in the form of AGL or VSL.

Second, to specify the role of different neural substrates throughout the different phases of the learning process, a paradigm is necessary that allows continuous monitoring of sequence learning. For this purpose, SRT tasks are in principle well-suited. A continuous RT measurement of sequence and random trials provides a running index of learning and can be related to the time course of different neural regions. Classification tasks, on the other hand, are

not appropriate. As these tasks are typically used, learning is measured by means of structure judgments in the test phase, after the learning phase has been administered. And even if several judgment phases were embedded in the learning phase of the classification task, this would induce a serious threat to the implicit nature of the learning process.

Third, although the SRT task lends itself better for the latter purposes, the original version of the task entails a confound between the ordinal structure of stimuli (S-S) and response-related associations (R-R, S-R and R-S). In order to separate stimulus and response sequences, the original SRT task has to be modified. To date, no imaging study has succeeded in making such distinction. On a behavioral level, several SRT studies have tried to disentangle stimulus versus response sequence learning. A critical review of the behavioral SRT literature, though, reveals no suitable tool for reliable comparison between implicit perceptual and motor sequence learning. Moreover, conclusive evidence demonstrating independent perceptual learning using an SRT paradigm is lacking. The following three problems are the main factors preventing a straightforward conclusion on whether or not stimulus information alone can support sequence learning during SRT tasks: (1) the possibility that perceptual sequence learning is based on the sequential structure in eye movements that may accompany the processing of stimuli at sequentially regular spatial locations (e.g., in studies of Howard et al. 1992; Mayr 1996; Remillard 2003; Seger 1997), (2) the absence of a clear dissociation between stimulus and response sequence during acquisition (in transfer studies of, e.g., Stadler 1989; Cohen et al. 1990) and (3) the possibility that lower order frequency information of the separate stimulus elements accounts for the learning effects (Frensch and Miner 1995).

In sum, the SRT task is in principle the best available paradigm to dissociate perceptual from motor learning and to compare the two forms of sequence learning. But whereas conditions that create motor sequence learning have been realized by means of the SRT task, perceptual sequence learning has never unequivocally been demonstrated with the SRT task despite frequent efforts to achieve this. The major goals of the current study are threefold. First, to develop a refined SRT task to unambiguously separate perceptual from motor learning and, at the same time, to allow continuous monitoring of sequence learning both at the behavioral and neural level. And finally, to use this version of the SRT task to investigate whether or not perceptual learning actually occurs in the context of the SRT task.

We developed a 'serial color matching task' (Fig. 1) in which participants are instructed to match the colors of three centrally presented small squares with the color of a large square that appears afterwards. Participants are asked to press the first button of a response box if none of the

colors of the small squares matches the color of the large square, the second button if the color of one small square matches, the third button if two small square colors match and the fourth button if all the colors of the small squares match the color of the large square. In the perceptual version of this task (Experiment 1), the sequential structure is imposed on the successive large target squares, whereas the colors of all small squares are selected randomly from trial to trial. Because the colors of the first small squares are randomly determined, subjects cannot predict which response has to be selected and hence all response-related associations (R-R, S-R and R-S) occur on a random basis. By presenting the sequence information in the center of the screen, oculomotor sequence learning is avoided. In the motor version (Experiment 2), the colors of the small squares as well as the color of the large square are randomly selected, with the restriction of eliciting a fixed response sequence. The presence of a sequential structure in the target colors (perceptual version) or finger responses (motor version) is not mentioned to the subjects.

To assess the process of learning continuously over time, short blocks of sequence trials are alternated with short blocks of random control trials. Pilot work showed that continuously introducing random trials with the same color set as the sequence trials disrupted sequence learning. This was probably caused by delearning of the sequence and the initiation of new learning each time the random trials were introduced. This problem was circumvented by assigning another color set to the random trials.

In sum, the serial color-matching task introduced in the present paper, satisfies the three constraints (as described above) that are needed to study and compare the neural basis of perceptual and motor learning: (1) it makes use of an identical task to explore differences between stimulus and response learning, (2) it allows for a continuous measurement of sequence acquisition over time and (3) it avoids the confounds that occurred in previous SRT studies through the complete disentangling of perceptual and motor learning. Experiment 1 tested the perceptual sequence version of the task and Experiment 2 the motor sequence version.

Experiment 1

Method

Participants

A total of 21 students (11 men, 10 women) of Ghent University were paid to participate in this study. All subjects had normal or corrected-to-normal vision and did not suffer from color-blindness.

Serial color-matching task

The experiment was controlled using the Tscope software (Stevens et al. 2006) running on a Pentium 4 computer with a 17″ color screen. As shown in Fig. 1a, on each trial, three small colored squares (2 × 2 cm; 1.91° × 1.91° of visual angle) appeared centrally on the screen against a white background. These small squares were presented with a
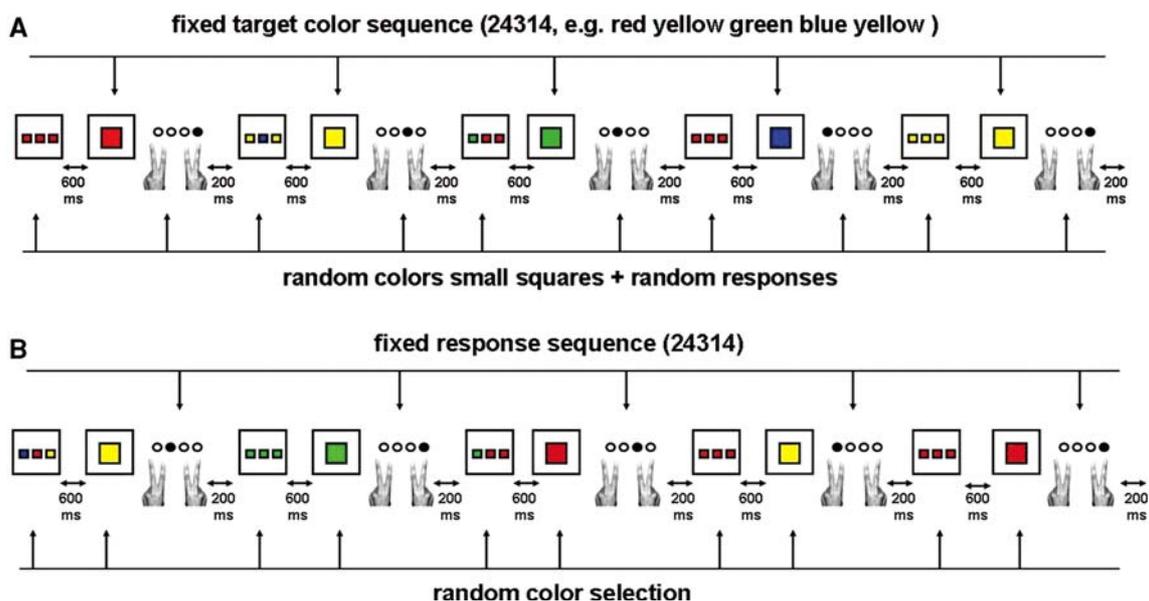

Fig. 1 a Experiment 1, the perceptual version of the serial color-matching task with presentation of 1 sequence of target colors and 5 trial possibilities. **b** Experiment 2, the motor version of the serial color-matching task with presentation of one sequence of responses and five trial possibilities

small gap (0.48° of visual angle) in between. After 600 ms, they disappeared from the screen and were replaced by a large colored square (side = 17 cm, 15.82° of visual angle). Instructions were given to the participants to observe carefully the colors of the small squares and match them with the color of the large square. Responses were collected via a response box and participants were asked to rest their index and middle finger of each hand on the four buttons. Button 1 corresponded to the condition were none of the colors of the small squares matched the color of the large square; button 2 to the condition where only 1 color of the small squares matched the color of the large square; button 3 if two colors of the small squares matched the color of the large square and button 4 if all three colors of the small squares matched the color of the large square. As soon as a response was given or after a maximum time of 3,000 ms, the large square was removed and the next small squares appeared after a 200 ms presentation of a black fixation cross. The stimuli were presented on a computer screen positioned at approximately 60 cm from the participants.

Procedure

All participants were tested separately and began with a short practice session of 60 trials with feedback on accuracy on each trial. Subsequently, the task consisted of 30 alternations of 25 sequence trials and 25 random trials with a short instruction screen of 3 s between each block of 25 trials. Half of the participants started with a sequence block, the other half of the participants with a random block. The experiment was divided into 10 runs consisting of 6 blocks with a rest break of minimum 15 s in between each run. During the break, average RT and error rate of the last block, together with the number of the next block, was projected on the screen.

The same eight colors (yellow, green, red, blue, brown, purple, magenta and cyan) were used for all subjects, but for each individual, four of these colors were randomly assigned to the color set used for the presentation of sequence trials, the other four colors were used to generate the random trials. By using randomly selected color sets for all subjects, we excluded the possibility that learning effects are due to differences between color-specific color sets. The colors of the small squares were randomly determined. During each sequence block, a five element sequence 2-4-3-1-4 defined the colors of the large target square and was repeated for five times. This sequence structure consists of three first order contingencies (the occurrence of color 4 can be uniquely predicted by the previous occurrence of color 2 or color 1; the occurrence of color 1 can be uniquely predicted by the previous occurrence of color 3) and two second order contingencies (the occurrence of color 3 can only be predicted by the occurrence of the two preceding

colors 4 and 2; the occurrence of color 2 can only be predicted by the occurrence of previous two colors 4 and 1). Sequence elements 1, 2, 3 and 4 were assigned to the colors of each individual color set and fixed for the whole course of the experiment. Each random block consisted of a randomly generated succession of the other four colors for the large target square but with the restriction of having the same simple frequency information inherent in the repeated structure of the sequence blocks: no repetitions of colors and the color assigned to abstract element 4 occurred twice as much as the colors assigned to element 1, 2 and 3. This constraint ascertained that learning effects were attributable to the sequential structure only and not to unequal frequency of occurrence. Moreover, sequence and random blocks were equated in terms of response alternatives: each of the four possible color combinations occurred with the same frequency in each block.

Awareness questionnaire

After the final block, subjects completed a questionnaire to assess explicit knowledge of the sequence. Following Mayr (1996), the participants were informed about possible between subjects conditions in the experiment and were then asked to which group they thought they belonged: group 1 the color of the large square followed a repeating sequence, group 2 the responses followed a repeating sequence or group 3 both sequences were random. If they indicated to belong to group 1 or 2, they were obliged to specify whether the serial pattern was present in color set 1, color set 2 or both color sets. The subjects were urged to think seriously about this question and to carefully reflect on the experienced task. Hereby, we intended to avoid fast and inconsiderate guessing behavior.

Next, all participants were informed about the presence of a repeating sequence structure in the colors of the large target square for their particular color set and asked to generate this pattern. Because it has been claimed that correct generation not only reflects explicit awareness but can be contaminated by implicit knowledge (Goschke 1998), a more sensitive measure was used in combination with the free generation task: the process dissociation procedure. This method was introduced to dissociate automatic and intentional processing (Jacoby 1991). We used a procedure specifically adapted for sequence learning (Destrebecqz and Cleeremans 2001). First, participants were told to produce a color sequence of 30 elements long that resembled the sequence structure as much as possible (free generation under inclusion condition). Next, subjects were instructed to generate a sequence of the same length using the same color set that differed as much as possible from the sequence structure (free generation under exclusion condition). In both conditions, they were instructed to avoid

repetitions of colors. The proportion of triplets consistent with the sequence, under inclusion and exclusion instructions, was computed. The rationale underlying this method is that conscious learning allows controlling the acquired knowledge. Consequently, the production of more sequence consistent triplets in the inclusion than the exclusion condition indicates conscious knowledge of the sequence.

Results and discussion

Accuracy

The mean error rate per block amounted to 5.96% (SD = 3.42). A positive correlation between mean RT and error rate demonstrated that there was no speed-accuracy trade off present ($r = 0.87$, $p < 0.001$).

Reaction times

To determine sequence learning, RTs of correct trials were considered. Because erroneous responses often disrupt performance, trials that followed incorrect responses were discarded as well. Median RTs of the remaining trials (88.75%) were calculated for each participant per block and the means of the individual medians per run and per order were used for subsequent statistical analyses. These RT data were submitted to a repeated measures ANOVA with run (10 levels) and order (2 levels sequential vs. random order) as within-subjects variables. This analysis revealed a significant main effect of order [$F(1,20) = 5.28$, MSE = 2,666, $p < 0.05$] reflecting slower responses on random trials than on sequential trials and indicating an overall sequence learning effect (Fig. 2). The main effect of run [$F(9,180) = 54.33$, MSE = 4,172, $p < 0.001$] and the run × order interaction ($F(9,180) = 2.21$, MSE = 1,040, $p < 0.05$) were also significant, indicating, respectively, a general improvement
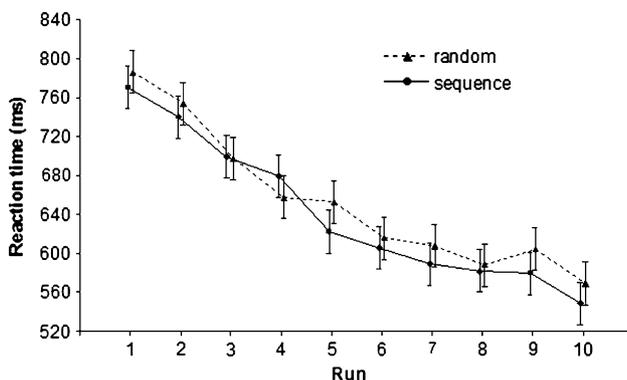

Fig. 2 Experiment 1, mean of median reaction times in each run, for sequence and random trials separately, *error bars* represent the within-subject 95% confidence intervals (Loftus and Masson 1994)

of performance and an additional performance increase in sequence blocks. Post hoc contrasts showed a significant difference between random and sequence trials occurring for the first time on run 5 ($p < 0.01$). But at this time, the learning effect was not yet robust. This is evidenced by the fact that it lost significance over the following runs 6, 7 and 8 (all $p > 0.1$). In the last two runs (9 and 10), the learning effect was fully established (both $p < 0.01$).

Next, we investigated whether learning differed for first order (where trial n can be predicted by trial $n - 1$) and second order associations (where trial n can only be predicted taking previous two trials into account) composing the sequence structure. Because another color set was assigned to the random target colors, RTs for separate color associations cannot be compared with a reliable reference, i.e., their baseline (random) RT. Therefore, for each participant, median RTs on correct responses were computed for each of the three first order associations (e.g., for first order association 2-*4*, the median RT on color 4 preceded by color 2, is computed) and for each of the two second order associations (e.g., for second order association 2-4-*3*, the median RT on color 3 preceded by color 4 and 2, is computed) over all sequence trials. By averaging over all participants, this measure cannot be confounded by perceptual effects as for each association colors are assigned in a random manner, nor by motor effects as each association could be linked with any response. The median RTs were then averaged for both types of associations. A paired t test revealed no significant difference in RT on trials defined by first order versus second order predictability [$t(20) = 0.79$, $p = 0.44$].

Additionally, control analyses were performed to make sure that the higher RTs on random trials were not confounded by a higher frequency of reversal trials in the random condition than in the sequence condition. Previous studies using the SRT task have shown that responses on so-called reversal trials (e.g., 4-2-*4*, a trial where the same element reappears after only one intervening trial) tend to be slower than non reversal trials (e.g., 4-2-*3*) (Vaquero et al. 2006; Anastasopoulou and Harvey 1999). These findings together with the notion that random blocks naturally contain more reversals than sequence blocks can contaminate the RT measure of learning. In our experiment, random blocks contained 19% more reversal trials than sequence blocks, but this finding could not explain the effect of order because responding to random reversal trials ($M = 652.45$ ms, SD = 136.16) did not differ from responding to random non reversal trials ($M = 653.19$ ms, SD = 131.97, $t(20) = .13$, $p = 0.89$).

Sequence awareness

Most participants (16 out of 21) were convinced that neither the colors of target squares, nor the manual responses could

have followed a repeating sequence. The other five participants incorrectly guessed to belong to a group where responses followed a fixed sequence. None of the subjects indicated correctly to belong to the group where the colors of the large square were sequential. This result, implying no sequence awareness, was also reflected in the free generation data, as shown in Fig. 3. There was no significant difference in the mean proportion of correctly recalled triplets under the inclusion ($M = 0.136$, SD $= 0.109$) versus exclusion instructions [$M = 0.134$, SD $= 0.126$, $t(20) = 0.05$, $p = 0.96$]. Moreover, generation performance did not differ from chance level (which is 0.139, computed by 5/36 as no repetitions were allowed) for both the inclusion [$t(20) = -0.08$, $p = 0.94$) and exclusion condition ($t(20) = -0.13$, $p = 0.89$). These results on the free generation task indicate that knowledge of the color sequence was acquired implicitly, a conclusion that is further corroborated by the fact that there was no reliable correlation between sequence learning scores (averaged RT difference between random and sequence trials) and explicit knowledge scores (generation under inclusion minus exclusion instructions) ($r = 0.11$, $p = 0.63$).

In sum, the results of the first experiment show that participants implicitly learned the perceptual sequence structure of colors without any support from the motor learning system. Moreover, additional analyses ascertained that simple frequency information cannot have contributed to the observed effect. The particular manipulation in this experiment, namely alternating sequence with random trials, enabled us to investigate the learning process closely over time. RT data evidenced that knowledge of a serial pattern in colors develops gradually and only reflects on performance when the sequence has been repeated for more than hundred times. RT comparison between first order and second order associations indicates that the perceptual learning system encodes more complex second order predictable relations as well.
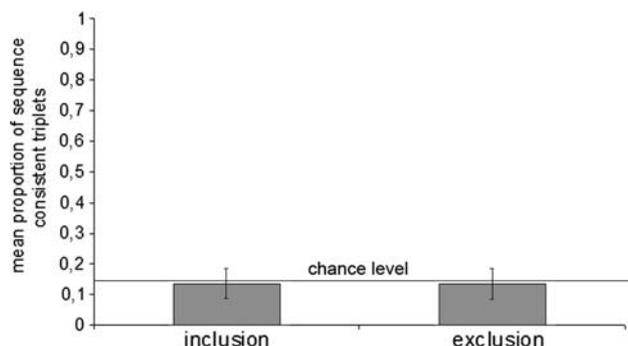

Fig. 3 Experiment 1, mean proportion of sequence consistent generated triplets, under inclusion and exclusion conditions, *error bars* represent the within-subject 95% confidence intervals (Loftus and Masson 1994)

Experiment 2

The new paradigm, presented in this paper, elicited clear evidence of learning a sequence structure of a pure perceptual form (Experiment 1). The purpose of the second experiment was to replicate this finding for motor sequences and to compare both learning processes.

Method

Participants

Seventeen paid volunteers (4 men, 13 women) participated in this study. All of them reported normal or corrected-to-normal vision and color perception, and did not participate in the previous experiment.

Serial color-matching task

Learning a response sequence was tested using identical task demands as the previous experiment.

Procedure

The procedure used in Experiment 1 was replicated for this study, except for the following aspects. For the sequence blocks, an identical abstract sequence structure was used as for the perceptual sequence (i.e., 2-4-3-1-4) but was now assigned to the responses instead of the colors of the large square. The colors of small as well as large target squares were now randomly generated but with the constraint of producing the above fixed response sequence. The color set used in response sequence blocks and the color set for random blocks were again randomly determined out of the pool of eight colors.

Awareness questionnaire

The same questionnaire was used as in Experiment 1 except that in the free generation test, participants were now informed of the presence of a response sequence in one color set and asked to generate the response sequence under inclusion and exclusion instructions.

Results and discussion

Accuracy

The mean rate of erroneous responses across all runs and all participants was 5.09% (SD $= 2.33$) and the pattern of errors correlated positively with the RT pattern ($r = 0.71$, $p < 0.001$) reflecting no speed-accuracy trade off.

Reaction times

Incorrect responses (5.09%) and the first following responses (4.72%) were discarded from analyses of the RT learning effects. One subject showed extremely large variability in correct response times (>2.5 SD from the mean variability) and was therefore excluded from the analyses. Further RT analyses were performed on the remaining 16 participants, in the same way as in Experiment 1. An analysis of variance (ANOVA) with run (10 levels) and order (2 levels sequential vs. random) as within repeated measures factors, revealed a significant main effect of run ($F(9, 135) = 27.63$, MSE = 3,090, $p < 0.001$) reflecting general learning (Fig. 4). A significant main effect of order ($F(1,15) = 40.88$, MSE = 1,114, $p < 0.001$) demonstrated that participants learned the motor sequence. The absence of a significant run × order interaction ($F(9,135) = 1.43$, MSE = 944, $p = 0.18$) implies that sequence learning occurred fast and did not change much over time. Paired contrasts showed that serial knowledge had a first significant effect on RT on run 4 ($p < 0.05$) and remained to have a reliable effect except for run 9 ($p = 0.24$).

To determine whether first and second order associations equally contributed to the observed learning effect, RT analyses were performed for each association separately. In contrast with Experiment 1, median RTs for each response association can be compared with a reliable reference, namely the median RTs of the same responses generated during the random blocks. Per subject, these difference scores (e.g., median RT on random response 4 minus median RT on response 4 in the sequential trials, i.e., when preceded by previous response 2) were computed for each association and then averaged for first and second order associations. No significant difference in learning first and second order contingencies was found [$t(15) = -1.22$, $p = 0.24$].
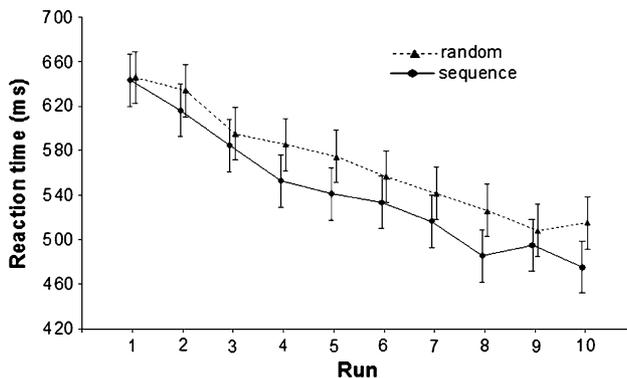
Additional analyses on the effect of reversal trials indicate that the learning effect is not due to the presence of more reversal trials (on average 20%) in random blocks. Averaged individual median RT of correct random non reversal trials was 590.69 ms (SD = 138.53), whereas averaged individual median RT over correct random reversal trials was significantly faster ($M = 491.84$ ms, SD = 154.83, $t(15) = 9.09$, $p < 0.001$) suggesting that the RT measure of learning might even be an underestimation.

Sequence awareness

Three out of the sixteen subjects reported more fluent responding on sequence blocks. Figure 5 shows the proportion of sequence consistent triplets when asked to include (inclusion) and to avoid the sequence structure (exclusion). Paired *t* test revealed no significant difference between both conditions [$t(15) = 0.72$, $p = 0.48$]. Moreover, the mean inclusion score [$t(15) = 0.89$, $p = 0.39$] as well as the mean exclusion score [$t(15) = -0.31$, $p = 0.76$] did not differ from chance level (0.139). Correlation analysis between learning scores (averaged RT difference between random and sequence trials) and explicit knowledge scores (generation under inclusion minus exclusion instructions) confirmed the above findings: learning of the response sequence was not influenced by awareness ($r = -0.13$, $p = 0.62$).

In sum, clear evidence is found for learning response-related deterministic serial associations with this new developed serial color-matching task. The predictable character of the responses in sequence blocks quickly influenced performance, however significance was reached only from the fourth run on. No influence of awareness or type of association was found on the amount of sequence learning.
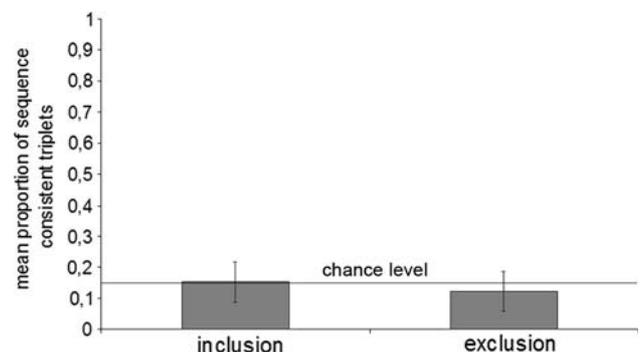

Fig. 4 Experiment 2, mean of median reaction times in each run, for sequence and random trials separately, *error bars* represent the within-subject 95% confidence intervals (Loftus and Masson 1994)

Fig. 5 Experiment 2, mean proportion of sequence consistent generated triplets, under inclusion and exclusion conditions, *error bars* represent the within-subject 95% confidence intervals (Loftus and Masson 1994)

Perceptual learning versus motor learning

Perceptual and motor learning behavior in Figs. 2 and 4 demonstrates clear differences, as confirmed by the statistical analyses of both experiments separately. Significant learning effects occurred earlier for motor sequences (from 4th run on) than for perceptual sequences (from 9th run on). To explicitly compare the time course of both learning processes, we analyzed the random minus sequence difference scores (dRTs) that were computed for each run and for both learning processes (Fig. 6). These dRTs show a comparable time course of learning for the second half of the experiment (run 6–10), but a clearly different time course for the first half of the experiment (run 1–5). Therefore, regression analyses were performed separately for the first and second half of the experiment following the procedure described by Lorch and Myers (1990). This was done by computing separate regression equations for each subject in the perceptual learning task and each subject in the motor learning task, hence evaluating the linear relation between learning scores and time (number of run).

For the first five runs, the slopes differed reliably from zero for the motor learning group [$M = 7.75$, SD = 13.77, $t(15) = 2.25$, $p < 0.05$] but not for the perceptual learning group [$M = -0.60$, SD = 15.16, $t(20) = -0.18$, $p = 0.86$], hereby confirming gradual learning in the motor group but no linear increase of learning for the perceptual group. Regression slopes also differed between groups [$t(35) = 1.73$, $p < 0.05$ one-tailed).

For the last five runs, slopes of the motor ($M = 2.23$, SD = 16.99) and the perceptual group ($M = 2.75$, SD = 14.43) did not differ reliably from zero ($t < 1$), nor from each other [$t(35) = -0.10$, $p = 0.92$].

General discussion

Learning the serial order of events or actions, without the intention to learn, is part of every day life and essential for

normal functioning. The neurocognitive mechanisms underlying this important human ability have therefore attracted research interest for many years. However, it remains an unresolved question whether neural systems of implicit sequence learning overlap when operating on motor versus perceptual serial information. Because imaging studies have used different paradigms (SRT and AGL tasks), developed with a focus on one learning process (motor or perceptual), task contexts have differed considerably and, consequently, the results of both paradigms cannot be compared meaningfully.

In this study, we developed a novel serial color-matching task that modifies the classical SRT task in three important ways: (1) identical task demands can generate independent perceptual and motor sequence learning, (2) the specific design allows for a continuous assessment of the learning process, hence providing an ideal tool for comparing time course difference between both types of sequence learning and future fMRI studies and (3) confounds that affected previous investigations of perceptual sequence learning with the SRT task (e.g., influences of oculomotor learning and simple frequency learning) are avoided.

Using the serial color-matching task, Experiment 1 and 2 provided clear evidence for implicit sequence learning both at a perceptual and motor level. These findings contribute to the domain of implicit sequence learning in the following three ways.

Perceptual SRT learning

Classification paradigms in the implicit learning domain such as the AGL tasks and VSL tasks demonstrated evidence of successful retrieval of perceptually acquired serial information (Knowlton and Squire 1996; Turk-Browne et al. 2008).

However, in the SRT literature, the existence of a pure independent perceptual learning mechanism has remained

Fig. 6 Random minus sequence difference scores (dRTs) reflecting learning effects for each run, *error bars* represent 95% confidence intervals (Loftus and Masson 1994)
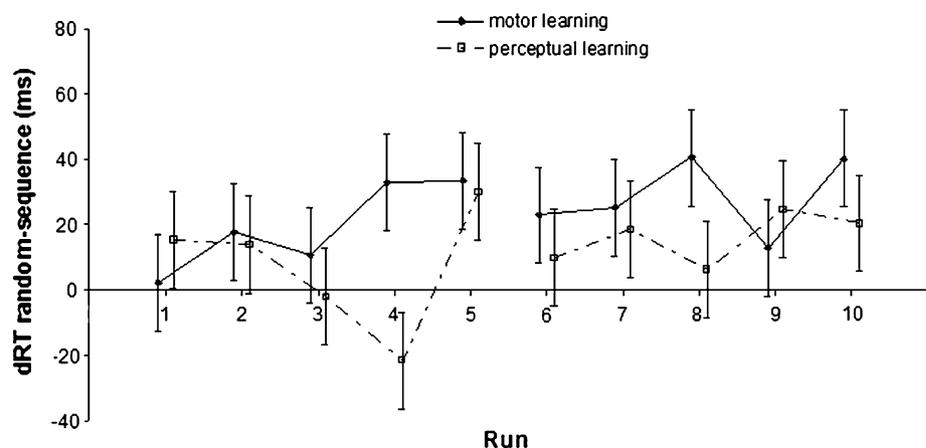
questionable. Although many studies have been published on this issue, none of them could unambiguously demonstrate evidence of learning sequential information independent of motor influences. To support this claim, we summarize these studies by organizing them in five classes of paradigmatic approach.

Observational studies

Some studies demonstrated that sequence learning can occur when the ordered stimuli are merely observed (Howard et al. 1992; Seger 1997). However, when overt responses are allowed in the first trials of the acquisition blocks, observational learning can involve further covert responding engaging the motor system (Jeannerod 2001). Moreover, since most of the observational sequence learning tasks used spatially organized stimulus sequences, one could argue that what is learned is not so much a perceptual sequence but rather a sequence of oculomotor responses. This is supported by some studies demonstrating that indeed participants learn a sequence of saccades while performing the SRT task (Albouy et al. 2006; Karatekin et al. 2007; Marcus et al. 2006).

Studies using separable response-relevant and response-irrelevant stimulus dimensions

Several prior studies created a perceptual learning condition by using stimuli with two independent dimensions: a response-relevant and a response-irrelevant dimension. Sequential perceptual regularity is imposed in terms of a stimulus attribute that is irrelevant to the response, namely the position of the stimulus on the screen. The response-relevant stimulus dimension on the other hand (e.g., color) is used to create an irregular series of responses. Some of these studies reported evidence showing that learning occurred for these ordered stimulus locations (Deroost and Soetens 2006; Mayr 1996; Remillard 2003). However, as outlined above, one can never be sure that learning the sequence of spatial positions is a purely perceptual way of learning because of the involvement of oculomotor learning.

Transfer studies

In transfer studies, perceptual learning is tested by looking at the effects of changing stimulus or response conditions after several blocks of serial visuo-motor learning have been performed (Stadler 1989; Cohen et al. 1990; Fendrich et al. 1991). Perceptual learning is assumed to have occurred in either of two situations. First, if the expression of sequential knowledge is reduced or eliminated after stimulus contingencies have been changed (while the response sequence has been retained). Or second, if the expression of sequence knowledge remains at the same level after the response mode (e.g., fingers to arm movements) or response sequence has been changed (while keeping an identical stimulus sequence). However, as Nattkemper and Prinz (1997) already pointed out, transfer tests are difficult to interpret. During sequence acquisition, a stimulus sequence is still linked to a specific action sequence. Consequently, if sequence knowledge has not been transferred in a subsequent task with altered stimulus modalities, this does not allow for the conclusion that stimulus information alone has induced sequence learning during acquisition. Similarly, finding preserved learning with the same stimulus sequence despite a change in effectors cannot be taken as evidence for pure perceptual learning. At most, it indicates that learning is not effector specific, but it does not rule out response-based learning on a more abstract level of motor planning. One study applied this method in combination with fMRI (Bischoff-Grethe et al. 2004). However, subjects in the unaware perceptual group were not able to retrieve the previously trained stimulus sequence.

Studies with a many-to-one stimulus–response mapping

Studies using many-to-one S-R mappings typically assign pairs of stimuli to the same response (Clegg 2005; Nattkemper and Prinz 1997). After a learning phase, a deviant stimulus is occasionally inserted in the repeating sequence leading to a preserved or disrupted response sequence. If subjects learn the sequential structure of the stimuli, then deviant stimuli should lead to RT increase whether or not the response sequence is violated. However, this paradigm cannot reveal conclusive evidence in favor of learning independent S-S relations. When considering such paradigm (e.g., stimulus 1 and 2 mapped to response 1 vs. stimulus 3 and 4 mapped to response 2) with a deterministic sequence (e.g., 31242341), the stimulus is still linked to a specific response on each trial, therefore the stimulus sequence has not been isolated from a response sequence (e.g., 21121221). In addition, even when probabilistic stimulus sequences are used (e.g., stimulus 1 is followed with a 50% chance by stimulus 2 and with a 50% chance by stimulus 4) and no predictions about R-R associations can be made, other response-related associations (S-R and R-S) cannot be ruled out: a stimulus can still be associated in a structured way with its corresponding response and vice versa. The latter was evidenced by Ziessler and Nattkemper (2001).

Studies using a matching task

Although aimed at a different issue, Frensch and Miner (1995) developed a perceptual matching task. On each trial,

a letter or graphic symbol appeared at the center of the screen following a 10-item sequence. The subjects were asked to match the target letter with one of the four letters appearing underneath the target and press the corresponding key. Because the locations of the four matching letters changed randomly from trial to trial, the response sequence was randomly determined. Unfortunately, the relative frequency of occurrence of each single stimulus was not equal in the sequence structure and this unequal frequency was not explicitly matched in the test blocks. Therefore, knowledge of the different frequencies of each single stimulus might have been sufficient to speed up responses in the sequence blocks (Hoffmann and Koch 1998). The fact that the acquisition of frequency information alone can account for learning in SRT tasks (i.e., if it is not explicitly controlled for in the experimental design), has been convincingly demonstrated by Shanks et al. (1994).

The present study contributes to the domain of perceptual SRT learning by presenting a new tool that avoids all confounds occurring in earlier studies. Motor influences in the form of sequential eye movements or key presses were removed and the possibility of simple frequency information eliciting the learning effect was prevented. A motor contribution in the form of (sub)vocalization also seemed very unlikely. One might only experience a benefit for task performance by (sub)vocalizing the first colors in order to hold them in working memory. These colors were, however, randomly selected. Moreover, if (sub)vocalization of only the target colors did occur, this would quickly have lead to high levels of explicit knowledge because of the very simple sequence structure. Not one of the participants, though, experienced any awareness.

The results of Experiment 1, showing independent perceptual learning, contradict previous studies claiming that perception alone cannot contribute to sequence learning (Kelly and Burton 2001; Nattkemper and Prinz 1997; Russeler et al. 2002; Willingham 1999; Willingham et al. 1989). When considering those earlier studies one could question why this present task resulted in learning and others did not? Which experimental conditions are necessary for independent perceptual learning to arise?

First of all, stimulus learning in the present study was investigated with simple deterministic material. Previously, it has been demonstrated that perceptually based learning is vulnerable to sequence complexity (Dennis et al. 2006; Deroost and Soetens 2006). The complexity limit of perceptual sequence learning is a matter of further investigation.

Secondly, in the current serial color-matching task, the stimulus sequence is attended and actively processed, with the involvement of a working memory component in the task (remembering the first three random colors). Note that this load was renewed on each trial and that it was dissociated from the actual sequence learning material. Potentially, it had a positive impact on learning by creating effortful processing of the target color sequence. Previous work has proven that attentive and effortful processing of the sequential information facilitates learning (Hartman et al. 1989; Hsiao and Reber 1998). On the other hand, introducing working memory load to the task can help to avoid explicit awareness. In our task, both factors are in good balance: focused attention to the color sequence was necessary to give the appropriate response but this attention was shared with the random colors of the small squares, which was then helpful to obscure the presence of a sequence and to avoid awareness.

Next, it is possible that in order to demonstrate learning effects, predicting the next item in sequence should have a benefit. In other words, learning the sequence structure should improve the performance of the task: e.g., being able to predict that the next large square will be red, one can anticipate the next response based on the colors of the first random small squares and speed up RT. Future experiments comparing the present stimulus presentation (with an SOA of 600 ms in between the random and target colors) with a stimulus presentation of 0 ms SOA could test this idea.

Perceptual versus motor learning processes

Using an identical task, procedure and sequential structure enabled us to advance the understanding of how different types of sequential information are processed. Results suggest that when the sequential structure is located at a motor level, this has a fast impact on performance. Sequence knowledge increased significantly in the beginning of the process and remained stable over time. Conversely, when the serial structure is located at a perceptual level, learning needs many more repetitions of the same structure before it robustly influences behavior.

It is worth noting that, although response-related learning influenced performance faster, it did not evoke higher levels of explicit knowledge. No explicit awareness of the serial structure was needed whatever type of information guided the learning process during this serial color-matching task.

The neural basis of implicit sequence learning

Several neuroimaging studies have adopted the standard SRT task to explore the brain mechanisms of implicit serial learning (Destrebecqz et al. 2005; Peigneux et al. 2000; Rauch et al. 1997; Schendan et al. 2003; Seidler et al. 2005). Motor areas such as primary and premotor cortex, supplementary motor area, basal ganglia and cerebellum have been reported to play a role in SRT learning. It is

unclear, though, whether the role of these motor areas can be generalized or whether their function is restricted to motor sequences. To gain insight into the specific brain systems underlying the acquisition of modality specific (motor vs. perceptual) representations or general sequence representations, the standard SRT task falls short whereas the serial color-matching task can help to advance this issue.

As evidenced in our behavioral study, the serial-matching task involves a large amount of non sequence specific learning, e.g., learning the stimulus–response mapping. In order to describe the sequence specific learning process adequately, future imaging studies with this serial-matching task will have to take this basic task learning into account. Including a practice session with random trials before the experimental session starts would be a straightforward possibility.

In a similar way, this study offers important implications for future clinical studies. A number of studies have demonstrated impaired implicit sequence learning in patients (Parkinson: Deroost et al. 2006; Ferraro et al. 1993; Jackson et al. 1995; cerebellar lesions: Gomez-Beldarrain et al. 1998; Molinari et al. 1997; dyslexia: Stoodley et al. 2006). However, these studies did not specify what type of learning was impaired as no dissociation was made between the different kinds of sequential information. Using the original SRT task gives a general but not a complete and detailed picture of the impairments affecting the patients. Positive performance outcome on the SRT task can still raise the question whether impaired learning of one type of sequence representation could have been masked by learning another intact format of serial information, or vice versa. The method we presented in this paper allows making this dissociation.

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