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Abstract

The capacity to acquire motor skills through repeated practice of a sequence of movements underlie many everyday activities. Extensive research in humans have dealt with the importance of spatial and temporal factors on motor sequence learning, standing in contrast to the few studies available in animals, particularly in non-human primates. In the present experiments, we studied the effect of the serial order of stimuli and associated movements in macaque monkeys overtrained to make arm reaching movements in response to spatially distinct visual targets. Under different conditions, the temporal structure of the motor sequence was varied by changing the duration of the interval between successive target stimuli or by adding a cue that reliably signaled the onset time of the forthcoming target stimulus. In each condition, the extent to which the monkeys are sensitive to the spatial regularities was assessed by comparing performance when stimulus locations follow a repeating sequence, as opposed to a random sequence. We observed no improvement in task performance on repeated sequence blocks, compared to random sequence blocks, when target stimuli are relatively distant from each other in time. On the other hand, the shortening of the time interval between successive target stimuli or, more efficiently, the addition of a temporal cue before the target stimulus yielded a performance advantage under repeated sequence, reflected in a decrease in the latency of arm and saccadic eye movements accompanied by an increased tendency for eye movements to occur in an anticipatory manner. Contrary to the effects on movement initiation, the serial order of stimuli and movements did not markedly affect the execution of movement. Moreover, the location of a given target in the random sequence influenced task performance based on the location of the preceding target, monkeys being faster in responding as a result of familiarity caused by extensive practice with some target transitions also used in the repeated sequence. This performance advantage was most prominently detectable when temporal prediction of forthcoming target stimuli was

optimized. Taken together, the present findings demonstrate that the monkey's capacity to make use of serial order information to speed task performance was dependent on the temporal structure of the motor sequence.

Keywords

Sequential movements, Response speed, Temporal predictability, Monkey

Introduction

Many skilled behaviors, like driving a car or playing a musical instrument, are organized as a series of individual movements automatically executed following extensive practice. The processes involved in the learning and performance of automatic sequential behaviors are essential for everyday activities as well as being clinically relevant in a number of neurodegenerative disorders. How subjects learn to extract regularities in sequential events and to execute corresponding sequences of actions has been the subject of extensive research (Hikosaka et al. 2002; Keele et al. 2003; Willingham 1998), a considerable effort being devoted to identify the contribution of distinct forms of learning in mediating various kinds of sequential behavior (Ghilardi et al. 2009). There is therefore a great interest in developing experimental situations through which to get insight into the anatomical substrate involved in the learning and performance of skilled motor sequences. Animal studies can provide important contributions to this research field by allowing to study sequential behavior in conjunction with simultaneous invasive neurophysiology. However, there have been few studies of the ability of monkeys to use serial order information in sequential motor tasks. Within the limits of differences in testing procedures, these studies reported that monkeys are generally faster in responding when the order of stimuli and associated movements followed a repeating sequence of three or four elements (Matsumoto et al. 1999; Matsuzaka et al. 2007; Nixon and Passingham 2000; Procyk et al. 2000) or even ten elements (Lee and Quessy 2003) and their responses slow if stimulus locations varied randomly. Such findings are not unique to monkeys, but have also been reported in sequential motor tasks with rodents (for a review see Schwarting 2009).

There are a number of factors that can play a role in determining the subject's ability to profit from a repeating sequence of movements. In particular, it has been reported that performance of sequential motor skills requires the integration of both the serial order and the

timing of the elements in the sequence. In humans, studies using serial reaction time (SRT) tasks have provided evidence that motor sequence learning may be compromised by varying the temporal regularity of stimulus presentation (Destrebecz and Cleeremans 2003; Dominey 1998; Stadler 1995; Willingham et al. 1997) which was interpreted as evidence that temporal relationships between successive task events are part of the content of sequence learning. These results indicate an impact of temporal as well as spatial regularities in stimulus presentations on the learning and performance of sequential movements. To date, work in monkeys has focused on determining whether subjects can learn about sequences of spatially distinct stimuli, some of them dealing with the effects of the length of the sequence (Procyk et al. 2000) or the degree of practice on the development of skilled sequential motor behavior (Matsuzaka et al. 2007) but the influence of temporal factors on performance of learned sequential movements, in this species, has not been explored. In addition, the question of how serially organized behavior in monkeys can be represented has been rarely investigated. In particular, little is known regarding the extent to which monkeys are sensitive to relations among successive stimuli or responses. Two kinds of serial knowledge have been distinguished in human SRT studies (Cohen et al. 1990; Reed and Johnson 1994; Remillard and Clark 2001). The first is based on the knowledge of relationships between adjacent target stimuli, each target location consistently predicting the next location (i.e, “first-order associations”); the second is based on more complex relationships between non-adjacent target stimuli, the prediction of any target location requiring knowledge about more than of the immediately preceding location (i.e, “second-order associations”). Compared to humans, there is little information on how serial patterns are encoded in monkeys.

The aim of the present study is to examine the manner in which the temporal features of a repeating series of spatial locations may affect the monkey’s capacity to make use of serial order information to speed performance in a sequential arm reaching task. Monkeys

were already overtrained to perform a predictable sequence of movements and their knowledge of the sequence order was assessed by a decrease in the speed of performance when the well-practiced repeating sequence was replaced by a random sequence. We examined the behavioral expression of such sequence learning under different conditions in which the temporal structure of the task was varied, either by shortening the time interval between successive target stimuli or by providing a cue on the timing of the upcoming target stimulus. We also employed a sequential task in which the limited number of locations made elements of the sequence fully predictable based on unique pairwise associations, each target location in the sequence consistently predicting the next location. We therefore provide evidence for the influence of organization in time of sequential events on the monkey's ability to show a performance advantage when the order of stimuli and associated movements followed a repeating sequence in comparison to a random one.

Materials and methods

Animals

Three male macaque monkeys (*Macaca fascicularis*), P, R, and B, weighing between 5 and 9 kg, served as subjects in these experiments. All animals were trained to perform arm-reaching movement towards a target to obtain a liquid reward. Two of them (monkeys R and P) were used for single-neuron recording in the striatum (Deffains et al. 2010). After overtraining in different versions of the task, the monkeys were implanted with a stainless steel device embedded into an acrylic cap to permit head stabilization during the subsequent experimental sessions. In the same surgery session, two horizontal pairs of Ag-AgCl electrodes were permanently implanted in the bony extraorbital ridges for recording electrooculograms (EOGs). Surgical procedures were performed using aseptic techniques under general anesthesia. After a postoperative recovery period of 10 days, monkeys were trained gradually

to accept restraint of the head during the experiments. The monkeys were trained on weekdays and obtained their daily amount of liquid on these days during the testing sessions. Over the weekend, they had free access to water in their home cage. All surgical and experimental procedures complied with National Institutes of Health guidelines and the French laws on animal experimentation.

Behavioral procedures

The experimental setup was the same as previously described (Deffains et al. 2010). Briefly, monkeys were seated in a restraining box in front of a panel equipped with three bicolored (red/green) light-emitting diodes (LEDs) and three metal knobs arranged horizontally at a distance of 10 cm from each other, at arm's length and at eye level of the animal. A resting key is mounted in the lower part of the panel below the central LED and knob. Trials were initiated when the monkey kept the hand on the key until the presentation of a red light at one of the three locations, acting as a trigger for movement. The animal's task was to reach the knob below the illuminated LED, the light remaining on the panel until the target contact. Correct target contact was accompanied by the delivery of a liquid reward (0.3 ml) through a tube positioned in front of the monkey's mouth. After target contact, the monkey returned to the key in preparation to the next trial, which did not begin until the total duration of the current trial had elapsed. We measured the reaction time (RT) of each movement as interval between trigger onset and movement onset (key release) and the movement time (MT) as interval between movement onset and target contact. The latency of movements after trigger onset and the time allowed to contact the target were limited to 300 ms (monkey R) or 500 ms (monkeys P and B) to force the monkeys to respond quickly. If the monkey failed to keep its hand on the key until the trigger onset, the trial was aborted and the same trial was repeated. If upper limits of RT and MT were exceeded, the trigger stimulus disappeared and the trial

continued until the end of its total duration. These trials were recorded as errors. All three monkeys performed the task with the right arm.

The monkeys were first trained for a period of several months on a protocol where the trigger stimulus followed a repeating series of three locations (right-center-left for monkeys P and R and left-center-right for monkey *B*). In this sequence, locations are fully predictable based on unique pairwise associations, i.e., location A is always followed by location B, location B by location C, and location C by location A. After this training period, a condition in which the stimuli are presented in a pseudorandom order was added to the protocol so that the monkeys were tested concurrently with the repeated and random sequences. The two types of sequence were conducted in distinct blocks of 40-60 trials, their order being counterbalanced across sessions, and there were no external signal indicating the transition to one sequence block to the other. Each daily training session involved practice of 4-6 blocks of the repeated sequence and 1-2 blocks of the random sequence.

Before recordings of the experimental data started, the monkeys were extensively trained with both the repeated and random sequences in one (monkey B), two (monkey R), or three temporal versions of the task (monkey P) illustrated schematically in Figure 1. Task requirements were similar in all three conditions that differed only by the trial duration and the insertion of a cue before the trigger stimulus: (1) the uncued 4-s condition in which the trial duration was 4 s; (2) the uncued 2-s condition in which the trial duration was shortened to 2 s; (3) the cued 4-s condition had the same temporal structure as uncued 4-a condition but trigger stimulus presentation was preceded by the illumination of the central LED with a green light on each trial. This additional cue was presented for 0.5 s and was followed by a fixed 1 s delay before the trigger onset. The various temporal conditions were run in separate blocks of trials and the change in condition was not indicated by any external signal.

Data analysis

Each monkey's performance in the task was recorded excluding incorrect responses. Performance was assessed by calculating the mean of RT and MT of all correct responses (RTs < 100 ms not included), for each stimulus location, separately for temporal conditions and trial sequences. The data were subjected to ANOVA using the means calculated over trials pooled from several sessions, the factors being the stimulus location (left, center, right), the trial sequence (repeated, random), and/or the temporal condition (uncued 4-s, uncued 2-s, cued 4-s). The factors included in each ANOVA are given in the Results section. If the effect of a factor was significant, pairwise comparison was performed by post-hoc tests with a Bonferroni adjustment for multiple comparisons.

Quantitative analysis of EOG data was made off-line by single-trial analysis using horizontal components of the eye position digitized at 200 Hz and stored during each block of trials. Ocular movements were classified into two groups depending on whether they were predictive or reactive (Deffains et al. 2010). They were considered to be predictive if animal's gaze was already on the correct target before the trigger stimulus appeared or if saccade latencies to the trigger onset were shorter than 50 ms, and to be reactive if they occurred between 50 and 250 ms. We calculated the ratio of predictive eye movements, separately for temporal conditions and trial sequences, regardless of stimulus location, as a function of the total number of trials. The effects of experimental factors on percentage of predictive saccades were assessed by using the $\chi 2$ test. The mean latency of reactive eye movements directed toward trigger stimuli, i.e., saccadic reaction time (saccRT), was calculated for each stimulus location, separately for temporal conditions and trial sequences, using the total numbers of reactive eye movements. An ANOVA was used for comparison of saccRTs pooled from several sessions separately for stimulus locations, trial sequences, and temporal conditions, the factors being specified in the Results section.

Results

Arm movements

The percent correct performance of the monkeys was as high (> 95%) in the random sequence as in the repeated sequence, regardless of the temporal structure of the task. Figure 2 shows the mean RTs and MTs in the different versions of the task for the three animals. **With each monkey, we used a two-factor ANOVA performed with stimulus location (left, center right) and trial sequence (repeated, random) as primary source variables.** A main effect of stimulus location was generally observed on task performance, but not always. In monkey R, RTs to stimuli presented contralaterally to the moving arm were significantly longer than RTs to ipsilateral stimuli irrespective of temporal features of the task ($P < 0.01$), whereas, in monkey P, a similar effect was evidenced in the uncued 4-s and cued 4-s conditions ($P < 0.01$) but not in the uncued 2-s condition. There was also a significant effect of stimulus location on RTs in monkey B ($P < 0.01$) with the RT for ipsilateral stimuli being longer than the RT for contralateral ones. The MTs of all three monkeys varied significantly by stimulus location ($P < 0.01$), being longer for the leftward movements than for the rightward movements, except for monkey B. The effect of stimulus location on the speed of the initiation and execution of movement is consistent with previous findings showing that reaches directed at contralateral targets are performed more slowly than reaches directed at ipsilateral targets (Ravel et al. 2006; Deffains et al. 2010), with the exception of monkey B which apparently adopted a particular response strategy leading to optimize RT performance for contralateral targets. These differences among stimulus locations were generally maintained whether or not the sequence was repeated or random, as indicated by the lack of significant interaction between stimulus location and trial sequence observed in almost all cases (see below).

In the two monkeys tested in the uncued 4-s condition (Figure 2 left), **the results of 2-way ANOVAs revealed that trial sequence was not a significant factor** on RTs (monkey

P: F (1, 2404) = 3.61, P > 0.05; monkey R: F (1, 1168) = 0.84, P > 0.05) and MTs (monkey P: F (1, 2408) = 0.03, P > 0.05; monkey R: F (1, 1171) = 0.0001, P > 0.05). Moreover, the factor stimulus location did not interact significantly with trial sequence, except on MTs for monkey R (F (2, 1171) = 9.89; P < 0.01). In the uncued 2-s condition (Figure 2 middle), there was a significant main effect of trial sequence on RTs in monkey B (F (1, 2461) = 142.64, P < 0.01), reflecting faster responses to repeated versus random sequences, but not in monkey P (F (1, 2381) = 0.78, P > 0.05). There was no significant difference in MT between repeated and random sequences in monkey B (F (1, 2499) = 1.44, P > 0.05), whereas such a difference was detected in monkey P who took less time to execute movements in random than in repeated sequences (F (1, 2388) = 16.50, P < 0.01). There was no interaction between stimulus location and trial sequence on RTs and MTs for both animals, except on RTs for monkey B (F (2, 2461) = 3.44, P < 0.05). In the cued 4-s condition (Figure 2 right), there was a significant main effect of trial sequence on RTs in monkey P (F (1, 2846) = 58.23, P < 0.01) and in monkey R (F (1, 2856) = 9.88, P < 0.01), RTs being shorter in the repeated sequence than in the random sequence. The MT showed no significant difference between sequences in monkey R (F (1, 3495) = 0.43, P > 0.05), whereas a significant difference was evidenced in monkey P (F (1, 2884) = 31.94, P < 0.01), MT being shorter in the repeated sequence compared to the random sequence. No interaction between stimulus location and trial sequence were significant.

In sum, a serial order effect was evident on the speed of the initiation of movement, resulting in a shortening of RT when trigger stimuli were presented in a repeating order, as opposed to a random order. In comparison, the execution phase of movement was seldom influenced by the serial order of task events. The improvement in RT performance was consistently detected when a temporal cue preceded the onset of the trigger stimulus and, in one out of two monkeys, when trial duration was shortened so that the trigger stimulus was

presented every 2 s. This suggests that sequence learning was more efficiently expressed when monkeys in some way are better at predicting the time at which the trigger stimulus is to be presented.

Eye movements

Eye movements were recorded during task performance in all three monkeys, with the exception of monkey R in the uncued 4-s condition in which EOG data were lost due to technical difficulties. As illustrated for monkey P in Figure 3A, reaching movements were preceded by eye movements classified into two types, predictive and reactive (see Methods). We focused on the frequency of predictive saccades as an indication that the monkeys apparently anticipate forthcoming locations of the trigger stimulus. In the uncued 4-s condition, there was no significant difference in the frequency of predictive eye movements between the repeated sequence (27%) and the random sequence (22%) in monkey P ($\chi 2$ = 3.15, df = 1, P > 0.05). In the uncued 2-s condition, the frequency of predictive eye movements was significantly higher in the repeated sequence (40-47% in monkeys P and B, respectively) than in the random sequence (31-32% in monkeys P and B, respectively) (monkey P: $\chi 2$ = 10.93, df = 1, P < 0.01; monkey B: $\chi 2$ = 22.72, df = 1, P < 0.01). We also found that the frequency of predictive eye movements decreased in the cued 4-s condition when passing from the repeated (30-60% in monkeys P and R, respectively) to the random sequences (24-38% in monkeys P and R, respectively) in monkey R ($\chi 2$ = 47.92, df = 1, P < 0.01) and, to a lesser extent, in monkey P ($\chi 2$ = 4.50, df = 1, P < 0.05).

Mean latencies of reactive eye movements (i.e., saccRTs) in the different conditions are shown in Figure 3B. **Stimulus location x trial sequence ANOVAs were performed separately for each monkey.** There was a significant effect of the location of the trigger stimulus, reflecting longer saccRTs when the stimulus was located on the side contralateral to the moving arm, than when it was ipsilateral (P < 0.01), except for monkey B in which the

shortest saccRT was for contralateral stimuli, and for monkey P in the uncued 4-s condition in which no significant difference was detected between ipsilateral and contralateral stimuli. In the uncued 4-s condition (Figure 3B top), no significant main effect of trial sequence was found on saccRTs for monkey P ($F(1, 895) = 0.29$, $P > 0.05$), whereas such an effect was detected in the uncued 2-s condition in monkeys P and B (Figure 3B middle), with saccRTs being shorter in the repeated sequence than in the random sequence (monkey P: $F(1, 762) = 8.60$, $P < 0.01$; monkey B: $F(1, 667) = 21.73$, $P < 0.01$). SaccRTs that were sensitive to serial order were also observed in the cued 4-s condition (Figure 3B bottom) in monkey P ($F(1, 592) = 7.37$, $P < 0.01$), but not in monkey R ($F(1, 522) = 0.50$, $P > 0.05$). In all three monkeys, there was no significant interaction ($P > 0.05$) between stimulus location and trial sequence, except for monkey R in the cued 4-s condition ($F(2, 522) = 3.31$, $P < 0.05$).

In summary, the analysis of oculomotor behavior revealed that the presentation of trigger stimuli in a repeating order reduced latencies of reactive eye movements and increased frequencies of predictive eye movements, in comparison to a random order, when monkeys could better predict the timing of the next target stimulus. These changes in oculomotor behavior may contribute to the behavioral improvement manifested in shorter arm movement latencies when the stimulus locations and movements followed the repeating sequence, as opposed to a random order.

Comparison between temporal conditions

We compared performance of monkey P which was tested in all three temporal conditions. For this analysis, data were pooled from all stimulus locations, separately for each temporal condition and each trial sequence and we focused on the arm and saccade latencies (Figure 4). A two-way ANOVA with trial sequence and temporal condition as factors was carried out on these data. We found a significant main effect of both trial sequence ($F(1, 7643) = 7.35$, $P < 0.01$) and task condition ($F(2, 7643) = 105.67$, $P < 0.01$) on the RTs, as well as significant

interaction between these two factors ($F(2, 7643) = 27.79$, $P < 0.01$) indicating that the serial order effect changed according to the temporal structure of movement sequences. Comparisons between the repeated and random sequences in each task condition revealed significantly faster RTs in the repeated sequence in the cued 4-s condition (one-way ANOVA, $P < 0.01$ adjusted with the Bonferroni correction method), whereas the difference was not significant in the two other conditions, indicating that the serial order effect was greater for precued movements than it was for uncued movements, even when the intertrigger interval was shortened to 2 s. We also found significant main effects of trial sequence ($F(1, 2250) = 9.34$, $P < 0.01$) and task condition ($F(2, 2250) = 54.56$, $P < 0.01$) on the saccRT and the interaction between these two factors was significant ($F(2, 2250) = 3.77$, $P < 0.05$). Comparing the saccRTs between the repeated and random sequences in each task condition yielded significant shorter saccRTs in the repeated sequence in the cued 4-s condition ($P < 0.05$) and the uncued 2-s condition ($P < 0.05$), but not in the uncued 4-s condition. This analysis confirms that, as shown by our initial analyses of RT data, the effect of trial sequence was influenced by the temporal structure of the task. In the monkey tested in all three temporal conditions, it is only in the cued 4-s condition that a significant serial order effect on RT was detected. However, in this monkey, an improvement in oculomotor performance was observed in both the uncued 2-s and cued 4-s conditions, suggesting that the sensitivity to serial order was less for arm movements than it was for eye movements.

Within-random block analysis of performance

A key feature to consider in our experimental design is the presence, in the random sequence, of possible transitions of target stimuli identical to that of the repeated sequence (see Methods). There is therefore a possibility that monkeys more quickly react to the trigger stimulus when it occurred in the same serial location, as compared to the trials in the repeated sequence. We addressed this concern by analyzing task performance for random trials divided

into two types: those in which stimulus locations follow familiar transitions, as opposed to unfamiliar transitions. This analysis was performed for all correct trials pooled from all stimulus locations for each condition in each monkey (Figure 5). Also included in this analysis are data from the repeated sequence. One-way ANOVAs were conducted on the RT with trial type (familiar, unfamiliar, repeated) as the factor. We found a significant trial type effect in the cued 4-s and uncued 2-s conditions in all monkeys ($P < 0.01$) and, at a lesser extent, in the uncued 4-s condition only in monkey P ($P < 0.05$). Post-hoc pairwise Fisher's tests revealed significantly shorter RTs ($P < 0.01$, corrected) on familiar transitions versus unfamiliar transitions in monkey P in the uncued 2-s and cued 4-s conditions, but not in the uncued 4-s condition, in monkey R in the cued 4-s condition, and in monkey B in the uncued 2-s condition. These differences may reflect the fact that the memory for pairwise associations experienced in the repeated sequence still remained in the random sequence and resulted in an earlier movement initiation on familiar transitions. However, advantages of the familiar transitions were detectable in the uncued 2-s and cued 4-s conditions, but not in the uncued 4-s condition, suggesting a dependency on the temporal structure of the task.

Comparing the RTs obtained in familiar transitions with those in the repeated sequence yielded significant shorter RTs ($P < 0.01$) on familiar transitions in the uncued 4-s and uncued 2-s conditions in monkey P, and significant longer RTs on familiar transitions in the cued 4-s (monkey P) and uncued 2-s conditions (monkey B). On the other hand, RTs did not differ significantly between familiar transitions and repeated trials in the uncued 4-s and cued 4-s conditions in monkey R. Therefore the comparison between the familiar transitions and repeated trials indicates that, in some instances, the monkeys could acquire information other than just a simple association between consecutive target stimuli.

The same kind of analysis for familiar and unfamiliar transitions was also performed on saccRTs and revealed a significant effect of trial type in the uncued 2-s condition in

monkey B ($P < 0.01$) and, at a lesser extent, in monkey P ($P < 0.05$) and in the cued 4-s condition in monkey P ($P < 0.05$) (data not shown). However, paired comparisons between familiar and unfamiliar transitions indicated that saccRT did not differ significantly in any case, except for one animal (monkey B) in which saccRTs on familiar transitions were shorter than those in unfamiliar transitions. It therefore appears that the presence of familiar transitions in random blocks had little effect on saccRTs, compared to those observed on arm RTs.

In summary, it is worth noting that, depending on certain temporal requirements, RT on familiar transitions was consistently shorter than on unfamiliar transitions, suggesting that the monkeys could prepare for the initiation of the next movement based on their extensive practice in the repeated sequence with pairwise associations.

Discussion

The present experiments were designed to test for the influence of extensive practice of a repeating sequence of arm reaching movements on task performance under conditions where the temporal organization of stimuli and their associated responses was manipulated. Although there has been previous indication that monkeys could produce movements that were sensitive to serial order, no study has specifically investigated the influence of temporal factors on the behavioral expression of sequence learning in non-human primates. This led us to examine the effects of shortening the time interval between successive target stimuli or providing the monkey with a cue on the timing of the upcoming target stimulus. We found that both manipulations improved the ability of monkeys to produce movements that were sensitive to serial order.

Assessment of serially organized movements with an arm-reaching task in monkeys

In the present study, we showed that, under certain temporal conditions, target stimuli presented in a repeating order result in an earlier movement initiation with respect to those occurring in a random order. In contrast, there was less advantage on the movement execution side when target stimuli, regardless of the temporal condition, indicating that the sequence learning effect did not speed up overall reaching performance but, rather, had a selective impact on movement initiation. As we have pointed out in the introduction, few studies have explored motor sequence learning in monkeys (Lee and Quessy 2003; Matsumoto et al. 1999; Matsuzaka et al. 2002; Nixon and Passingham 2000; Procyk et al. 2000). Although methodological differences exist in the ways animals were tested, the findings generally provide support for the view that monkeys could learn about the serial order of target stimuli and organize their responses to them. In these experiments, sequence learning was assessed in terms of response time defined as the interval between the presentation of a given stimulus and the time when the corresponding target is acquired (Matsumoto et al. 1999; Nixon and Passingham 2000; Procyk et al. 2000). A notable exception is the study of Lee and Quessy (2003) who made a separation of processes related to the initiation of movements from those implicated in their execution. They showed that the increase in response time (i.e., the sum of RT and MT) observed when a repeating sequence of target stimuli turns to a random order, is not equally reflected in initiation (RT) and execution (MT) processes. In particular, MTs on repeating stimulus sequences decreased in the course of practice, whereas RTs were shown to be relatively unaffected, suggesting that MTs displayed sequence learning effects more sensitively than RTs. To our knowledge, only one study in humans has attempted to separate the initiation of movements from their execution in a SRT task involving arm reaching movements (Moisello et al. 2009). The findings indicated that the RT can be taken as a reliable index of the degree of sequence learning, whereas the MT is more closely related to

skilled execution of movements regardless of their serial order. This suggests that the changes in sequence order are not equally reflected in speed of movement initiation and execution.

In our task, the shortening of arm movement latency was accompanied by an increase in the fraction of predictive eye movements and a reduction in the latency of saccadic eye movements with the repeated sequence, indicating that sequence learning takes place through eye as well as arm movements. Thus, consistent with other observations in behaving monkeys (Miyashita et al. 1996), changes in the oculomotor behavior can serve as an index for learning sequential regularities. In humans, a serial order effect within the context of oculomotor behavior has also been reported in SRT studies involving saccade sequences (Albouy et al. 2006; Marcus et al. 2006) and saccade latency was increased when a well-practiced sequence of eye movements is replaced by a new sequence (Grosbras et al. 2001; Kawashima et al. 1998).

A number of factors are supposed to influence motor sequence learning in a sequential task, in humans as well as in monkeys, including the level of motor skill attained by subjects and task features, such as the length and complexity of the serial pattern and the duration of the interval between sequence elements. Another obvious factor in animal studies is the schedule of reinforcement. In most studies, monkeys are required to acquire all successive targets of a given sequence to receive reward (Lee and Quessy 2003; Matsumoto et al. 1999; Nixon and Passingham 2000). However, sequence learning may be confounded by a motivational factor because faster responses were consistently observed in relation to the prediction of the rewarded element of a series of movements (Bowman et al. 1996; Procyk et al. 2000; Shidara et al. 1998). In the present study, we diminished the influence of this potentially confounding factor by using a task design in which each sequence element was rewarded thus ensuring that the serial order effect could not be attributed to increasing reward proximity.

Influence of temporal structure of the task on the performance of sequential movements

So far, studies involving monkeys have never addressed the influence of the temporal factors on motor sequence learning. In the present experiment, we showed that the improvement in performance under repeated sequence, as compared to random sequence, clearly depended on the temporal structure of the task. In particular, we observed no specific gain in performance in the repeated sequence when target stimuli were not precued and the time interval separating sequence components was relatively long. This indicates that the monkeys' ability to express their representation of serial order can be facilitated by the prediction of when the upcoming target stimulus will be presented. This finding is consistent with the idea that organization in time of sequential events is indeed an influential factor determining the speed of performance. In our uncued 4-s condition, individual sequence elements were separated from each other by relatively long time intervals, the monkey being required to move back to the bar after target contact and wait for the total duration of the trial to elapse before a new trial began. It is possible that a difficulty for the monkey to build temporal predictions of forthcoming events may interfere with the behavioral expression of its knowledge of the sequence. Experiments with rats have also emphasized this point in suggesting that brain electrical stimulation used as a reinforcer for the performance of serially ordered nose-pokes can facilitate sequence learning by avoiding pauses for reward between sequence elements (Christie and Dalrymple-Alford 2004). A number of SRT studies in humans have demonstrated the importance of temporal structure of the task in sequence learning (Destrebecqz and Cleeremans 2003; Dominey 1998; French and Miner 1994; Stadler 1995; Willingham et al. 1997). Stadler (1995), for instance, pointed out that insertion of unusually long time intervals between a response and the subsequent stimulus presentation reduces sequence learning. Other studies have investigated the effects of changes in the temporal presentation of target stimuli by varying the durations between events during sequence training. It therefore appears that the

time available between successive elements of motor sequences is critical in determining the extent to which sequence knowledge is incorporated to SRT performance (Destrebecqz and Cleeremans 2003).

At least in one out of two monkeys, we reported that the sensitivity to serial order became detectable when the intertrigger interval was shortened from 4 to 2 seconds, indicating that animals could take advantage of the shorter interval to improve the temporal prediction of the forthcoming trigger stimulus and its associated response. This suggests that evidence for sequence learning could only be obtained if intervals were within a specific time range. Interestingly, when the interval was shortened, performance was significantly faster than with the long interval but not as effective as when a predictive temporal cue was added at the beginning of each trial, suggesting that the availability of such a cue results in maximum efficiency of the serial order effect. On the contrary, to predict the moment of the upcoming target stimulus under the uncued condition, monkeys must internally time when the stimulus will occur in relation to the preceding stimulus presentation and their readiness to respond could be better timed for short time intervals than for longer ones because of imperfect time keeping ability (Gibbon and Church 1990).

What do monkeys learn about sequence structure?

Our findings point out that the monkeys' ability to make speeded motor responses depended on which stimulus location previously occurred. We designed our sequential task so that monkeys encountered during the random blocks a subset of target transitions that were part of the sequence used in the repeated blocks, thus allowing for faster responding on these well-practiced transitions. It therefore appears that monkeys were faster in responding on familiar transitions than on unfamiliar ones indicating that they tended to reproduce the sequential associations experienced with the repeated sequence. In humans, previous research has provided evidence that sequence learning could be based on knowledge of relationships

between adjacent elements of a sequence (Cohen et al. 1990; Stadler and Neely 1997). Compared to humans, the question of how serial patterns are encoded has been rather poorly investigated in the few studies which have used sequential motor tasks in monkeys. The present data provide support for the view that the serial order effect observed in our experimental situation may be the expression of first-order transitions experienced in the repeated sequence, each element of the sequence consistently predicting the location of the succeeding element. The results showed, however, that there was a variability in the data of our monkeys, although all could produce faster responses specific to the familiar transitions, their level of performance on these transitions did not always sustain the level of performance attained in the repeated sequence. Thus, in some cases, RTs for familiar transitions increased compared with those of the corresponding transitions in the repeated sequence, whereas in other cases RTs were shorter in familiar transitions than in repeated sequence trials. These results suggest that the monkeys may get more information than just a sequence of stimulus-response relationships between successive stimuli and movements. It would be interesting to determine in future experiments whether monkeys can extract higher-order information in a sequence learning situation using more complex sequence structures than the first-order three-element sequence used here.

Conclusion

This is the first systematic study on the influence of temporal structure on performance of sequential movements in monkeys. Our results show that the ability of monkeys to make use of serial order information is dependent on certain temporal requirements in order to be effectively manifest in task performance. It therefore appears that both spatial and temporal aspects of sequential events are indeed effective factors for the production of motor sequences. Among the questions addressed by neuroimaging research examining the neural mechanisms of learned sequential behaviors in humans, there is a growing interest in the

contribution of brain regions involved, at cortical and subcortical levels, in the processing of spatial and temporal properties of sequential information (Bengtsson et al. 2004; Bortoletto et al. 2011; Garraux et al. 2005; Ullén and Bengtsson, 2003). Our recent single-unit electrophysiological study in monkeys (Deffains et al. 2010) has shown that neurons in the striatum may change their activity flexibly when the spatial organization of sequential movements is modified. By varying the temporal structure of a sequential motor task, as described in the present study, we will be able to manipulate separately temporal and spatial relationships between sequence elements in order to investigate the changes in neuronal activity specifically related to processing of either aspect of motor sequence learning. This will allow to link neurophysiological research on processing of sequential information in non-human primates to brain imaging studies in humans.

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Figures

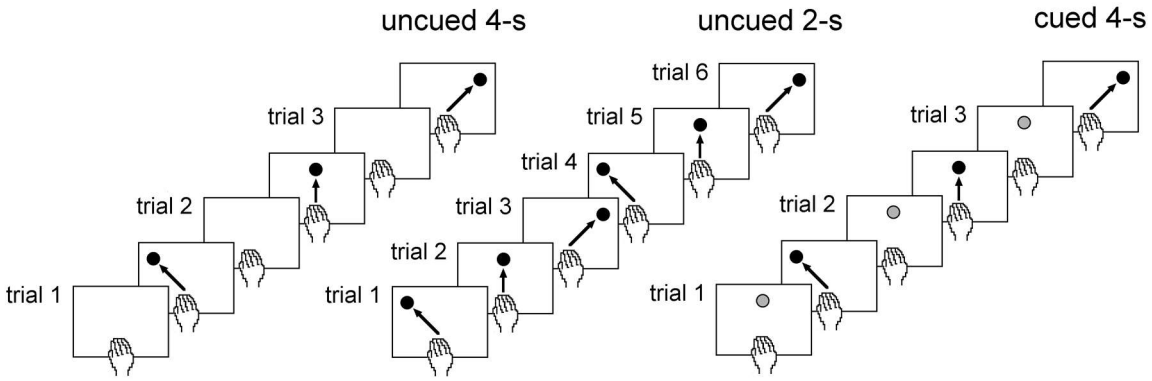

Figure 1. Schematic illustration of the temporal sequence of events in the three versions of the reaching task.

The task consisted of reaching movements on three target stimuli aligned horizontally on a panel. The target stimulus followed a repeating sequence of three locations or was presented in a pseudorandom order in distinct blocks of trials. A liquid reward was delivered after each correct reach. Under different conditions, the temporal sequence of task events was varied. In the uncued 4-s condition, the interval between two successive target stimuli was 4 s. This interval was shortened to 2 s in the uncued 2-s condition. The interval between two successive target stimuli was maintained at 4 s in the cued 4-s condition, but an additional visual stimulus serving as a temporal cue was presented 1.5 s before the target stimulus. The repeated and random sequences were tested under each of these three conditions of the task. Black circles indicate the location of the target stimulus and grey circles the location of the temporal cue. The arrows indicate the direction of reaching movement. The monkeys were extensively trained on one or more of these task conditions before the collection of experimental data.

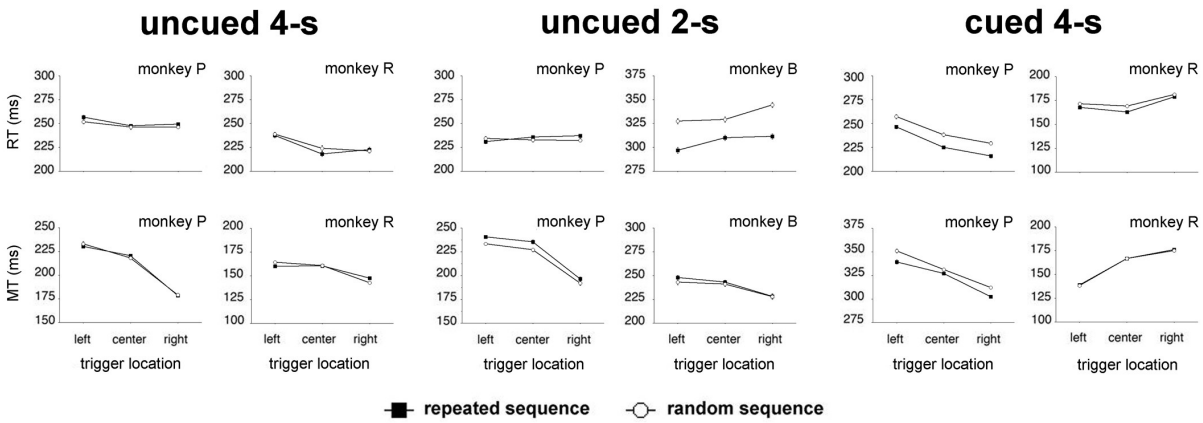

Figure 2. Effect of varying temporal structure of the task on reaching performance.

For each monkey, values of reaction time (RT) and movement time (MT) are means ± SE for the three locations of the target stimulus in the repeated and random sequences. Mean RTs and MTs were calculated by averaging all trials in each temporal condition (numbers of trials: monkey P, 1177-1344; monkey R, 570-1454; monkey B, 1221-1246).

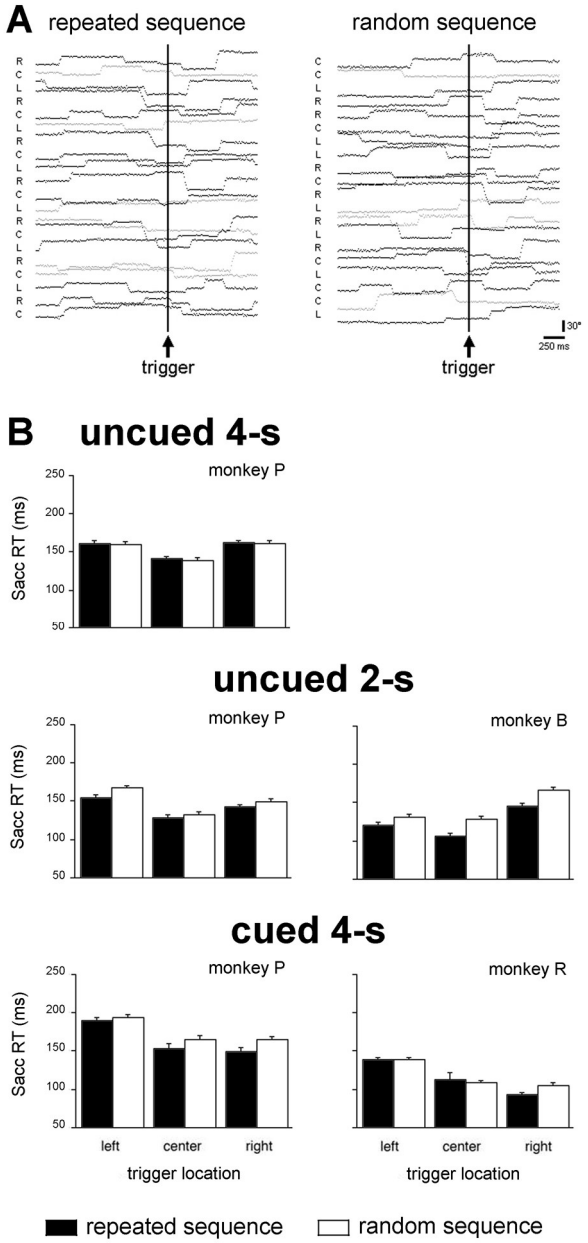

Figure 3. Effect of varying temporal structure of the task on oculomotor performance.

A. Examples of eye movements recorded during arm reaching toward target stimuli in the cued 4-s condition for monkey P. Traces of horizontal electrooculograms were aligned to the onset of the trigger stimulus in the repeated and random sequences. Black and grey traces denote the occurrence of reactive and predictive eye movements, respectively. The vertical alignment of letters in a row indicates the location of the target stimulus for each trial (R, right; C, center; L, left).

B. Latencies of saccadic eye movements directed toward the trigger in the different task conditions. Values of saccadic latency (saccRT) are means ± SE for the three locations of the target stimulus in the repeated and random sequences calculated from the total numbers of reactive eye movements in each temporal condition (numbers of trials: monkey P, 272-462; monkey R, 220-308; monkey B, 310-368). Data from the uncued 4-s condition for monkey R were not available.

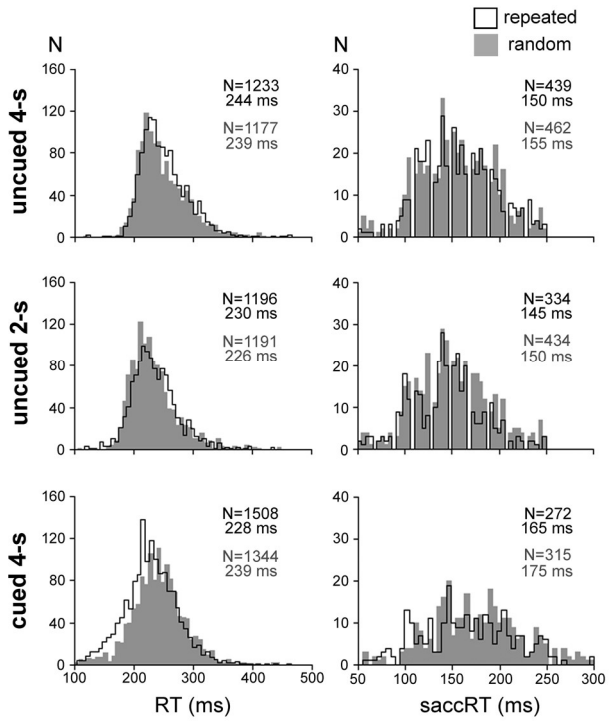

Figure 4. Histograms of latencies of arm and eye movements during performance in the three task conditions.

The data were taken from the monkey P who was tested in all three temporal versions of the task. Distributions of RTs and saccRTs are shown for the different conditions in the repeated and random sequences. Numbers of trials (N) and medians are indicated for each distribution.

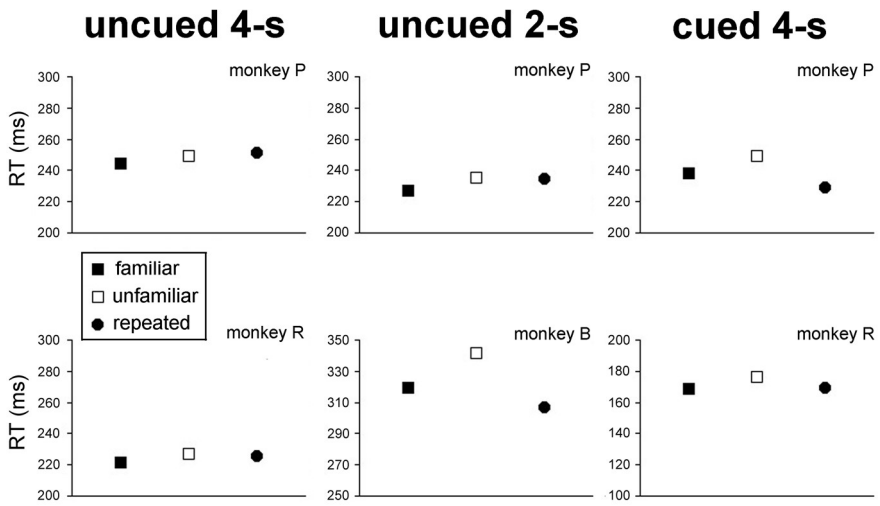

Figure 5. Effects of types of transition between adjacent target stimuli in the random sequence on the latency of arm movements.

Data points indicate means obtained from all trials pooled from all three spatial locations for each of the three conditions (familiar transitions: monkey P: 361-747, monkey R: 223-464, monkey B: 439; unfamiliar transitions: monkey P: 802-1416, monkey R: 356-939, monkey B: 759). Data from the repeated sequence were indicated for comparison.