

Movement chunking during sequence learning is a dopamine-dependant process: a study conducted in Parkinson's disease

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Abstract Chunking of single movements into integrated sequences has been described during motor learning, and we have recently demonstrated that this process involves a dopamine-dependant mechanism in animal (Levesque et al. in *Exp Brain Res* 182:499–508, 2007; Tremblay et al. in *Behav Brain Res* 198:231–239, 2009). However, there is no such evidence in human. The aim of the present study was to assess this question in Parkinson's disease (PD), a neurological condition known for its dopamine depletion in the striatum. Eleven PD patients were tested under their usual levodopa medication (ON state), and following a 12-h levodopa withdrawal (OFF state). Patients were compared with 12 healthy participants on a motor learning sequencing task, requiring pressing fourteen buttons in the correct order, which was determined by visual stimuli presented on a computer screen. Learning was assessed from three blocks of 20 trials administered successively. Chunks of

movements were intrinsically created by each participant during this learning period. Then, the sequence was shuffled according to the participant's own chunks, generating two new sequences, with either preserved or broken chunks. Those new motor sequences had to be performed separately in a fourth and fifth blocks of 20 trials. Results showed that execution time improved in every group during the learning period (from blocks 1 to 3). However, while motor chunking occurred in healthy controls and ON-PD patients, it did not in OFF-PD patients. In the shuffling conditions, a significant difference was seen between the preserved and the broken chunks conditions for both healthy participants and ON-PD patients, but not for OFF-PD patients. These results suggest that movement chunking during motor sequence learning is a dopamine-dependent process in human.

Keywords Parkinson · Dopamine · Striatum · Motor learning · Movement · Sequence learning

Introduction

Motor sequences are used in simple movements such as reaching and grasping an object as well as more complex skills such as using a tool or playing a music instrument. Learning of such skills is thought to involve the combination or the grouping of single movements into fluent sequences, leading to coordinated actions (Seidler 2006; Verwey and Eikelboom 2003). According to such a view, motor skill would contain multiple groupings of simpler movements. With practice, those groupings consolidate into chunks, which optimize the automatic execution of movements. Sakai et al. (2003) have demonstrated that different chunks can be created by different persons for a

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given sequence of movements. These authors have also found that motor learning was faster when chunks of previous movement sequences can be incorporated into new ones. In other words, it seems that motor sequence learning involves a reorganization, or a new chunking of already known movements, or groups of movements.

There is evidence suggesting that movement chunking involves the nigrostriatal dopaminergic system. In primates, selective and unilateral lesion of this neurochemical system has prevented the learning of a motor sequence task with the contralateral arm, but not with the ipsilateral one (Matsumoto et al. 1999). Monkeys were not able to add new individual movements to old and well-established ones. In rodents, dopamine (DA) dysfunction within the striatum was also studied on natural sequential behaviours, such as grooming. This behaviour always follows predictable patterns, with up to 25 forelimb strokes and body licking movements into a four-phase syntactic chain of actions that always lasts around 5 s. Striatal excitotoxic lesion or a lack of the D1a receptors in mutant mice was found to disrupt the serial structure of this chain pattern, without disrupting the execution of single movement components of the grooming behaviour executed outside a sequential context (Berridge and Whishaw 1992; Cromwell et al. 1998).

Results obtained recently by our team have demonstrated a chunking deficit during motor sequence learning in primates treated with raclopride, a specific dopamine D2 receptor (D₂R) antagonist (Levesque et al. 2007). The drug had no effect on the sequences that were well executed before the injection, but learning of new sequences was significantly affected. Monkeys were not able to join a new movement portion to a well-learned one, and this was maintained throughout the treatment with raclopride, which lasted many weeks. More recently, we have shown that this raclopride-induced deleterious effect on chunking can be prevented by a pre-administration of sumanirole, a highly selective D₂R agonist (Tremblay et al. 2009). This suggests a primary role of dopamine in the chunking process of isolated movements into integrated motor sequences.

In human, motor sequence learning has also been found to be affected in clinical conditions characterized by nigrostriatal DA lesions, such as Parkinson's disease (PD) (Siegert et al. 2006). However, it is not clear whether such a motor deficit involves a movement chunking process disturbance. To our knowledge, this has never been directly investigated. Boyd et al. (2009) have used patients with basal ganglia strokes, mainly in the putamen, to show an impaired ability in chunking single movements into organized sequences. Although dopamine was not directly involved in this study, the results reinforced the view of a primary role of the striatum in movement chunking.

The aim of the present study was to assess movement chunking during motor sequence learning in PD patient and

to specifically verify whether DA levels in the striatum play a primary role in this process. We also measured how previous movement chunks can be reintegrated into new motor sequences. PD patients were tested with (ON) and without (OFF) their usual treatment with levodopa. They were also compared with age-matched control subjects. We hypothesized OFF-PD patients as being unable to develop new chunks as efficiently as when they are ON, or as control subjects.

Materials and methods

Subjects

Eleven patients with Parkinson's disease (PD) were compared to twelve age-matched healthy participants. Patients were recruited at the André Barbeau Movement Disorder Unit of the "Centre Hospitalier de l'Université de Montréal" (CHUM). The diagnosis of PD was done by an experienced neurologist based on the presence of an akinetic-rigid syndrome, with or without resting tremor. The diagnosis was corroborated by a positive reactivity to levodopa, as defined by a minimum of 30% symptoms change between the ON (with levodopa) and OFF (without levodopa) conditions, using the Unified Parkinson's Disease Rating Scale (UPDRS) motor subscale. All patients had to be treated with levodopa in monotherapy for at least 5 years before their enrolment in the study. Patients treated with dopamine agonists, anticholinergic agents, or amantadine were not enrolled. PD severity ranged from mild to moderate, corresponding to stages II to III on the Hoehn and Yahr (1967) scale. Patients with wearing-off, prominent axial symptoms, or autonomic disturbances were excluded to avoid any treatment complication or atypical parkinsonian syndromes.

Depression was ruled out in every participant by a score over 11 in the Geriatric Depression Scale (GDS). Dementia was also excluded using the Mini-Mental State Examination (MMSE) (Folstein et al. 1975), which had to be over 28. History of stroke, head injury, neurosurgery, or drug abuse was also considered as exclusion criteria. Among the whole sample, 21 participants were right handed, and two were left handed. All had normal or corrected-to-normal vision and were naïve as to the experiment design or purpose. Everyone signed an informed consent form approved by the University (UQAM) and hospital (CHUM) Research Ethic Boards.

Experimental procedure

Parkinson's disease patients were seen while under levodopa/carbidopa medication (ON state) and after a 12-h drug

washout (OFF state). According to the product monograph, such a 12-h delay is sufficient to allow a complete blood withdrawal. Drug condition was counter balanced over the 2 days of testing (cross-over design), in that half of the patients were seen ON first, and OFF on the second day, while the reverse order was done for the other half. Age-matched participants were also assessed on two consecutive days in order to control for the test–retest effect. Testing always occurred at the participant's home, between eight and ten o'clock in the morning.

Motor sequence chunking was assessed with a modified version of the 2×5 task (see Fig. 1) developed by Hikosaka et al. (1995), and Sakai et al. (2003). In this task, participants had to press successively, in the correct order, and as fast as possible, a series of keyboard buttons. The keyboard buttons were in the same spatial position as visual stimuli (1×1 cm squares) presented on a computer screen. Two stimuli (called a set) were presented at a time, and buttons had to be pressed one at a time, with the dominant index finger only. Subjects had to learn, by trial and error, the correct order of the two buttons to press and had a maximum of 3-s to respond. If the response was given in the correct order, a new set of two stimuli immediately appeared on the screen. A series of seven consecutive sets (14 stimuli) constituted a complete trial and was called a hyperset. The latter determines the specific stimuli included within a sequence, but not their respective rank. Any variation of the sets rank within a hyperset will create different sequences.

The subjects' responses to each set of stimuli were recorded as reaction times (RT), corresponding to the delay between the stimuli onset and the first pressed button. Therefore, a hyperset always generated seven RTs. At any time within a hyperset, if RT exceeded 3-s, or if an error occurred in the order of responses, the following message appeared: *"You have made an error: The whole sequence will now be restarted from the beginning"*. RTs associated with errors were not kept for analyses, and a sequence had to be completed without error for 20 consecutive trials to be considered well executed. This constituted a complete block of trials. Blocks were therefore of varied duration, depending on the participant's number of errors.

During a testing session, the first three consecutive blocks of trials consisted of the same repeated sequence, used to assess motor learning. After each block of 20 trials, there was a 5-min rest period. This learning stage was followed by a fourth and a fifth blocks of 20 trials each. In these two last blocks, the same hyperset (series of 7 sets) was used, but two new sequences were created by shuffling the 7 set positions within the series. Shuffling was done according to the participant's own performances and was based on two possibilities; "preserved chunks" or "broken chunks" (see Fig. 1c). In the former, the hyperset learned at

the end of the first three blocks of trials was split at two locations between the pairs with the longest RT (thus creating three chunks). We then shuffled these three chunks by switching the first with the third one. A new sequence was thus created, which kept intact the chunks naturally made by the participant. In the broken chunks condition, the hyperset was also split, but at the point of the shortest RT, disrupting therefore the chunks naturally made by the participant. We then shuffled the first and the third movement units to create a new sequence made of broken chunks.

In order to control for a practice effect, there were two parallel versions of the hypersets to learn (see Fig. 1b). The two forms were counterbalanced between the 2 days of testing in that half of the sample received one version on day one, and the other version on day two, while the reverse was done for the other half of the sample. This was done for both control subjects and PD patients. As another control, the "broken chunk" and "preserved chunk" conditions were also counterbalanced between the fourth and fifth blocks of trials in both PD patients and control subjects. Finally, before beginning a testing session, all participants familiarize themselves with the task by performing a short hyperset of six stimuli (only three sets), which had to be completed without error for ten consecutive times.

Outcome measures

In each subject, general performance was estimated from the sequence execution time (ET), corresponding to the time elapsed between the onset of the first pair of stimuli (1st set) and the last button press for a complete hyperset (14th button). ET was averaged over the 20 successful trials of each block. Sequence learning was assessed by comparing the average ET for block 1 and block 3. The larger this ET difference between blocks 1 and 3, the better was the learning.

A chunking score was also created for each block of trials, in every subject, from the Eta Squared (η^2). The latter was computed from the RTs of each set (pair of stimuli), taken separately (RT to set #1, RT to set #2, ..., RT to set #7) and averaged over the successive trials of a given block (thus creating seven mean RTs per block). η^2 varies between 0 and 1 and corresponds to the ratio of the sum of square between, over the sum of square total (SSb/SS_t), taken from the ANOVA table of these seven mean RTs obtained in a given individual. The chunking score for a given block of a given individual can therefore be operationally defined as the portion of the RT total variability attributable to the inter-set RT variability occurring as a consequence of the movements grouping. The larger this score, the better was the ability to group movements into chunks.

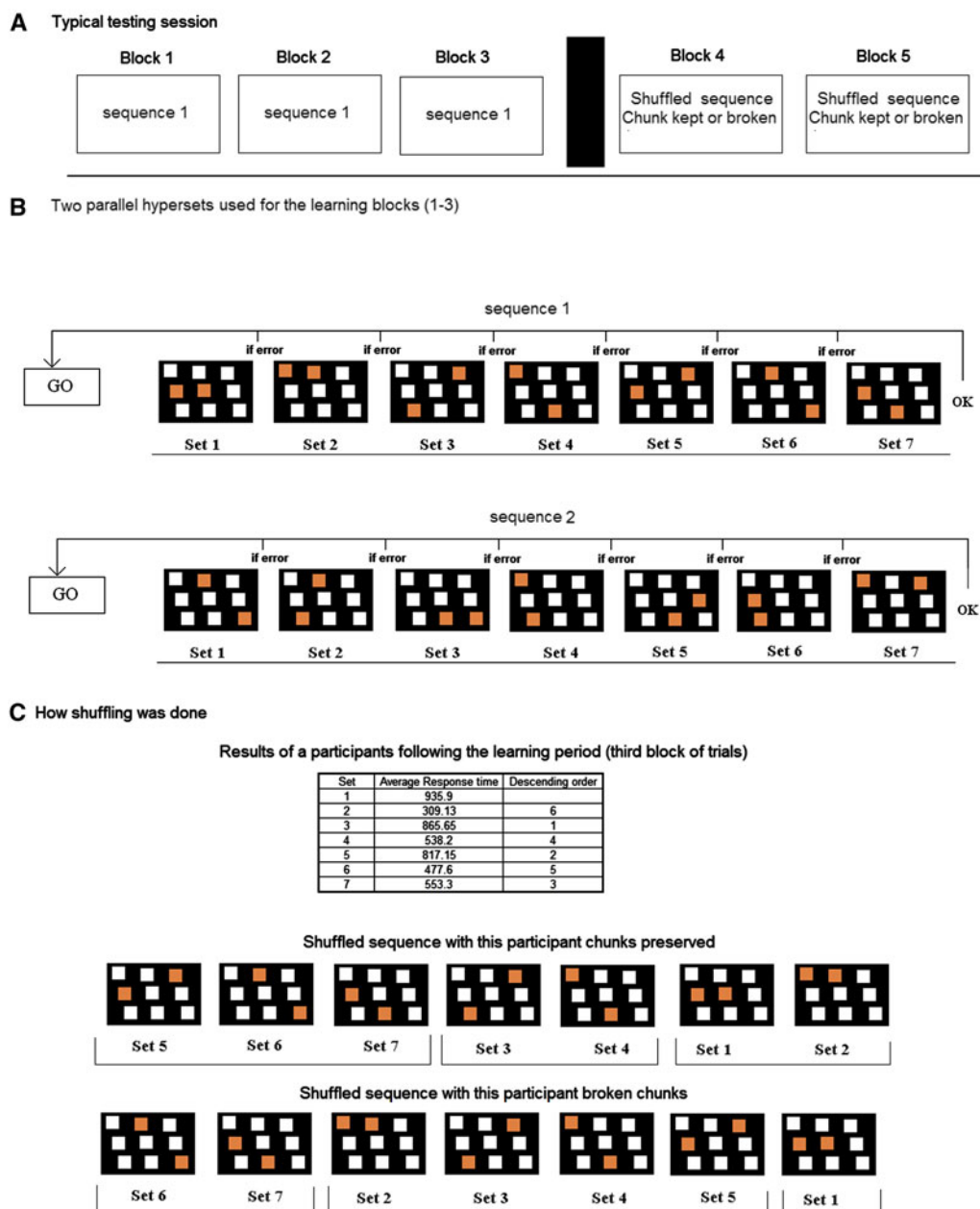


Fig. 1 Schematic representation of the task. **a** Illustration of a testing session by using one of the two hyperset versions. Motor sequence learning occurred during blocks 1–3. Each block requires to succeed in 20 consecutive trials. The sequence remained the same throughout the three blocks of trials. Movement chunking takes place during this learning stage. In the fourth and fifth blocks of trials, the hyperset remained the same, but the sequence (rank of sets) was changed according to the subjects' own chunking, in that one of these blocks includes a sequence with "preserved chunks", while the other block includes a sequence with "broken chunks". Such a testing session was repeated on the following day, using the other parallel hyperset version. **b** Illustration of the two parallel hypersets used in the current study. Participants were facing a computer screen presenting nine stimuli in a 9×9 matrix and a keyboard pad that includes nine buttons placed in the same spatial position. The task requires the participant to press the keyboard button corresponding to the same location as the stimulus presented on the screen. Two out of nine stimulus were illuminated at

a time. Participants had to learn, by trial and errors, the correct order of the two buttons to press. After a correct response, another pair was immediately presented. If an error occurred in any pair of buttons, a warning message appeared and the participant had to start the sequence from the beginning. A pair of stimulus corresponds to a set. A series of seven consecutive sets (hyperset) were presented within a complete sequence. **c** At the end of the third block of trials, the mean RT was computed for each set taken separately. The sequence was then split at two different points, corresponding to the two longest RTs. This creates three segments of sets corresponding to the chunks naturally produced by the participant. We then shuffled these three chunks by inverting the first and the third segments to obtain a new sequence made of "preserved chunks". The same procedure was done to get the "broken chunks" condition, with the exception that sequence was split at the two shortest RTs, disrupting therefore the chunks naturally made by the participant. Sequences made of preserved and broken chunks were executed during blocks 4 and 5

Statistical analyses

Control subjects and PD patients were compared first on the MMSE and GDS scores, using Student's *t* tests. Learning effect was estimated by comparing block 1 and block 3 in the two subsamples separately (controls and PD patients), using paired sampled *t* tests on each outcome measure (ET and chunking scores), for the 2 days of testing taken together. Smaller ET and higher chunking scores in block 3 were both compatible with a sequence learning effect.

Equivalence of the two hyperset parallel versions was verified with 2×2 ANOVAs for repeated measures (Blocks 1 and 3) \times (Hypersets 1 and 2), performed separately on the ET and chunking scores of the total sample (Controls and PD patients taken together). Test–retest effect was verified over the 2 days of testing, in control subjects and in PD patients separately, using 2×2 ANOVAs for repeated measures (Blocks 1 and 3) \times (1st day and 2nd day), performed on the ET and chunking scores.

Effect of treatment on the sequence learning was analysed with 2×2 ANOVAs for repeated measures (Blocks 1 and 3, treatments ON and OFF), performed separately on the two outcome variables. Control subjects were also compared with both ON- and OFF-PD patients using two separate 2×2 ANOVAs for repeated measures and independent samples (Blocks 1 and 3, Controls and ON-PD), (Blocks 1 and 3, Controls and OFF-PD). Significant interactions were further assessed with paired sampled *t* tests comparing blocks 1 and 3, within each subgroup separately (Controls, On-PD, and OFF-PD).

The ability in using chunked movements into a new sequence was assessed from the fourth and fifth blocks of trials, corresponding to the shuffled hypersets in which the subject's chunks have been either preserved or broken. The treatment effect was assessed with three separate 2×2 ANOVAs for repeated measures between ON- and OFF-PD patients, as well as between control subjects and either ON- and OFF-PD patients. Interactions were further analysed using paired sampled *t* tests comparing the two shuffling conditions, within each subgroup separately (Controls, ON-PD, and OFF-PD).

Results

Parkinson's disease patients and control subjects were not differed on age ($t = .52$, $P > .05$), nor on the MMSE ($t = .13$, $P > .05$), or GDS ($t = -.08$, $P > .05$). Scores in these two latter scales were in the normal clinical range for the two subgroups (see Table 1). The men/women ratio was 10/1 in PD and 10/2 in the control group.

Table 1 Sociodemographic and clinical features of the sample

	Age	MMSE	GDS
PD	65.9 (5.3)	29.4 (.8)	3.4 (3.1)
Controls	68.4 (6.1)	29.4 (.7)	3.3 (3.2)

Task learning and test–retest consistency

In the control subjects, the 2×2 ANOVAs for repeated measures (Blocks 1 and 3, Day 1 and 2) showed a block effect that confirms a sequence learning for both the ET [$F(1, 11) = 110.99$, $P < .05$] and the chunking scores [$F(1, 11) = 16.59$, $P < .05$]. There was, however, no day effect [ET score: $F(1, 11) = .908$, $P = .36$; chunking score: $F(1, 11) = 1.215$, $P = .29$], and no day by block interaction [ET score: $F(1, 11) = .27$, $P = .61$; chunking score: $F(1, 11) = 2.14$, $P = .17$]. The same tendencies were observed in PD patients, with a block effect statistically significant on ET [$F(1, 10) = 23.3$, $P < .05$] and near significant on the chunking scores [$F(1, 10) = 4.343$, $P = .06$]. There was no day effect either in these PD patients [ET score: $F(1, 10) = .001$, $P = .98$; chunking score: $F(1, 10) = .302$, $P = .59$], nor any day by block interaction [ET score: $F(1, 10) = .15$, $P = .708$; chunking score: $F(1, 10) = .116$, $P = .74$].

Parallel forms equivalence

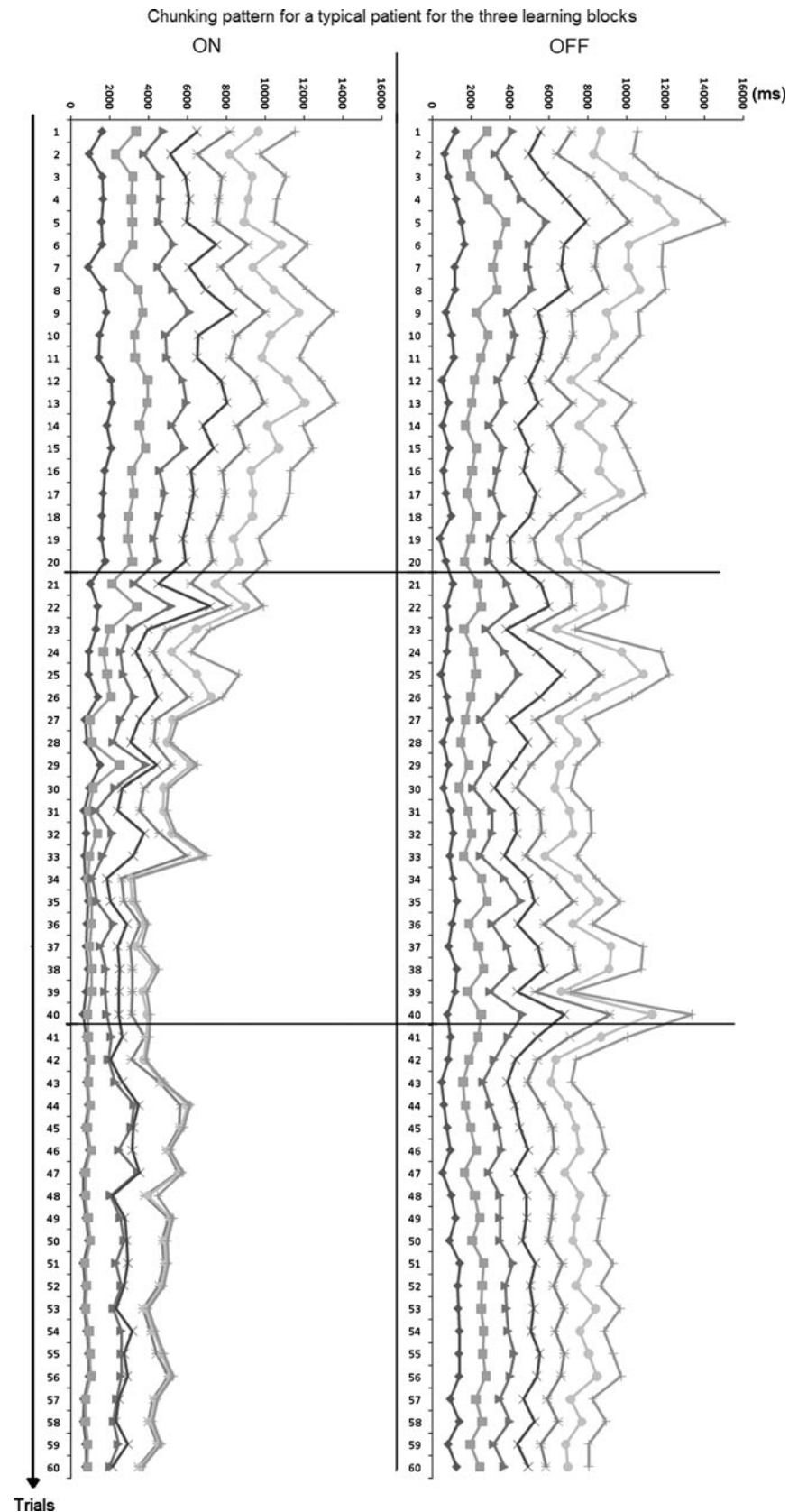
There was no significant difference between the two parallel versions of the hypersets used. More specifically, the ANOVAs revealed a significant sequence learning effect between blocks 1 and 3 [$F(1, 22) = \text{ET score: } 86.32$, $P < .05$; chunking score: 17.045 , $P < .05$], but no effect of the hyperset versions [$F(1, 22) = \text{ET score: } .721$, $P = .41$; chunking score: 1.826 , $P = .19$], nor any interaction [$F(1, 22) = \text{ET score: } .169$, $P = .69$; chunking score: $.416$, $P = .53$].

Treatment effect

Figure 2 shows the responses pattern of a typical PD patient in the ON- and OFF-levodopa conditions along the three blocks of trials. Chunking seems to take place in the ON- but not the OFF-levodopa condition.

Figure 3 shows the averaged values of ET and chunking scores for the first and third blocks of trials, in ON- and OFF-PD patients as well as in control subjects. The ON–OFF comparisons on the ET variable using the general 2×2 ANOVA for repeated measures (Blocks 1 and 3, PD–ON and PD–OFF) showed a block effect [$F(1, 10) = 23.30$, $P < .05$], and no drug effect [$F(1, 21) = 1.02$, $P = .34$], with a significant interaction [$F(1, 10) = 6.15$, $P < .05$].

Fig. 2 Progressive chunking in a typical PD patient in the ON- and OFF-levodopa conditions. *Vertical line* delineates the three blocks of 20 trials. Values correspond to cumulative RTs (ms) for each of the seven sets included in the hyperset. At the end of the third block of trials, three chunks can be clearly seen in the ON- but not in the OFF-levodopa condition



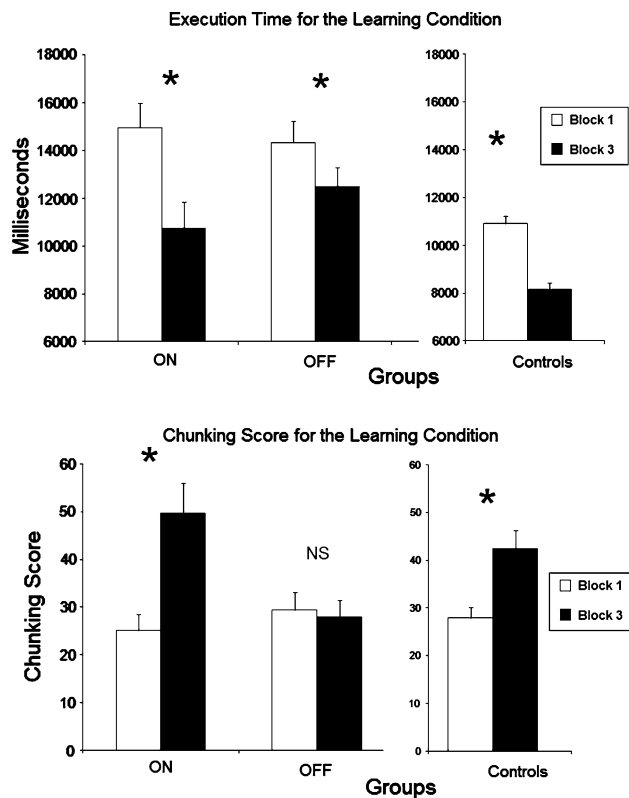


Fig. 3 Comparison of the mean execution time (ET) and the chunking score between the first and third blocks of trials, for the PD patients and control subjects. Vertical bars correspond to standard error

The same analysis performed on the chunking score revealed a near significant block effect [$F(1, 10) = 5.58$, $P = .06$], a significant drug effect [$F(1, 21) = 23.61$, $P < .05$], and a significant block by drug interaction [$F(1, 10) = 5.58$, $P < .05$]. These interactions on the two variables were further assessed by the paired sample t tests performed separately in each subgroup. In ON-PD patients, a significant learning effect was found for both the ET [$t(1, 10) = 4.499$, $P < .05$] and chunking scores [$t(1, 10) = -2.629$, $P < .05$], while in the OFF-PD patients, significance was found only for the ET score [$t(1, 10) = 4.737$, $P < .05$] but not for the chunking score [$t(1, 10) = .951$, $P = .36$].

The ANOVAs comparing ON-PD patients with control subjects showed similar patterns of sequence learning for the two outcome measures in both groups, although performances remained better in controls than in ON-PD patients. This was evidenced by a block effect [$F(1, 21) = \text{ET score: } 45.54$, $P < .05$; chunking score: 16.36 , $P < .05$], a group effect [$F(1, 21) = \text{ET score: } 12.94$, $P < .05$, chunking score $.39$, $P = .53$] and no interaction [$F(1, 21) = \text{ET score: } 1.99$, $P = 17.31$, chunking score: 1.06 , $P = .31$].

Comparisons between OFF-PD patients and control subjects revealed effects similar to those described above with the ON–OFF comparisons, although the strength of these new effects was weaker because of the variance induces by

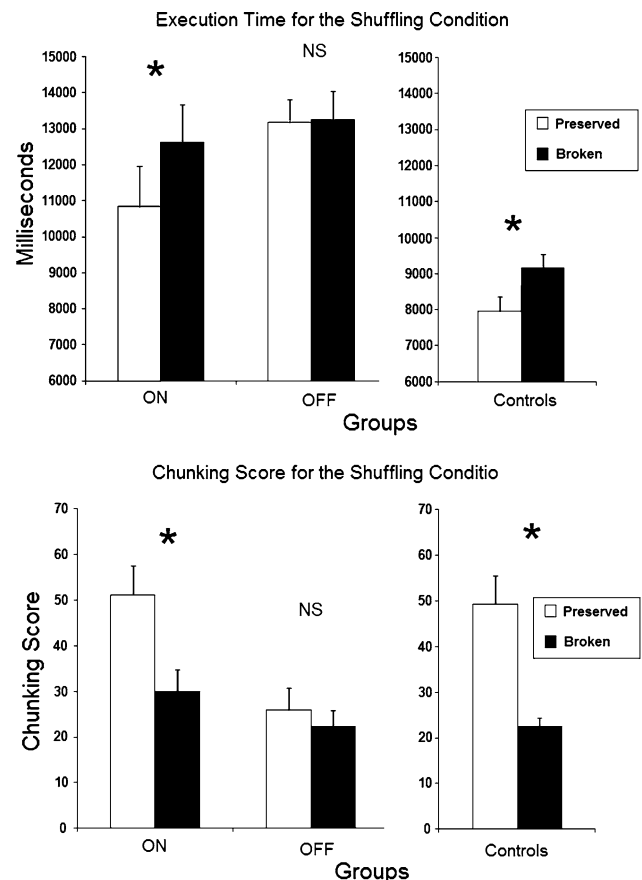


Fig. 4 Comparison of the mean execution time (ET) and the chunking score between the preserved and broken chunks conditions (fourth and fifth blocks of trials), for the PD patients and control subjects. Vertical bars correspond to standard error

the two independent samples. The block effect was significant for the ET score [$F(1, 21) = 98.73$, $P < .05$], but not the chunking score [$F(1, 21) = 3.77$, $P = .07$]. A group effect was also significant for the ET score [$F(1, 21) = 21.06$, $P < .05$], but not the chunking score [$F(1, 21) = 3.74$, $P = .07$]. As for the interaction, the effect was near significant for the ET score [$F(1, 21) = 3.70$, $P = .06$] and clearly significant for the chunking score [$F(1, 21) = 5.63$, $P < .05$]. Detailed analyses of the interactions were presented above for ON- and OFF-PD patients. In the control subjects, just like the ON- but not the OFF-PD patients, significant differences were found between blocks 1 and 3 on both the ET score [$T(1, 11) = 10.535$, $P < .05$] and the chunking score [$T(1, 11) = 4.074$, $P < .05$].

Treatment effect on shuffling

Figure 4 shows the averaged ET and chunking scores for the broken and preserved chunks conditions in the three separate subgroups. The ON–OFF comparisons with the 2×2 ANOVA for repeated measures (broken chunks and

preserved chunks, ON-PD and OFF-PD) carried out on the ET score showed no drug effect [$F(1, 10) = 3.42, P = .09$], no shuffle effect [$F(1, 10) = 2.96, P = .12$], but a significant drug by shuffle interaction [$F(1, 10) = 5.87, P < .05$]. The same analysis carried out on the chunking score showed a drug effect [$F(1, 10) = 7.27, P < .05$], a shuffle effect [$F(1, 10) = 16.75.09, P < .05$], and a significant interaction [$F(1, 10) = 4.54, P = .05$]. Further analyses with paired sample t tests in ON-PD patients revealed better performances in the preserved than in the broken chunk condition for the two outcome measures [ET score: $t(1, 10) = 2.48, P < .05$; chunking score: $t(1, 10) = 3.45, P < .05$]. However, in OFF-PD patients there was no difference between the broken and preserved conditions for any of the two outcome measures [ET score: $t(1, 10) = .137, P = .89$; chunking score: $t(1, 10) = .95, P = .36$].

The ANOVA comparing ON-PD patients with control subjects revealed a group effect on ET score [$F(1, 21) = 8.95, P < .05$], but not on the chunking score [$F(1, 21) = .65, P = .43$], a shuffle effect on both the ET score [$F(1, 21) = 14.58, P < .05$] and chunking score [$F(1, 21) = 30.09, P < .05$], and no interaction on either the ET score [$F(1, 21) = .52, P = .48$] or the chunking score [$F(1, 21) = .42, P = .53$]. Paired t tests performed in control subjects revealed results similar to the ON-PD patients; that is, better performances in the preserved than in broken chunks conditions, for both the ET score [$t(1, 11) = 3.331, P < .05$] and chunking score [$t(1, 11) = 4.322, P < .05$].

Comparison between OFF-PD patients and control subjects revealed the tendencies already described above between OFF- and ON-PD patients. The ET and chunking scores were better in controls than in OFF-PD patients as indicated by the group effects [$F(1, 21) = \text{ET score: } 40.20, P < .05$; chunking score: $5.15, P < .05$]. The shuffle effect was also statistically significant for the chunking score [$F(1, 21) = 16.71, P < .05$] and near significant for the ET score [$F(1, 21) = 3.84, P = .06$]. As for the interaction, it was significant for the chunking score [$F(1, 21) = 9.69, P < .05$], but not for the ET score [$F(1, 21) = 2.98, P = .18$].

Discussion

This study is the first to directly investigate the effect of dopamine depletion in the chunking process occurring during motor sequence learning in human. The results indicate that, in spite of better general performances in control subjects than in PD patients, motor sequence learning occurs in all groups, as revealed by the ET improvement from block 1 to 3. However, while control subjects and ON-PD patients also improved on the chunking score, OFF-PD patients do not. Therefore, in the latter group, there seems to have a difficulty in grouping isolated movements into

integrated motor sequences. In other words, motor sequence learning would involve a movement chunking process when DA levels are preserved or pharmacologically restored in the CNS, but not in case of DA depletion such as in untreated PD patients.

Part of our results also replicate those obtained by Sakai et al. (2003) on the capacity to use chunks previously learned in order to integrate them into new motor sequences. Using old movement chunks into new motor sequences improved significantly the performances as measured with ET. This phenomenon was observed in both control subjects and ON-PD patients. However, this was not possible in OFF-PD patients given their primary deficits in producing chunks. In these OFF-PD patients, whether the new motor sequences included chunks that were identified as broken or preserved, ET performances remained poor (see Figs. 4, 5). Such a result could be related to the movement chunks measurement in the present study (Two shortest RT lengths between the seven sets included in a hyperset). This method, taken from Sakai et al. (2003), has the advantage of extracting the three most prominent chunks of a motor sequence, but in OFF-PD patients, it may create artificial chunks, because RT does not vary much throughout an entire hyperset. In these circumstances, new motor sequences would be performed the same, whether any artificial chunks are defined or not.

The main finding of the current study is therefore the clear demonstration that dopamine is essential for movement chunking during motor sequence learning. The strict patient selection used here allows us to be confident in this

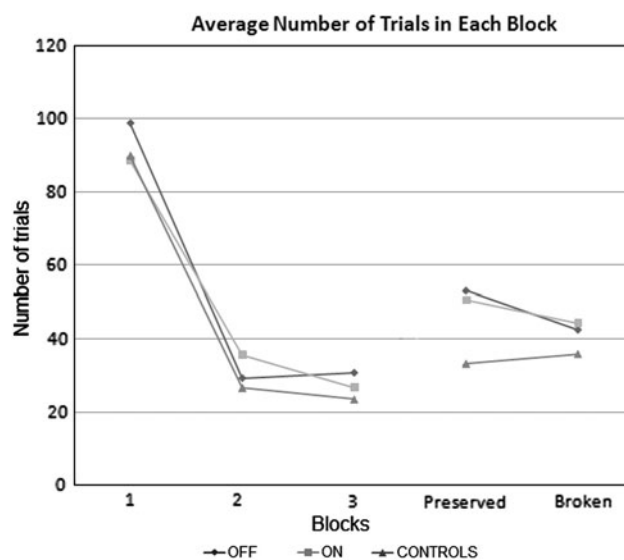


Fig. 5 Mean number of trials within each block. Values were similar between the three subgroups. No group receives therefore more rehearsal than any other. A block was completed after 20 consecutive successful trials

conclusion. The diagnosis was defined with standardized criteria, and patients were not treated with any medication other than levodopa, thus reducing any unspecific explanation for these results. Actually, it is thought that parkinsonian symptoms occurring after a 12-h levodopa washout mainly result from a striatal DA deficit (Contin et al. 2001). Therefore, although we cannot completely rule out the role of other neurochemical or anatomical systems known to be affected in PD, we strongly believe that the nigrostriatal DA system plays a primary role in the current results.

Brain imaging studies conducted by Badgaiyan et al. (2007, 2008) have directly shown a DA release in the dorsoposterior part of the putamen and caudate during a sequential motor learning task in human. In rat, 6-OHDA lesion of the striatum was recently found to produce a deficit of sequential behaviour, especially during acquisition (Eckart et al. 2010). Our own results obtained in primates have also demonstrated that DA antagonists and agonists can respectively induce or prevent movement chunking deficits during sequential learning (Levesque et al. 2007; Tremblay et al. 2009). The concept has also been successfully simulated in a computational model (Suri and Schultz 1998), showing that DA-neuronal signal can allow the striatum to efficiently learn longer motor sequences by facilitating the grouping of single elements.

Although movement chunking during sequential learning can be affected by DA depletion, it should be stressed that OFF-PD patients in the current study were nevertheless able to learn the motor sequences. ET score improved throughout the trials in these patients, even though the chunking score remains unchanged. In control subjects and ON-PD patients, these two scores improved in parallel. This may indicate that OFF-PD patients use a different strategy of motor sequence learning, not related to a chunking process. Such a view would explain why PD patients do not have the same brain network activated during sequence learning, when compared with normal subjects (Nakamura et al. 2001). In the latter, activations were observed in the dorsolateral prefrontal cortex (PFDl), rostral supplementary motor area (preSMA), anterior cingulate cortex, and the striatum. However, in PD patients (mostly untreated), motor sequence learning was associated with PFDl activation, together with the ventral prefrontal and rostral premotor regions. Hence in PD, successful motor sequence learning may be accomplished by deploying a brain network involving abnormal cortical–cortical interactions instead of a cortical–subcortical one. This might constitute the anatomical substrate for the motor learning process that does not involve movement chunking.

Other imaging studies in PD have demonstrated that, compared to healthy controls, PD patients show increased activity in many cortical areas while performing automatic movements (Wu and Hallett 2005; Wu et al. 2010),

whereas no activation in the striatum was found. This may indicate that the usual shift from cortical to subcortical areas when controlled processing switch towards automatic processing does not, or not sufficiently, take place in PD patients. It appears, in these patients, that skill learning remains on a consciously controlled level, while the more implicit procedural learning that should follow in the course of automatization does not sufficiently occur (Koerts et al. 2009). DA depletion would thus reduce the ability to automatize processes including movement chunking. Such a phenomenon has also recently been described in normal elderly (Verwey 2010), who showed, in contrast to young adults, no evidence of motor chunks and an excessive dependency on voluntarily controlled cognitive processes. One may suggest that the age-related DA cell reduction in the striatum (Stark and Pakkenberg 2004; Erixon-Lindroth et al. 2005) might be involved in such a loss of automation.

In the last decade, a growing body of evidences have pointed towards a central involvement of DA in automatic motor habits or skills (Graybiel 2008). DA-depleted animals such as 6-OHDA-treated rats (Domenger and Schwarting 2008) or MPTP-treated monkeys (Matsumoto et al. 1999) are able to learn a motor sequence but never reach a performance that can be qualified as automatic. Seidler et al. (2007) also found this disabled ability to reach automatic performance using SRT in PD patients that withheld their morning dopaminergic medication. Patients without treatment showed good learning capacities of the implicit sequential order (less error through learning) but had difficulties in translating this to rapid automatic motor performances.

At a neuronal level, striatal DA was found to be required for many firing patterns associated with learning (Aosaki et al. 1994; Matsumoto et al. 1999). For instance, DA nigrostriatal cell firing is thought to allow cortico-striatal plasticity, through long-term potentiation (LTP) and long-term depression (LTD) (Charpier and Deniau 1997; Centonze et al. 2001; Reynolds et al. 2001; Pisani et al. 2005). Moreover, DA affects the synchrony and oscillatory cell behaviours, thought to be associated with the cortico-striatal network selection and reinforcing during learning (Costa 2007). This could play a primary role during movement chunking, through a gradual shortening pool of movement possibilities, and the reinforcement of the network underlying the specific movement sequence. As the chunks appear, the underlying networks consolidate and no longer require DA. Consistent with this hypothesis, changes in DA signalling brought about by sensitization with dopamine agonists mimic the transition from goal-directed to habit-based instrumental performance (Tremblay et al. 2009; Wickens et al. 2007). However, such a perspective arose mainly from animal studies. The current study is the first one showing human results compatible with this view.

One might argue that the poor chunking improvement observed in the OFF-PD patients is a consequence of bradykinesia, a well-known dopa sensitive symptom in PD. Even though we cannot rule out this possibility, it is clear that our results do not simply reflect bradykinesia. The chunking scores were similar between ON- and OFF-PD patients at the beginning (block 1). This suggests that dopamine or bradykinesia has no direct impact on the chunking score per se. However, since the latter improved between the first and third blocks in the ON- but not in the OFF-PD patients, it is possible that whether dopamine depletion or its related symptom can explain the results. Therefore, although bradykinesia does not seem to affect the chunking score per se, it might indirectly influence its change during motor sequence learning.

Given that participants had to look at the computer screen and then translate the stimulus position onto the keyboard, a concern can be raised about the sensorimotor adaptation required in our version of the task. PD patients have been found to be impaired in sensorimotor adaptation tasks (Contreras-Vidal and Buch 2003; Messier et al. 2007), and this impairment has been found to be DA sensitive (Paquet et al. 2008). This could have increased the level of difficulty in the first learning blocks and consequently delayed the chunking process in OFF but not in ON-PD patients.

Another limitation of the current study would be the small sample size. Inclusion criteria such as L-dopa monotherapy greatly reduced the number of eligible patients. This could account for the statically near significant results obtained in some comparisons. However, the strongest tendencies have been well detected and described and allow the conclusion of a primary role of DA in movement chunking during motor sequence learning in human.

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