The role of the contingent negative variation in chunking. Evidence from a go/nogo discrete sequence production task.

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#### Abstract

In the present EEG study we examined the effect of differing lengths and complexities of learned motor chunks on the contingent negative variation (CNV). Participants learned two different three-key sequences (A and B/1x3 sequence) in a go/nogo discrete sequence production (DSP) task in the practice phase. In the test phase, the two sequences had to be executed in combinations of two (with four possible combinations, either two times the same sequence, AA or BB/2x3 sequences, or different sequences in succession, AB or BA/1x6 sequences). Comparisons were made between execution of the short (1x3) versus the long (2x3 and 1x6)sequences and between execution of the 2x3 (less complex) versus the 1x6 (more complex) sequences. The effects on the CNV were assessed with regard to amplitude at three different electrode locations (Fz, Cz and Pz). With the exception of interaction effects indicating that only at Pz negativity was more pronounced for long than for sort sequences, differences in CNV response of none of the other comparisons were significant. We concluded that the results were probably due to either the present application of particular assumptions about the CNV, to the bad spatial resolution of EEG measurement, to differential task demands in the practice and the test phase, or to concurrent processing during execution. Clearly, more research is needed in order to disentangle the complex relationships between chunking, motor preparation, and the CNV.

### Introduction

Many of our everyday behaviors consist of series of small single actions. With some practice, we learn to execute them in rapid succession, which enables us to meet the demands of our everyday lives. Even seemingly trivial and simple acts, which belong to the basics of human behavior, such as speaking, lacing shoes or riding a bike consist of an extraordinary number of small motor acts, which have to be executed in the right serial order and sufficiently rapid succession in order to succeed (Cohen, Ivry & Keele, 1990). This capability to learn and produce sequential actions is hypothesized to be a hallmark of human cognition and a key element of voluntary behavior (Willingham & Bullemer, 1989). It allows among others language and logical thinking, thereby constituting a prime indicator of human intelligence, which is evidently extraordinary compared to that of other species (Corballis, 1991; Lashley, 1951).

It has been suggested that our skilled performance with this motor behavior stems from the ability to execute a series of movements as if they were only one movement (Miller, 1956). This is possible because, with extensive practice (Inhoff, 1991), we form some kind of integrated and unified memory representation of the sequence or part of the sequence, called "motor chunk" (Verwey, 1994). These units of comprised knowledge are highly efficient because they reduce the demands on memory storage and retrieval capacity (Jones, 1981; Gallistel, 1980). It is common knowledge that, with extensive practice, the execution of motor sequences becomes faster. That this can be attributed to the formation of chunks, is shown by the fact that longer motor sequences are performed with specific patterns of timing, thought to correspond to the organizational representation of the sequence. Longer time intervals between single movements indicate chunk boundaries, separating the whole sequence into groups of subsequences, or chunks (Rosenbaum, 1983). With still more practice, these motor chunks can, in turn, be integrated and used to build higher order chunks, comprising the smaller chunks. Thereby, a hierarchical organization of chunks develops (Ericsson, Chase, & Faloon, 1980; Verwey, Lammens, & van Honk, 2001).

When learning the execution of serial movements from externally presented visuomotor sequences, the placement of chunk boundaries can be externally specified (Sakai, Kitaguchi, & Hikosaka, 2003). Through repetition, transposition, insertion of elements, or pauses during presentation (Koch & Hoffmann, 2000), the sequences might inherit a unique rhythmic pattern of elements (Cohen, Ivry, & Keele, 1990; Stadler, 1993). In these cases, the given structure of the

sequence determines the chunking behavior, which is similar for everyone. When no such external structure is imposed on sequences, chunking occurs spontaneously and differs from person to person for identical sequences (Fendrich & Arengo, 2004), with the most common and seemingly most efficient chunk-size of 3 to 4 items (Klemmer, 1969; Fendrich & Arengo, 2004).

A number of theories have been proposed trying to explain the exact mechanisms underlying motor learning of sequential action. One of them, proposed by Verwey (2001), states that discrete motor learning depends on two different processing systems, a cognitive system and a motor system. This distinction is based on the influential additive factors method, first proposed by Sternberg in 1969 and applied and supported by, amongst others, Sanders (1980; 1990). This approach assumes that cognition takes place in a series of distinct and separable processing stages, two of which are denoted as "response selection" and "motor processing". Those two may, according to Verwey (2001), be carried out by the two independent processing systems, the cognitive and the motor system, respectively. The cognitive processor is responsible for initially planning and selecting a symbolic representation of an action sequence. The motor processor then loads this representation into some kind of motor buffer and executes it subsequently (Verwey, 2001; MacKay, 1982). According to this model, the formation of motor chunks reduces the load on planning and organization and, accordingly, the demands on the cognitive system, because motor chunks can be selected at once with the use of stimulusresponse rules in working memory. The motor system is unaffected by the formation of motor chunks, because the loading of the motor buffer and the subsequent execution are thought to be independent of learning (Verwey, 2001). That this dual processor model holds in a variety of circumstances, has been shown in various studies on motor and sequence learning (De Kleine & Van der Lubbe, 2009).

Moreover, it is consistent with the influential notion of hierarchical movement control (Lashley, 1951). The notion of hierarchical organization of action execution has been supported many times in the last decades. For example, in simple RT tasks the initiation time of a sequence becomes longer when the sequence itself is longer or more complex (sequence length effect), which can be ascribed to the "unpacking" of hierarchical plans into their constituents (Sternberg et al. 1978; Collard & Povel, 1982) prior to execution. Plenty of research (Jax & Rosenbaum, 2007; Rosenbaum et al., 1993) indicates that action sequence hierarchies are planned in advance and with regard to goal postures, thereby requiring the preparation and planning of the whole

movement before execution. As already mentioned, hierarchical action planning can also be seen with chunking, as with practice chunks might become concatenated or even unified and integrated into one single superordinate chunk (Ericsson, Chase, & Faloon, 1980; Verwey, Lammens, & van Honk, 2001). The dual processor model of motor learning can account for the abovementioned findings on hierarchical action control, as the cognitive processor can accomplish the hierarchical organization and advance planning of the sequences, thereby being responsible for the concatenation of motor chunks, as well.

Further support for this distinction of the two systems operating as processors in cognition comes from a neurophysiological model of sequence production, which, too, proposes two separate systems (Verwey, Lammens, & van Honk, 2002). Some subcortical structures, namely the basal ganglia, are thought to be more involved in the work of the cognitive processor, implementing movement plans by initiating each of the to be executed elements via a relatively slow thalamo-cortical motor loop (Hayes, Davidson, Keele, & Rafal, 1998). The motor processor, on the other hand, is probably based on frontal cortical structures, namely the supplementary motor area (SMA), the primary motor cortex (M1) and the premotor cortex, which work together to execute each individual element (Verwey, Lammens, & van Honk, 2002). The neurophysiological distinction between the two processing systems, however, is not a clear one. Those cortical and subcortical structures are known to communicate with each other constantly and to work with repeating loops of activity, which makes it difficult to entirely separate their contributions.

Other neurophysiological models of motor learning adopt a different viewpoint on motor learning. Those models are more concerned with the hierarchical control of motor behavior and not with the distinction of two processing systems. One of them, proposed by Koechlin and Jubault (2006), states that areas extending from premotor to the most anterior prefrontal regions of the cerebral cortex govern the temporal organization of behavior (Koechlin & Jubault, 2006; Fuster, 2004). The more anterior regions are responsible for the hierarchically higher levels of action control, thus higher-order integration and concatenation of motor chunks into superordinate chunks. This view does not contradict a dual processor model, it simply tries to explain motor learning from a different point of view, focusing on neurophysiological evidence underlying the hierarchical organization of behavior, rather than focusing on methods within the framework of the additive factors tradition.

There are a number of psychophysiological techniques, which can be used to study the contributions of certain cognitive functions to movement control. One of them, the electroencephalogram (EEG), is especially useful for measuring brain activity following or preceding certain events, such as movement, by assessing event-related potentials (ERPs). One ERP, namely the contingent negative variation (CNV), is thought to reflect the preparation of signaled movement (Cui, Egher, Huter, Lang, Lindinger, & Deecke, 2000). The CNV, first described by Walter et al. (1964), is a slow negative voltage change peaking at mostly central brain locations. It can be seen in the interval between a warning stimulus and a signal requiring a motor response (Jentzsch & Leuthold, 2002; Verleger, Vollmer, Wauschkuhn,). Two subcomponents of the CNV have been distinguished: an earlier wave, representing stimulus orienting, and a later wave, associated with the expectation or preparation of the response (McCallum, 1988; Rohrbaugh & Gailliard, 1983; Bareš, Nestrašil, & Rector, 2007), which might correspond to activity of the cognitive processor. In an earlier study, conducted by De Kleine and Van der Lubbe (2009), the CNV was decreased for execution of familiar compared to that of unfamiliar sequences. In line with the dual processor model (Verwey, 2001), which states that demands on the cognitive processor reduce with the formation of motor chunks, these results can be taken as support for the hypothesis that the CNV might reflect activity of the cognitive processor, as for the familiar sequences motor chunks could have been formed. This reduced the load on the cognitive processor, which in turn was reflected in a decreased CNV response. Which brain regions are responsible for the generation of the CNV, is not clear yet. Some have found out a contribution of the basal ganglia (Ikeda et al., 1997; Bareš & Rector, 2001), which would, given the assumed association with the cognitive processor, be consistent with the abovementioned neurophysiological dual processor model of motor learning (Verwey, Lammens, & van Honk, 2002). Other potential underlying sources of the CNV might be frontal and motor areas (De Kleine & Van der Lubbe, 2009), such as the SMA and the primary motor cortices (Yazawa et al., 1997), or a summation of multiple cortical potentials, having different functions and different origins, most of them in the frontal and prefrontal areas (Hamano et al. 1997; Ikeda & Shibasaki, 1995; Drake, Weate, & Newell, 1997).

Taking all these lines of research together, one can now hypothesize an association between the neurophysiological and behavioral findings. The cognitive processor, as mentioned earlier, might be responsible for planning whole sequential movements in advance (Sternberg et al., 1978), as well as later for the concatenation of chunks. When the preparatory demands increase, as for example with the planning of longer or more complex action sequences, which eventually requires concatenation of chunks, the escalated activity of the cognitive processor might be reflected in an increased CNV response.

In order to test this hypothesis, we implemented an EEG study, in which participants had to learn and execute action sequences with differing degrees of length and complexity. Following the line of investigation started by De Kleine and Van der Lubbe (2009) mentioned earlier in this paper, we used a go/nogo discrete sequence production (DSP) task. In a typical DSP task, participants respond to a fixed series of, normally, three to seven key-specific visual stimuli, thereby learning a limited number of distinguishable discrete sequences (mostly two) (De Kleine & Verwey, 2009). In addition, in a go/nogo DSP task, the whole sequence is presented prior to execution. The participants only respond when a go-signal is presented, and not when a nogosignal is presented. This is especially suitable for assessing activity of the cognitive processor, as the go/nogo signal is thought to separate action preparation from action execution (Rosenbaum, 1980; De Kleine & Van der Lubbe, 2009). Thereby concurrent preparation of action during execution is supposedly kept at a minimum. We proposed a task in which two different sequences (A and B) of 3-key length each, yielding a 1x3 sequence and representing a simple motor chunk, had to be learned in a practice phase. Each 1x3 sequence constituted a motor chunk, as with practice, the representation of the keys became more and more integrated into a unified whole (Verwey, 1994). In a following test phase the two sequences (or chunks) had to be executed in combinations of two, thus yielding four different new higher-order, concatenated chunks (AA, BB, AB, and BA). These sequences were believed to be of two different levels of complexity. Sequences of the form AA and BB required the repetition of the same chunks, creating 2x3 sequences, whereas sequences of the form AB and BA required the concatenation of two different chunks, producing 1x6 sequences, which might have put more load on the cognitive processor, as it is probably responsible for preparing the whole sequence in advance, thereby establishing a hierarchical action plan. This might be reflected in a greater CNV amplitude in 1x6 trials compared to 2x3 trials. Moreover, the CNV might also have a greater amplitude in the combined-sequence trials (1x6 and 2x3) than in the single-sequence trials (1x3), as the combination and concatenation of chunks requires more advance preparation. Also, longer sequences have been shown to result in a greater CNV amplitude (Schroeter & Leuthold, 2009).

In order to keep the taxing EEG recording time for the participants at a minimum, the task requirements in our study differed for the practice and the test phase. This could have influenced the results and the following discussion in an important way. It will be explained in detail later in this paper, but should be mentioned here in order to put the following parts in perspective.

## Method

*Participants.* Sixteen students from the University of Twente and Saxion University in Enschede participated in this study. They were either given course credits for participation or took part voluntarily. Seven of them were male and 9 female and their age ranged from 20 to 27, with a mean age of 22.8. Fifteen of the participants were right-handed and one was ambidextrous, as indicated by Annett's handedness inventory (1970). All of them had normal or corrected to normal vision and none had neural or motoric disorders. Futhermore, 7 of them played an instrument (piano). All participants signed informed consents and agreed with the conditions of the study.

Stimuli and task. Four horizontally aligned, whitely outlined squares with a length of 2 cm on each side were presented in the middle of a black background on a computer screen. Participants had their little, ring, middle and index fingers of their left hands on the C, V, B, and N keys of the keyboard, respectively, each of them corresponding to one of the squares on the screen in the same spatial arrangement. Each trial started with the presentation of a sequencespecific fixation letter above and in the middle of the four squares. This letter could be either an A or a B in the practice phase or a combination of the two in the test phase (thus AA, BB, AB or BA), indicating the name and identity of the following sequence. In the practice phase, after 1000 ms one of the squares filled white (stimulus presentation) for 400 ms, followed by a second and a third one. This presentation of three constituted a sequence. After the presentation of a sequence, a pause of 1200 ms followed. This presentation of the stimuli was left out in the test phase, where only the sequence-specific letter combination was presented for 1850 ms. Then in both, the practice and the test phase, the sequence-specific letter(combination) changed its color into either red or green. In the case of a red letter (nogo signal), participants were not supposed to react, whereas in the case of a green letter (go signal) they were supposed to react as quickly and correctly as possible and subsequently type with their fingers the keys, which corresponded to

the stimuli they just saw, in the corresponding order. Feedback about the number of wrong key presses was given after typing the whole sequence and feedback about incorrectly reacting after a nogo signal or about reacting too early, before presentation of any signal, was given immediately via an error message on the screen.

Each participant completed six blocks, each of which contained 168 trials (sequences), i.e. 160 go trials and 8 nogo trials. The first four blocks served as practice blocks, with two different three-key sequences, A and B. Four different versions of the task (named 1 -4) were designed, so that four different pairs of A and B were executed by participants. All sequences were built up of the key sequences vnc, bcn, cbv, nvb. This made sure that, across participants, each key had to be pressed an equal number of times, thereby eliminating finger-specific effects. There were no key repetitions within a single sequence. The last two blocks constituted the test phase, in which the participants had to type in combinations of the two sequences, i.e. AA, BB, AB, and BA. The order of presentation of each sequence was randomized in each block, but each sequence (or sequence combination) was presented an equal number of times, resulting in 84 repetitions of each sequence combination in each of the last two blocks, thus 84 repetitions for the test phase. The computer registered response times and number of errors made, whereby responding after a nogo signal counted three errors in the practice phase and six errors in the test phase.

*Procedure.* After being welcomed by the experimenter, participants signed the informed consent forms and were instructed to sit in front of the computer. Then the EEG and EOG electrodes were installed and the participants received instructions (in written form and orally) for the task and were asked to react as correctly and as quickly as possible after presentation of the go signal. Halfway through each block a short break of 20 seconds was provided, in which participants could relax. During this break and at the end of each block they received feedback about their mean reaction time and number of errors. Participants were allowed to take a 5-minute break after each block, where they could get a drink, offered by the experimenter. After the first two blocks, participants filled out Annett's Handedness Inventory and at the end of the experiment they filled out a questionnaire concerning their demographics, neural or motoric disorders, and the recognition and recall of the previously learned sequences. Each block lasted

approximately 11 minutes and the whole task took about 90 minutes. Taken together with the preparation time, the whole experiment lasted 180 minutes.

*Recording and Data Processings.* The presentation of the stimuli and the registration of the responses made by the participants during the task were controlled by E-Prime version 1.1 on a Pentium 4 PC running with Windows XP with a QWERTY keyboard. The participants were seated in front of a 17 inch computer screen with a viewing distance of approximately 60 cm in a room with normal daylight circumstances. EEG and EOG were amplified with Quick-Amp amplifier and recorded with Brain Vision Recorder (version 1.05) software. EEG was recorded from 64 Ag/AgCl ring electrodes arranged according to the extended 10/20 system and measures from the forehead served as reference. EOG was recorded vertically, above and below the left eye, and horizontally from the outer canthi of both eyes. Electrode impedance was kept below 20 k $\Omega$  and both EOG and EG data were sampled at a rate of 500 Hz. Filtering of the measured activity was accomplished by a digital online filter (low pass 140Hz, DC).

# Data Analysis.

*Response parameters.* Response time (RT) was defined as the time between onset of the go/nogo signal and the beginning of the first key press and as the onset time between two consecutive keypresses. The first two trials of every block and after each 20 s break within each block were excluded from analysis, as were trials with one or more errors. Trials in which the mean response time was more than three standard-deviations above the overall mean in each session were also removed from the according sessions. This eliminated 6.1% of the trials. The Greenhouse-Geisser epsilon correction for degrees of freedom was applied whenever the sphericity assumption of the F-test was violated. The percentage of errors was calculated for each block for each participant. The mean RTs and error percentages were evaluated statistically by analysis of variance (ANOVA) with repeated measures, with in the practice phase Block with 4 levels and Key with 3 levels, as within-subject factors and with in the test phase Block with 2 levels, Key with 6 levels, and Sequence with 2 levels (1x6 and 2x3) as within-subject factors. (For the analysis of the error percentages "key" was left out as a factor because of the small number of errors made at each single key.) Furthermore, practice phase and test phase were compared regarding mean RTs and percentage of errors.

*EEG parameters.* One participant was excluded from the EEG analysis because of huge fluctuations in response times during the experiment, which made analysis unfeasible. Only trials without errors were included. The interval between offset of the last stimulus and onset of the go/nogo signal was 1200 ms in the practice phase and 1850 ms in the test phase. The data was segmented starting 2000 or 1850 ms before the go/nogo signal and ending 400 or 500 ms after the go/nogo signal for the two phases, respectively. The segments for the practice phase started earlier before the go/nogo signal because due to the presentation of the key-specific stimuli, the trials were longer. They also ended earlier after the go/nogo signal because the typing of 3 keys was accomplished more quickly than the typing of 6 keys. Accordingly, baselines were set 2450-2350 ms before the go/nogo signal in the practice phase and 1200-1300 ms in the test phase, each reflecting the interval of 500-600 ms after beginning of the trial. Trials with errors and artifacts (eye movements and others) were removed and the data was corrected for further artifacts. Furthermore, it was corrected for ocular movement and filtered with a low cutoff of 0.53 Hz at 12 dB octave and a high cutoff of 8 Hz and 12 dB octave.

The CNV was computed by averaging EEGs for all the remaining trials from all electrodes. Averaged activity was determined at Fz, Cz, and Pz (Schröter & Leuthold, 2009) in 200 ms intervals from 1200 ms before to 0 ms before the go/nogo signal, on which statistical analyses were performed. The factors Time with 6 levels (6 intervals from 1200 before the go/nogo signal until the signal), Electrode with 3 levels (Fz, Cz, and Pz), and Sequence with 2 levels (Short and Long) were included in the ANOVA, allowing comparison of the practice phase (short sequences) and the test phase (long sequences). For the test phase (comparison of 2x3 and 1x6 sequences), Time with 6 levels, Electrode with 3 levels, and Sequence with 2 levels (2x3 and 1x6) were included in the ANOVA.

Results

*Practice phase.* There was a significant main effect of Block, F(3,45)=37.155, p<.01,  $\varepsilon$ =0.456. Post hoc Bonferroni corrected pairwise comparisons revealed that participants became faster with practice, reflected in significantly faster response times in later blocks than in earlier ones, p<.001. The main effect of Key was significant, as well F(2,30)=93.185, p<.01. The responses were faster for later Keys in the sequence, thus execution of Key 1 was significantly slower than both, Key 2 and Key 3, as shown by post hoc Bonferroni corrected pairwise comparisons, p's<.001. There was a significant interaction between Block and Key, F(6,90)=6.713, p<.01,  $\varepsilon$ =0.548. In Block 1 the slower execution of Key 1 (compared to both Key 2 and Key 3) seemed to be more pronounced than in Blocks 2 to 4. Other interaction effects were not evident.

For error percentages, only Block (4) was included in the analyses, because the error rates were too small (mean error rate <2.5%) to be analyzed separately for the different Keys. The effect of Block on percentage of errors, however, was not significant, F(3,45)=2.267, p>.1,  $\epsilon=0.698$ .

*Test phase*. All three main effects were significant and in the expected directions. As shown by the main effect

of Block, F(1,15)=8.473, p<.02, participants were faster in the second Block than in the first Block, reflecting practice effects. Moreover, as indicated by the main effect of Key, F(5,75)=60.963, p<.01, ε=0.41, significant differences RTs in between the Keys were found. Post hoc Bonferroni corrected pairwise comparisons



Figure 1. Mean RTs as a function of Key and Block in the test phase.

revealed that the first and the fourth Key were executed more slowly than the rest of the Keys, p's<.001, indicating initiation of the sequence and the chunk boundaries after the first motor chunk, as expected. The last main effect of Sequence revealed that the 2x3 Sequence was executed more quickly than the 1x6 Sequence, F(1,15)=17.734, p<.01. In addition, all three

interaction effects were significant. The interaction between Block and Key showed that the slowing of Key 1 and 4 was more pronounced in Block 1 than in Block 2, F(5,75)=8.721, p<.01,  $\epsilon$ =0.37 (Figure 1). The slower execution of the 1x6 Sequence was less visible in Block 2 than in Block 1, as shown by the interaction between Block and Sequence, F(1,15)=9.902, p<.01. Finally, the interaction between Key and Sequence revealed that the slow



Figure 2. Mean RTs as a function of Key and Sequence in the test phase.

execution of the first and fourth Key was greater for 1x6 Sequences than for the 2x3 Sequences, F(5,75)=4.512, p<.03,  $\epsilon=0.386$  (Figure 2).

As in the practice phase, the error percentages in the test phase were too small to analyze them for each Key separately (mean error rate < 5%). Thus, the analysis of the error percentages included the variables Block (2) and Sequence (2). The participants made significantly less errors in the second compared with the first Block, as shown by the main effect of Block on error percentage, F(1,15)=14.045, p<.05.

Comparison of practice and test phase. There were significant differences between mean RTs in the practice and in the test phase, F(1,15)=8.237, p<.02,  $\varepsilon=1.0$ . As expected, the participants had significantly longer response times in the test phase compared to the practice phase (means were 305.46 ms and 268.30 ms, respectively). The same was true for error percentages. More errors were made in the test phase as compared to the practice phase, F(1,15)=19.564, p<.01 (4.53% and 2.52%, respectively).

# EEG data.

*Comparison of 2x3 and 1x6.* The main effect of Sequence in this ANOVA was not significant, F(1,14)=1.16, p>.2. The main effect of Time, however, was significant, F(5,70)=7.415, p<0.01,  $\varepsilon$ =0.391. Post hoc Bonferroni corrected pairwise comparisons revealed that the interval from -200 to 0 ms showed significantly more negativity than the interval from -1200 to -1000 ms, p<.01, indicating more negativity closer to the go/nogo signal, which is in accordance with the development of an increasing negativity before the movement, corresponding to the CNV. The only significant interaction was between Time and Electrode, F(10,140)=26.938, p<.001,  $\varepsilon$ =0.336. At Fz the activity became more positive with time, whereas at both Cz and Pz activity became more negative with time, even more so at Pz than at Cz. In order to make sure that these results are not a reflection of the long time interval used to measure differences, a second analysis was performed with only the time interval closest to the go/nogo signal (thus from -200 ms to 0 ms) as measurement point. It is suggested that possible effects might be greater the closer in time to the actual movement.

factors Sequence (2x3 and 1x6) and Electrode (Cz and Pz) were included, as only activity at these two electrodes seemed to reflect increasing negativity representing the CNV. The only significant effect with this analysis was found Electrode, for F(1,14)=19.329, p<.01. Apparently, as shown by hoc Bonferroni post corrected comparisons, activity at Pz was significantly more negative than activity at Cz, p<.01.

Comparison of practice and test phase. The main effect of Sequence was not significant, F(1,14)=0.177, p>.6. The main effect of Time, however, was significant, F(5,70)=18.047, p<.001,  $\epsilon$ =0.332. Post hoc Bonferroni corrected pairwise comparisons revealed that the -200 to 0 ms interval showed significantly more negativity than all other intervals, all p's<.02. This, again, is in accordance with



Figure 4. Mean amplitude as a function of Time and Sequence for the three Electrode locations for the comparison of the practice and test phase.

For this analysis, the within-subject

0,2-

0,0

-0,2

-0,4

-0,6

-0.8

-1.0

Amplitude in µV

increasing negativity closer to the go/nogo signal, corresponding to the CNV. Except for the interaction between Sequence and Electrode, F(2,28)=1.707, p>.2,  $\varepsilon=0.515$ , all other interactions reached significance. The interaction between Time and Sequence, F(5,70)=6.045, p<.01,  $\varepsilon = 0.489$ , showed that for the Long Sequences, the negativity gradually increased over time, whereas for the Short Sequences, the slope showed more fluctuations, becoming more positive at the beginning, and veering off to strong negativity from about -600 ms to the end (Figure 3). The interaction between Time and Electrode, F(10,140)=26.551, p<0.001,  $\varepsilon=0.354$  showed that, as with the comparison of the 1x6 and 2x3 Sequences, activity at Fz became more positive over time. Activity at Cz and Pz became more negative with time, whereby Pz showed the most negativity of all Electrode locations. The second-order interaction between all three variables showed significance, too, F(10,140)=9.602, p<.001,  $\varepsilon=0.345$ , as demonstrated in Figure 4. At Fz activity for both the Long and the Short Sequences became gradually more positive over time. At Cz activity resembled the interaction between Time and Sequence (Figure 3). At Pz, activity for Short Sequences started out more negative than that for Long Sequences, but immediately became more positive and, despite an overall negative slope, stayed more positive than the Long Sequences to the end.

For these comparisons, too, analysis at only the -200 to 0 ms time interval was performed. The within-subjects factors included Sequence (Long and Short) and Electrode (Cz and Pz), because here, as for the comparison of the 2x3 and 1x6 sequences, only activity at these two electrodes seemed to reflect the CNV response indicated by increasing negativity towards the go/nogo signal. The main effect of Sequence was not significant, F(1,14)=0.009, p>.9. The main effect of Electrode was significant, F(1,14)=14.164, p<.01. Post hoc Bonferroni corrected comparison revealed that activity at Pz was significantly more negative than activity at Cz, p<.01. The interaction between Electrode and Sequence was significant, as well, F(1,14)=11.109, p<.01. At Cz activity of the Long Sequences was more positive than for the Short Sequences. The opposite was true for activity at Pz, which showed more negativity for the Long Sequences than for the Short Sequences.

### Discussion

The aim of this study was to investigate whether the execution of more complex or longer sequences is associated with increased load on the cognitive processor, as might be predicted on the basis of the dual processor model proposed by Verwey (2001). This would be due to preparation of the sequence in advance and concatenation of motor chunks. Furthermore it was tested whether this would be reflected in a heightened CNV amplitude. For this purpose, a go/nogo discrete sequence production (DSP) task was used. Participants had to learn two different short sequences (1x3) and were tested with these in combinations of either two times the same (2x3) or two different (1x6) sequences in succession. It was hypothesized that the CNV amplitude would be greater for the longer 2x3 and 1x6 trials than for the shorter 1x3 trials. Moreover, the CNV amplitude was thought to be increased for 1x6 trials compared to 2x3 trials due to more complex preparatory demands.

*Behavioral data.* The behavioral data show that the participants had learned the two sequences in the practice phase. The RTs significantly decreased with time, obviously reflecting learning effects. Furthermore, it was shown that the initiation of the movements took longer than the execution of the subsequent keys, as reflected in longer RTs for the first key of the sequences, probably an indicator for the successful formation of motor chunks, whereby the whole sequence is stored and executed as one unified whole. The faster execution of the second and third key was even more pronounced for later blocks, again supporting the notion of motor chunk formation, which takes time and requires practice.

Similarly, in the test phase participants became faster with time, indicating practice effects, as well. Here, too, the initiation of the sequence was slower than the execution of the subsequent elements, in accordance with the notion of motor chunk formation. Moreover, the execution of the fourth key was slowed, as well, revealing a chunk boundary at this position. This indicates that the two 3-key chunks, which had to be combined in the test phase, were actually concatenated. Participants kept the externally specified chunk boundaries and did not group the elements in a new manner. This phenomenon was found to be even more pronounced in the first than in the second block of the test phase, which shows that with extensive practice over time, chunk boundaries might shrink. It suggests that with even more practice, they might actually vanish completely, as the chunks become integrated into a superordinate chunk.

The comparison of the test and practice phase showed that the shorter sequences (1x3)were executed with faster mean response times than the longer sequences (2x3 and 1x6). This might be due to the longer and more complex structure of the longer sequences, which made it more difficult to execute them, resulting in longer response times. Whether this might be due to increased preparatory demands, is not clear. This would, according to our expectations, be reflected only in slower initiation times for the longer sequences and not in overall slower execution rates, because preparation is thought to take place before the movement itself. Also, the 2x3 sequences were executed more quickly than the 1x6 sequences. In line with the proposed hypotheses, the execution of the 2x3 sequences seemed to be less demanding, probably due to a less complex structure, because the 2x3 sequences consisted of the repetition of a sequence, unlike the 1x6 sequences, in which two different sequences had to be prepared and executed. This explanation is further supported by the fact that the slower execution of the 1x6 sequences compared to that of the 2x3 sequences was especially pronounced at the first and the fourth key position. Thus the initiation of the sequences and the concatenation of the chunks might have been the processes at which the two sequences differed in complexity and ease of execution. They might be responsible for the slower overall execution time of the 1x6 sequences, as the execution times of the remaining keys did not show significant differences between the two sequences. This suggests that the intended difference between the two conditions was actually successfully implemented. The two sequences did not differ in overall difficulty and complexity, but only at the intended key positions. Concluding, the behavioral data are in line with our expectations. They show that with practice, participant learned the sequences and formed motor chunks. In the test phase, these chunks were concatenated, which placed more demands on the executing resources, resulting in longer RTs. Finally, the intended complexity manipulation was shown to be successful, as the execution of the 1x6 sequences compared to that of the 2x3 sequences seemed to require more effort at the initiation and the concatenation of the chunks. After ensuring that the implemented task apparently met the required conditions for scrutinizing the proposed hypotheses, we will now turn to the EEG data in order to address this issue.

*EEG data*. The comparison of the 2x3 and 1x6 sequences showed that with time, negativity increased. This is in accordance with the notion of a CNV response as preparatory activity before the movement (Walter, 1964). However, no increasing negativity was found at Fz, indicating that the CNV might not have been reflected in frontal activity. Furthermore, shortly

before the go/nogo signal negativity was the strongest at Pz, as shown by the comparison between Cz and Pz at the -200 to 0 ms time interval. The CNV might thus have been strongest at more parietal locations. Contrary to our expectations, no difference in overall negativity was found between the 2x3 and the 1x6 sequences. Even when investigating only the -200 to 0 ms time interval, no significant difference could be made visible. One of our hypotheses, i.e. that the CNV would be greater for the 1x6 than for the 2x3 sequences, can thus be rejected.

Both the test and practice phase, thus the long and short sequences, also revealed increasing negativity towards the go/nogo signal, most likely corresponding to the CNV. Here, too, the CNV could not be measured at Fz, and the negativity was strongest at Pz when compared to Cz at the -200 to 0 ms time interval. Because similar results were obtained from the comparison of the 1x6 and 2x3 sequences, one might suggest that the findings might not be specific to one of the comparisons, but that general mechanisms and phenomena might have been revealed here. They might also apply to other contexts and tasks. This is in line with the existing literature, which states that, as mentioned earlier, the CNV is a negative voltage change occurring before movement (Walter et al., 1964). Moreover, slow waves such as the CNV, have been shown to be maximal over parietal regions when the study material has spatial properties, as in the present study (Rösler, Heil & Röder, 1997). What else can actually be seen from the comparison of the short and long sequences is that the CNV for the short sequences seems to start later and with a more negative slope than that for the long sequences. One intuitive interpretation might be that this later starting might be due to less preparatory demands before the execution of the short sequences, reflected in later onset of the CNV. However, no support for this explanation can be found in literature. Furthermore, the fact that the short sequences become more strongly negative over time than the long sequences, cannot be explained in terms of our underlying theoretical framework. They even seem to contradict our initial hypothesis. One case in which our hypothesis might actually be in part supported, concerns interaction effects. At Pz, in contrast to Fz and Cz, the long sequences do indeed show stronger negativity than the short sequences, as revealed by a second-order interaction between all three variables. Additionally, analysis at the last time interval showed a significant interaction between electrode and sequence, demonstrating that activity at Cz is most negative for the short sequences, whereas acitivity at Pz shows strongest negativity for the long sequences. Pz seems to be the electrode location at which one of our hypotheses might hold.

However, these results, with this one exception, do not generally confirm our hypotheses from the beginning; the CNV amplitude was not shown to be greater at the more complex (1x6) as compared to the less complex (2x3) sequences and it was not generally greater for the long (1x6 and 2x3) than for the short (1x3) sequences, with the exception of electrode location Pz, resulting from interaction effects. Now the question arises what could have led to our present findings.

Our previous underlying assumption that the CNV reflects motor preparation may not apply to our present study, as the CNV has also been hypothesized to reflect non-motoric processes, such as stimulus anticipation and visuo-spatial working memory (Brunia &Van Boxtel, 2001; Ruchkin et al., 1996). Interpreting the results from this point of view, one might mention that with regard to stimulus anticipation, all three sequence conditions did not differ greatly from each other. In each condition, participants had to wait for the go/nogo signal before they were allowed to respond. This might explain why no significant differences in negativity between conditions were found. However, this cannot explain the fact that at Pz, the long and short sequences did indeed seem to differ. It also seems unlikely that the CNV might have reflected visuo-spatial working memory, as the conditions did actually differ in that respect. The execution of the short sequences was preceded by presentation of the key-specific stimuli, which the participants had to learn. This presentation was omitted for the long sequences, which has presumably resulted in differential demands on the visuo-spatial working memory. Those were, however, not completely reflected in generally different CNV responses. Only at Pz, this difference seemed to be evident. It might thus be that at Cz and Fz, the intended effects of our study might not be visible at all. The reasons for that would not be clear but the findings revealed by interaction effects, that at Pz activity of the long sequences can indeed be more negative compared to that of the short sequences, are nevertheless, partially in accordance with our hypothesis that activity of the long sequences is more negative than that of short sequences (Schröter & Leuthold, 2009). It might be that due to the generally rather bad spatial resolution of EEG data our measurement of the signal location has been misleading and that the activity in fact does take place at broader areas of the brain than measured here. This explanation, however, would not clarify the results obtained from the comparison of the 1x6 and 2x3 sequences, where

our hypothesis was confirmed at none of the electrode locations. Another plausible alternative explanation of the results is nevertheless related to the fact that the presentation of the keyspecific stimuli was left out in the test phase, but not in the practice phase. As a consequence of this, similar to the mechanisms of effector-dependent and effector-independent preparation (Verwey & Wright, 2004), in the practice phase the participants might have prepared the sequences in a more motor-specific way than in the test phase, because the key-specific stimuli which corresponded to particular motor responses might have provided cues for immediate and specific motor preparation. In the test phase, by contrast, they might have prepared them on a more abstract level, independent of the specific motor responses, because no such specific motor cues were provided. Although the details of this explanation are quite speculative, it seems plausible to suppose that the CNV might not have reflected the preparation in the test phase in the same way it reflected the preparation in the practice phase, as both preparatory processes differed in their very nature. Further, this seems feasible, as so far no general consensus on the precise causes and sources of the CNV has been reached (Rohrbaugh, Syndulko & Lindsley, 1976; Drake, Weate, & Newell, 1997). A further issue in interpreting these findings might be the length of the to-be-executed sequences. It has been hypothesized that the cognitive processor might be responsible for the presetting of whole action sequences in advance. However, this might not be the case. The motor chunks, which have been formed in the practice phase, might have been prepared in the same way during the test phase, thus despite the required combination and concatenation (which has evidently occurred) the cognitive processor might have prepared only the first motor chunk before the go/nogo signal. The preparation of the whole sequence in advance might not occur until the two concatenated chunks are unified and integrated into one superordinate chunk. This seems plausible, but unfortunately does not explain the differences between the long and short sequences found at Pz. Still, the work of the cognitive processor might have proceeded during the execution of the sequence, thus the concatenation might not have taken place until the execution of the first chunk. Evidence of concurrent processing in the literature is numerous (Verwey, 1994; Hulstijn & Van Galen, 1983). This could also explain the finding that no differences were shown between the 1x6 and the 2x3 sequences by measuring the CNV before the go/nogo signal. The differences in processing demands might have occurred later. This is also in accordance with our behavioral findings, which revealed that the slower execution of the 1x6 sequences compared to that of the 2x3 sequences was especially

pronounced at the first and the fourth key position. The positions of increased slowness in response might also be the positions in the process, where the cognitive processor is most active. One last issue concerns the participants. Seven of them were proficient piano players, which might have influenced the results in a substantial way, as this included almost half of all participants. Because the task at hand resembled that of playing the piano, the proficient players might have had a different way of preparing such action sequences in advance. This factor was not taken into account during the analyses, as it would have extended the scope of this study, but it should nevertheless be mentioned as a possible confound.

Future research should consider the abovementioned alternative explanations. It could be tested whether the results are different when one uses the same presentation in both the practice and the test phase. Thus in test phase, the key-specific stimuli could be presented in order to keep both phases comparable. In our present study, this was not done in order to keep measurement time at a reasonable length. Presentation of the key-specific stimuli in the test phase would have led to a considerable increase in overall measurement time. This might have resulted in discomfort of the participants, which should be avoided for obvious reasons. For future studies, a more feasible possibility would be to ask the participants to learn the sequences in advance at home, so that already in the test phase, the presentation of the key-specific stimuli can be omitted, thereby making both practice and test phase comparable. Moreover, future research should address the issue of sequence length and advance preparation. It should be investigated under which circumstances action sequences might be prepared as a whole, and whether and how the concatenation of motor chunks with differing degrees of complexity is probably reflected in concurrent processing activity of the cognitive processor. In addition, other measurement devices, such as fMRI, could be used in order to be able to make stronger claims about the location of specific processes. It is also imaginable to measure ERPs other than the CNV (e.g. LRP) in order to explore the issue of motor preparation more in detail. Finally, it could be investigated whether and how the proficiency in piano playing contributes to changes in action sequence preparation on a cognitive level. Differences between participants, which might influence the results, should always be taken into consideration when analyzing the findings and interpreting the results.

In conclusion, with some caution and limitations regarding proper measurement, we dare to claim that our study partially supports the statement that for the preparation of longer sequences the CNV response might be more negative than for short sequences, and that this might be the case at more parietal locations. Furthermore, it clearly confirms the notion that small motor chunks become concatenated with practice. It further suggests that concurrent processing might be an issue in action execution and that longer sequences consisting of more than one chunk might not be prepared as a whole before they become executed. This study gives rise to ideas and inspiration for future research and for the exploration of matters concerning motor learning, chunking, and ERPs in order to paint the complex picture more clearly.

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