Exploring the impact of theta activity in Motor Sequence Learning in a mobile EEG Dance Sequence Task

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Abstract

Extensive research into motor sequence learning (MSL) has gathered insights into how learning can be conceptualised and presented in memory. Frameworks like the Cognitive Framework for Sequential Motor Behaviour 2.0 (Verwey et al., 2015; Verwey, 2023) have conceptualised the different phenomena that occur during MSL, namely the initiation, concatenation, and execution of movements. This research prompted the investigation into various brain oscillations to explore their role in facilitating or inhibiting MSL. Theta oscillations (4-8 Hz) have been identified as a key factor in working memory and the integration of movements into memory. This thesis explored the role of theta activity in a mobile EEG discrete dance sequence task. 13 participants learned a six-step sequence using a go/no-go discrete dance sequence task over six blocks. During movement execution, their performance was tracked alongside brain activity measured by a 22-channel EEG cap. Results showcased a significant decrease in response times over time that peaked around block five. Theta activity increased for the first three blocks and decreased significantly for blocks four and five. During the last block participants encountered an unfamiliar sequence. This caused an increase in both response times (i.e. slower reactions) and relative theta activity. These findings support the idea that learning new motor sequences initially increases the mental load required to process the new stimuli as indicated by increased theta activity. As movements become increasingly integrated into memory this demand seems to decline.

Artificial Intelligence Disclaimer: OpenAI's ChatGPT-4 was used to assist in programming and debugging Code in both Python and R. For the report itself artificial intelligence was only used to receive feedback on the structure or flow of the text.

Contents

1. Introduction

Most of our daily activities involve the acquisition and execution of movements in a specific sequence. Think of lifting your feet while walking or more complex behaviours like hitting a volleyball. The process of learning movements and performing them in a sequential order is referred to as Motor Sequence Learning (MSL). Theoretical frameworks like the Dual-Processor Model proposed by Abrahamse et al. (2013) and the more recently developed Cognitive Framework of Sequential Motor Behaviour C-SMB (Verwey et al., 2015, Verwey, 2023) provide insights into how motor learning is structured. Both models highlight the creation of motor chunks. They refer to movements that can be loaded into memory, initiated, and executed in order (Verwey & Abrahamse, 2012). The transitionary points between single motor chunks are called “concatenation points”. They are often visible in reaction times (RT) due to a somewhat slower reaction after the fourth input of a sequence. This slowdown has been attributed to mental processes like preparing upcoming motor chunks or strategic parsing (Verwey et al., 2010, Wymbs et al., 2012).

Recent research has started incorporating electroencephalography (EEG) to gain more insight into how motor learning processes are represented in the brain (Van der Lubbe et al., 2021, Zhao et al., 2022). One way to assess these brain waves is by analysing event-related potentials generated by internal or external events (Pfurtscheller & Lopes da Silva, 1999). These events can either take the form of an event-related desynchronization (ERD) or an event-related synchronization (ERS). During an ERD relative power in a given band decreases whereas it increases during an ERS.

1.1 Discrete Sequence Production (DSP)

A common research paradigm used to explain MSL is the Discrete Sequence Production (DSP) task (Verwey, 1999; Verwey, 2001). This computerized task measures the performance of participants during a key sequence pressing task on a keyboard, similar to how one would play keys on a piano. In the context of the DSP, participants are tasked to learn short key sequences between three to seven elements and undergo extensive training in these sequences. The training phase usually includes 500 to 1000 repetitions (Abrahamse et al., 2013). Afterwards, a testing phase measures the reaction time (RT) of participants to determine their performance. This way the paradigm can measure the otherwise innate building blocks of complex motor behaviour (Clarac et al., 2009; Gallistel 1980).

Motor execution of well-trained sequences consists of three different phases. Initiation, concatenation, and execution. The initial initiation of a motor sequence exhibits a slower reaction time compared to the following reactions. This is partly due to suboptimal anticipation of the upcoming motor reaction (Abrahamse et al., 2013). The initial reaction is also influenced by the length of the sequence. The sequence length effect is often attributed to the anticipatory loading of multiple separate movements into the motor buffer. The following inputs are usually very fast with reaction times below 100ms. Sequences longer than four key presses show a slow response halfway through the sequence (Verwey 2002, Brown & Carr 1989). Verwey and Eikelboom (2003) argued that fixed sequences are often divided into multiple motor chunks due to assumed limitations in the number of movements that can be represented in a motor chunk. For example, 6-item sequences are usually executed as two or more motor chunks in rapid succession. Concatenation is the process that allows the transition from one motor chunk to the next to ensure the smooth execution of more complex sequences. It can be seen as a sort of priming of the next motor chunk to be used (Abrahamse 2010). This preparatory activity requires substantial cognitive processing early in learning that is not necessarily needed with repeated practice (Abrahamse et al., 2013). When it comes to the execution phase, people start with a more general understanding of how to execute a sequence of movements. With extensive practice they become more reliant on specific body parts to use, this is referred to as effector-specific knowledge.

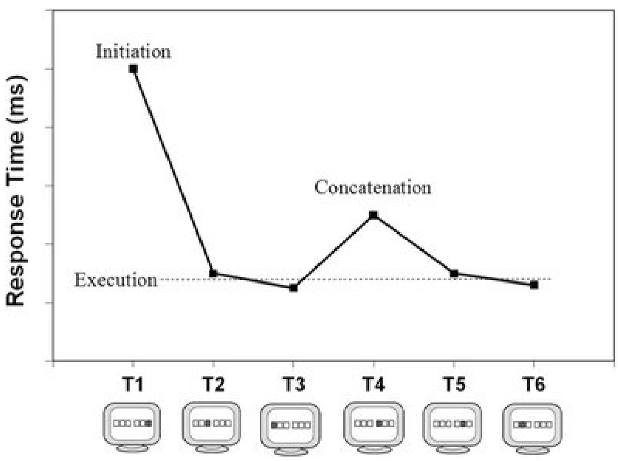

Figure 1. The three phases of motor sequence execution. From the slow first initiation to the execution of the following steps until the slowdown around the fourth input, the concatenation.

1.2 Cognitive framework for Sequential Motor behaviour 2.0 (C-SMB 2.0)

A significant part of research in MSL focuses on isolated body functions, like finger movements or elbow flexion/extension (Abrahamse et al., 2010; Park et al., 2004; Verwey et al., 2015). For instance, the original Dual Processor Model (DPM) by Verwey (1999) was built on a keyboard sequence learning task. This model proposed two different processors a "cognitive processor" and a "motor processor" that act together to translate stimuli and execute movements. To incorporate the insights of the last decades of research with the DSP a more comprehensive model, the Cognitive Framework for Sequential Motor Behaviour 2.0 (C-SMB 2.0), was developed (Verwey, 2023).

The C-SMB 2.0 extends the DPM by accommodating other assumptions of motor sequence learning models like the Additive Factors model and the bottleneck model for the Psychological Refractory Period task. Key assumptions of the Psychological Refractory Period include the existence of a central processing bottleneck and a separate responseproduction bottleneck for motor responses (Pashler & Christian 1994). The C-SMB 2.0 proposes the involvement of three processors in reaction time tasks. The "perceptual processor" translates external stimuli into a perceptual representation that is then interpreted by a "central processor". The processed information is then given to the "motor processor" (Verwey, 2015). The central processor is understood to not belong to a specific area of the brain but consists of a network of regions spread throughout the brain. Whereas the other two processors involve cortical and subcortical regions. More concretely, it reframes the motor processor to involve primary and supplementary motor areas.

Therefore, when we learn a new sequence, it first gets picked up by the perceptual processor in terms of bodily, visual, or even auditory information (Verwey, 2015). Then the result of this process, the perceptual representation, gets transferred to the central processor.

Short-term memory is used as the storage until further processing. Afterwards, it is matched to an existing motor representation from long-term memory, or a new one has to be created. Next, analysis of the motor representation determines what characteristics of it to load into the motor buffer. The motor processor starts executing the movements as soon as the motor buffer has sufficiently been loaded. According to the C-SMB 2.0, attention determines what details get passed through the short-term memory and turned into the multidimensional representations necessary for motor sequencing skills (Verwey, 2023).

1.3 Theta-band oscillations and MSL

One way of assessing attention and working memory in the human brain is by analysing theta activity (Sauseng et al., 2010). Theta is defined as sub-cortical brain activity in the range of 4-8 Hz. These brain oscillations have been linked to encoding and retrieval of new information as well as allocating attention to stimuli (Hsieh, 2011). For example, Sauseng et al. (2010) found evidence for the modulating role of theta activity in integrating new information for example in processes like concatenation. Theta activity plays a role in sequence acquisition and retention, in other words, motor sequence learning. Lum et al. (2022) used a Serial Reaction Time task to explore this relationship. Participants learned a motor sequence by repeatedly responding to a visual stimulus with finger movements. They found that with repeated practice reaction times decreased. At the same time, theta power decreased over the frontal and central electrode sides. Interestingly, when confronted with a random sequence theta power increased as performance worsened. The novel sequence seemed to require more attentional demand to process compared to the well-practised sequence. Increases in frontal-central theta power have also been associated with attention and processing of (new) sensory information (Clayton et al., 2015). Hence, this suggests that learning early on requires increased attentional resources. This attentional demand seems to level off with repeated practice. When a new sequence is encountered, theta activity increases to process the novel stimulus. A similar increase can be observed in theta power levels anticipating a new stimulus (Lum et al., 2022). However, Tzvi et al. (2016) saw a decline in theta power during sequence learning regardless of whether participants practice a sequence or just random motor movements. This probes new questions into the role of theta activity during motor sequence learning.

To explore this connection between theta activity and its influence on MSL this thesis conducted a whole-body motor sequence learning task experiment. Simultaneous tracking of sequence learning performance and electroencephalography was utilised to gain insights into the role of theta activity and behavioural phenomena, namely concatenation.

1.4 The aim of this thesis

The aim of this thesis is threefold. Firstly, it aims to confirm if concatenation will become apparent during the dance sequence task. In line with past research, we do not expect this to be the case. Secondly, theta activity will be analysed to determine if there are indicators for concatenation occurring like an increase in theta activity around the fourth step of a sequence. Lastly, the relationship between performance on the whole-body motor sequence learning task and theta oscillations will be explored further and recommendations for future research will be provided.

2. Methods and Materials

2.1 Participants

For this pilot study a total of 13 students were recruited (6 female, 7 male) with a mean age of 23.23 (*SD* = 2.49). A majority of them reported being right-footed (10 out of 13), two reported being left-footed and a single person stated they were equally proficient with both. All participants took part in one session lasting about 150 minutes. This time includes the setup of various hardware components (E-Prime®, EEG, Xsens) and the execution of the six blocks. We had to remove the first participant due to technical issues with the setup. This resulted in the final sample of 12 participants (6 female, 6 male) with an average age of 23.25 (*SD* = 2.60). Participant 13 was used to replace 1 as they had the same conditions to have a fully counterbalanced sample. All participants provided written informed consent about taking part in the study and their data being used for research purposes. Ethical approval was given by the Ethics Committee BMS/Domain Humanities & Social Sciences.

2.2 Dance-step Task and E-Prime®

E-Prime® was used to display and measure the performance data in terms of accuracy and reaction times of participants during the dance step task. It was run on a laptop connected to a large screen approximately 1.20m from the centre of the dance mat. The dance mat itself was fixed to the floor using tape to prevent it from slipping. Participants responded using the four arrows on the dance pad (↑, ←, ↓, →). They started each block by standing in the middle of the dance mat. The mat itself was connected to the laptop running E-Prime®. Inputs of the mat were converted to keyboard presses (w, a, s, d) using JoyToKey (https://joytokey.net/en/).

The task consisted of participants memorising and reproducing two different 6-step sequences using their legs. The steps were shown using a cross surrounded by four squares. The squares lit up in order of the steps the participants had to respond to the stimuli presentation. After the whole sequence was presented the colour of the cross, green for go and red for no-go-trial, was the cue for their response. Feedback was only provided after participants responded with six key steps. It took the form of either “good” if all six steps were correct or “wrong” followed by a notification of which steps were incorrect.

Pair of sequences	First Sequence	Second Sequence (up, down
1st AB	A: ← → ↑ ↓ → ←	B: → ↑ ← ↑ → ↓
2nd CD	C: ↓ ↑ ← → ↑ ↓	D: ↑ ← ↓ ← ↑ →
3rd EF	E: → ← ↓ ↑ ← →	F: ← ↓ → ↓ ← ↑
4th GH	G: ↑ ↓ → ← ↓ ↑	H: ↓ → ↑ → ↓ ←

Table 1. The list of sequences used for the experiment. Pairs of reoccurring steps in reverse order are marked in red for the first step and green for the second step.

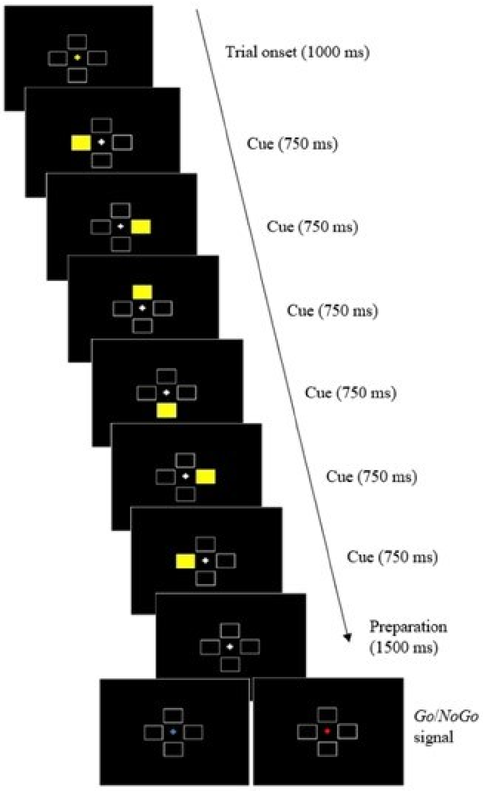

Figure 2. The stimuli presentation participants saw before trying to replicate the sequence. Only after six steps plus the go/no-go-signal were participants allowed to enter the sequence with their feet. Moving during the stimulus presentation resulted in a "too early" error message with them having to wait for the presentation to finish.

2.3 ANT Neuro eego™sports EEG

To capture brain activity during the task execution we used ANT Neuros eego™sports 22channel EEG caps. The electrodes of the cap are embedded within the fabric. The caps were then connected to an external amplifier which was connected to a tablet running the ANT Neuro software. Both the amplifier and the tablet were placed inside a backpack to grant the participants unrestricted movement. E-Prime®, Xsens and the tablet communicated over a wireless network to transmit event markers from the E-Prime® to the other channels. Event markers were tracked for steps, sequence display, (in-)correct completion of sequences, and the start/end of blocks.

2.4 Design

For this experiment, each participant had to complete all six blocks. Four training blocks and two test blocks. A single block spanned approximately 10 minutes. Each participant got assigned a pair of Sequences (AB, CD, EF, GH) to get familiar with during the first four blocks. The last two blocks were used to compare their performance on the two wellpractised sequences in block five against two unfamiliar sequences in block six. For example, participant 1 practised the sequences AB for blocks one to four. Blocks five and six were tested on the familiar sequence AB in block five and the novel sequence CD in block six. The sequence used for the sixth block was then used as the training sequence of the following participant to counteract potential sequence-specific effects. Within each block, participants encountered 48 go-trials and six no-go trials. The trials appeared in random order and were indicated by the colour of the cross between the squares. In total each participant completed 48x6 (288) go-trials throughout the entire experiment.

2.5 Procedure

The experiment started by welcoming participants at the entrance. Afterwards, they signed the consent form for taking part and agreed to their performance, EEG data, and movement data to be used in scientific research. After giving consent their head sizes were measured to use the appropriate size EEG caps. Next, blunt needles were used to bridge the gap between the electrodes and participants' scalps with gel. Gel was applied until the electrical resistance reached levels between 0-20kΩ.

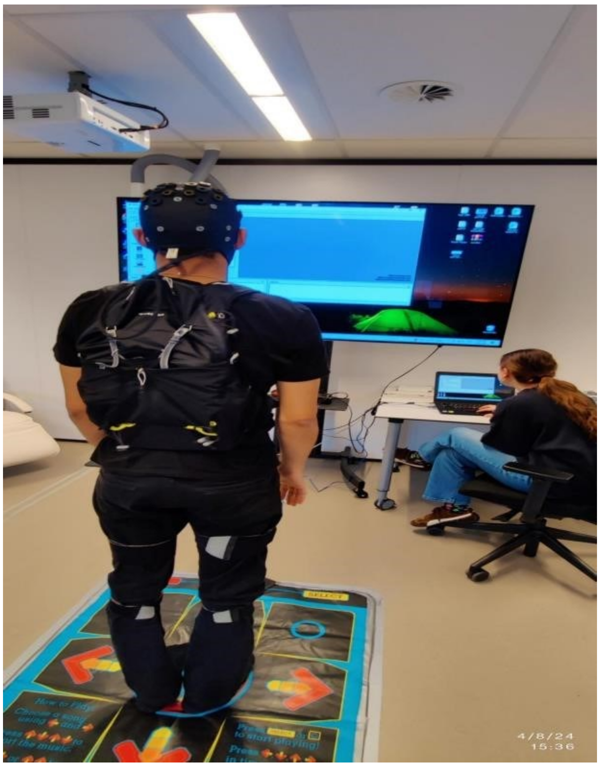

Figure 3. A participant during the dance sequence task. Wearing the 22-channel AntNeuro cap connected to the amplifier and

tablet inside the backpack standing on the dance mat while wearing the Xsens sensors.

In the meantime, participants filled out a questionnaire on the online survey platform Qualtrics (https://www.qualtrics.com/) about demographics like gender, age, height and how they rated their self-perceived exhaustion levels. The questionnaire was completed at the beginning, after blocks two and four and at the end of the experiment. After completion, the participants were equipped with a backpack containing the tablet the ANT Neuro software ran on and the amplifier. While wearing the backpack we attached the Xsens sensors to their body. One on the back of their pelvis, two on the outside of their thighs, two on the inside just below their knees and two above their ankles. After calibrating the Xsens system, by having the subject walk in a straight line of about 5m back and forth, they moved on to the Dance Sequence Task. They started the beginning of every block by standing in the middle of the dance mat. After every block, they had a two-minute break and were offered chocolate and water to keep their energy levels up. At the halfway mark, before block four, subjects could relax for up to 10 minutes.

3. Data analysis

3.1 Behavioural Data

Behavioural data was extracted from E-Prime® using a Python 3.0 script by Emma Wiechmann (Appendix A) and turned into a .xlsx file for further processing in R Studio. In R participant one was replaced by participant 13 as previously mentioned. Afterwards, we excluded every trial from the dataset with at least a single wrong step. Outlier removal was done using the Interquartile Range based on all participants' feedback response times (RTs). This method removed 43 trials in total, keeping 98.5% of the sequences.

3.2 EEG data

EEG data was analysed using Brainstorm Version 3.240503. Firstly, the reference electrode was re-referenced from FPz to the average of all electrodes. Secondly, a bandpass filter from 0.3-30Hz was applied. Next extended events markers were created to mark segments with correct trials. Then we sliced the data into training and test blocks based on the timestamp markers. After removing artifacts such as eyeblinks or heartbeats each block was ICA-corrected using the INFOMAX method. On average, per participant per block 1.7 components were removed. Then we epoched the data based on the correct trial markers to match the behavioural data. Epochs were cut from -200ms before the s27 marker (go-signal) to -100s after the s26 marker (good response). Time-frequency plots were created using the Morlet wavelets method for each correct trial. Those theta-frequency plots were then converted into xlsx files for further analysis in R.

The exported trials had different lengths because participants improved their performance over time. To standardise the epoch lengths, we applied a procedure similar to dynamic time warping (Yamauchi et al., 2015). All data points within an epoch were divided into 300 equally sized bins. The means of those 300 bins were taken as data points to compare trials. This method was applied to the values of the electrodes C3, C4 and Cz. Afterwards, the mean values were put into relation to the average value of the electrodes in the 200ms before the go-signal was displayed. This time interval was used as a baseline to report relative changes in theta activity over C3, C4, and Cz. Thanks to this method every trial regardless of its length was compressed down to a standardized amount of data points. Lastly, behavioural data was amended to the EEG dataset. This way comparisons and correlations of theta power and behavioural data were possible. One of the comparisons took the form of a correlation analysis between response times and relative theta power changes.

3.3 Theta ERD/ERS

We measured theta oscillations over the electrodes C3, C4 and Cz. To determine what models to use for further analysis of relative theta power we used the Akaike information criterion (AIC). Out of the three, the C3 model scored the lowest on the AIC metric. For explorative purposes, we proceeded with the model using Cz based on the assumption that due to the highest AIC score, it will contain the most variance and unique information

3.4 Linear mixed model analysis

Performance data was analysed using linear mixed-effects models using the lme4 package Version 1.1-35.3 in the RStudio environment Version 4.2.2. Two models were used to analyse performance data. For the first model, the outcome variable was the feedback response time in milliseconds with block as the predictor variable. This model was used to assess performance changes over blocks as an indication of learning. The second model also used feedback response times as the outcome variable. For predictor variables, it included block and step number. Both models used subjects as a random factor to allow for individual differences in the baseline of the outcome variable. To further explore interaction effects estimated marginal means were calculated using the package emmeans Version 1.10.2 in R.

The linear mixed models used for theta activity analysis used the same models only with relative theta power as the outcome variable. For clarity, the four linear mixed models used were the following: RT ~ block, RT ~ block x step number, Theta Power ~ block, and Theta Power ~ block x step number.

4. Results

4.1 Performance change over blocks

Feedback reaction times improved over the training phase from blocks one to four. Performance peaked during the testing phase in block five. For the last block, in which two new sequences were introduced, performance slowed considerably. Overall, the block participants were in predicted performance significantly χ^2 (5) = 1885.1, p < .001.

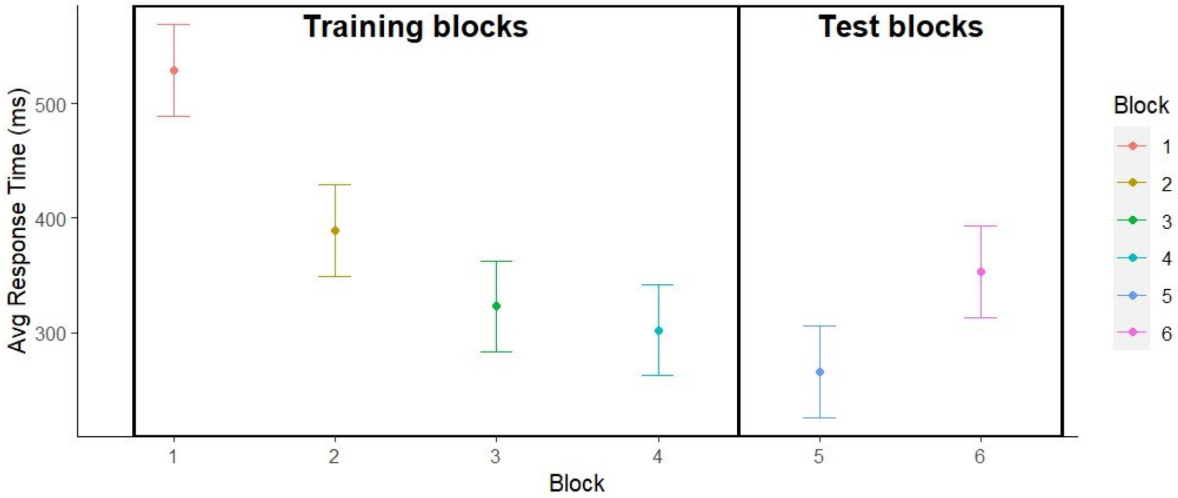

Figure 4. Average response times for all six blocks. Reaction times got faster until peaking in block five.

4.2 Concatenation analysis of performance data

Concatenation analysis using linear mixed models was used to test the effects of blocks and step numbers on feedback reaction times. Across all blocks, there was no evidence for a clear concatenation in terms of a meaningful decrease in reaction times around the fourth step. However, the effects of block χ^2 (5) =3254.44, p < .001, and step number χ^2 (5) =11603.40, p < .001 on feedback response times were significant. Block and step number interacted significantly χ^2 (25) = 689.29, p < .001. Estimated marginal means were computed to reveal more details about this interaction effect. This pairwise comparison using Emmeans showcased a significant slowing in response time from steps four to six for blocks two, three, four and five with -36.56 ms (SE = 11.7), z = -3.12, $p < .023$; -46.84 ms, (SE = 11.8), z = 3.96, $p < .001$; -67.27 ms, (SE = 11.9), z = -5.64, $p < .001$; -42.44 ms, (SE = 11.8), z = -3.59, $p < .005$ respectively. Within those blocks, response times declined on average by -48.23 ms (*SD* = 13.34) from steps four to six.

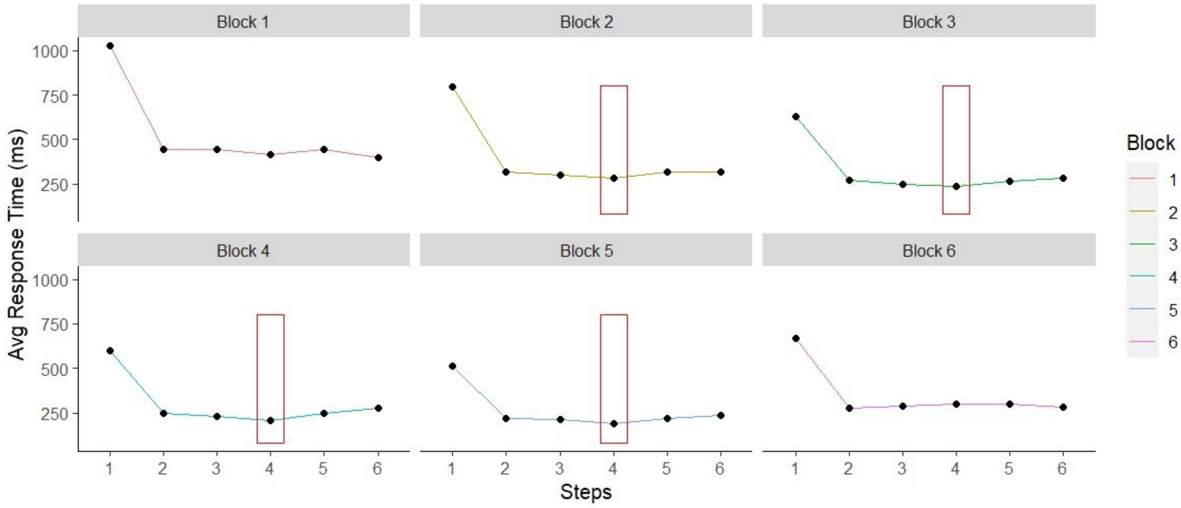

Figure 5. Linear model constructed with response times based on block, step, and their interaction. For blocks one to five the fastest step reaction time occurred around step four. In block six the quickest reactions occurred in steps one and six. Red rectangles indicate the fastest steps for blocks two to five.

The testing phase highlights the difference between the reaction time of steps from familiar sequences against unfamiliar ones. In block five performance peaked around step four and slightly declined. In block six peak performance was achieved at step two and stayed at a comparable level until the end.

4.3 Theta activity over blocks

Block meaningfully predicts theta power across all blocks χ^2 (5) = 64.88, $p < .001$. Relative theta power increased from block one to three. Then it sharply dropped for both four and five. On the last block, it increased to levels similar to the first block. More concretely, the contrast between blocks five and six showed a significant increase in relative theta power -223.10, (SE = 48.8), z = -4.57, p < .001. In other words, two newly introduced sequences in block six caused a meaningful increase in relative theta power compared to the previous block with the familiar sequence.

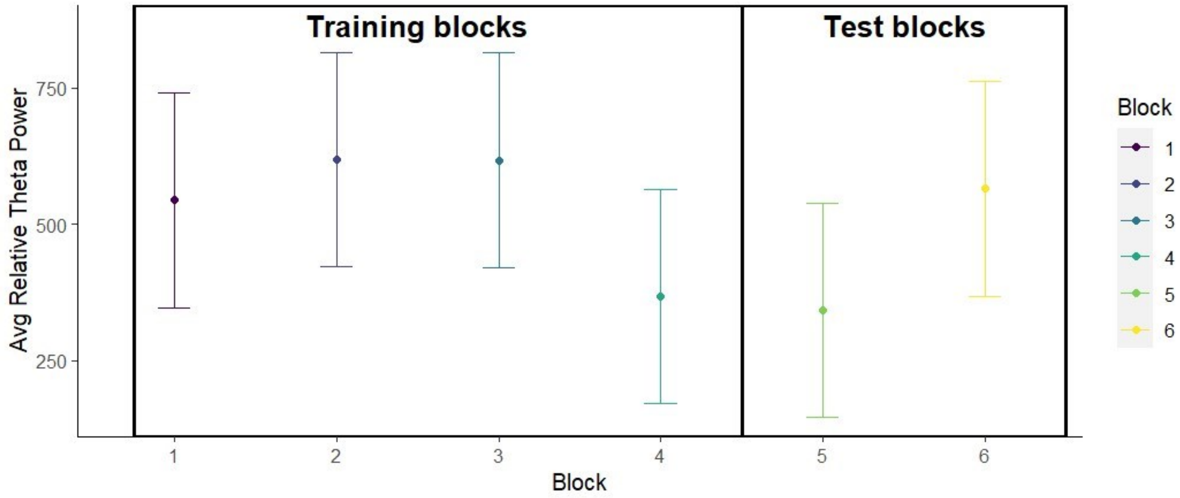

Figure 6. The relative changes in theta power over Cz across blocks. The testing phase shows a sharp decline after an initial increase. The testing phase highlights the difference between theta power of well-practised sequences and unfamiliar ones. The relative theta power changes were calculated towards a baseline of 100ms before each correct sequence.

4.4 Concatenation analysis for theta activity

Both block χ^2 (5) = 65.76, p < .001 and step number χ^2 (5) = 178.66, p < .001 were significantly predicted relative theta power changes across blocks. The interaction between both also noticeably affected theta power over Cz with χ^2 (25) = 50.86, p < .001.

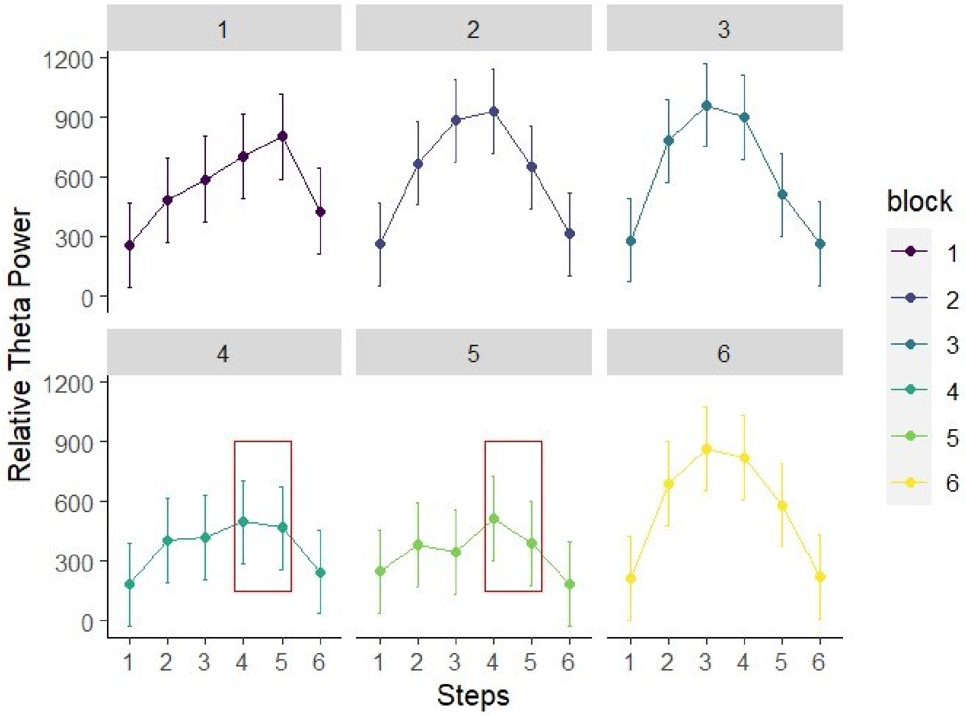

Figure 7. Relative theta power for every step over the six blocks. The theta activity for block six (novel sequence) looks similar to block two/three. Red squares highlight the increase in theta activity around the fourth/fifth steps in blocks four and five. This was the closest that could potentially resemble a concatenation point.

The peak theta activity seems to shift closer towards the beginning of the sequence. It went from initially peaking around step five (block one) to shifting towards step four (block two) and then towards step three (block three) until settling in around step four for both blocks four and five. Contrast analysis revealed a significant difference between the first step of blocks one (-547.56, (SE = 129), z = -4.26, $p < .001$), two (-671.17, (SE = 116), z = -5.78, $p < .001$), three (-681.89, (SE = 117), z = -5.82, $p < .001$) and six (-652.65, (SE = 120), z = 5.42, $p < .001$) and their respective peaks in relative theta activity. As well as a meaningful decline of theta power from that peak to the end of the sequences with 375.76, (SE = 129), z

= 2.92, $p < .04$, 619.20, (SE = 116), z = 5.33, $p < .001$, 700.38, (SE = 117), z = 5.97, $p < .001$, 642.33 (SE = 120), z = 5.33, $p < .001$ for blocks one, two, three and six.

In the testing phase, a considerable change in theta power was visible between blocks five and six. Post hoc pairwise comparison using estimated marginal means revealed a decrease of -223.10 (SE = 48.5), z = -4.60, p < .001 from five to six. Block five experienced its peak of relative theta power changes around step four, whereas in block six it was around step three. Lastly, correlation analysis between response times and relative theta power was significant r(16942) = -0.09, p < .001. In other words, faster reaction times correlated with higher relative theta power measurements.

5. Discussion

5.1 Concatenation analysis of performance data

No clear evidence for a slowdown around step four was found across all blocks that would resemble a concatenation point. These findings align with past motor sequence learning tasks (Chan et al., 2023). Stationary tasks like the keyboard sequence learning task by Verwey et al. (2015) were able to reliably show a slowdown between the fourth input on a motor learning task. In addition to our study, this preparatory slowdown was not evident in other whole-body sequence learning tasks like the one by Chan et al. (2023). They hypothesised that due to fewer joints and larger joints in the legs, it might be less taxing on the body to coordinate inputs using your legs than your fingers. This suggests that participants executed the six-step sequence without splitting the movements into multiple motor chunks. This may be due to the sequences being too trivial or too short to require multiple motor chunks for their execution.

5.2 Changes in theta activity over blocks

In the first three blocks, relative theta power changes increased compared to the baseline. As theta power is often associated with mental load (McFerren et al., 2021) this seems to indicate an increased need for attention or processing of sensory input. Alternatively, this could also indicate reading information from the short-term memory (Verwey, 2023). A higher mental load implies that initial learning requires significantly more mental resources compared to later blocks (Clark & Ivry, 2010). Central processor activity might explain this increase in theta activity (Verwey, 2023). This processor has to hold the perceptual representation of the stimuli in the working memory while either searching for existing central-symbolic representation or creating a new representation for the perceptual image and loading the motor representations into the motor buffer. Shifting one's attention has been shown to increase frontal/central theta power (Clayton et al., 2015).

Blocks four and five show a sharp decline in relative theta power. These results align with past findings from Lum et al. (2022). They experienced a decrease in frontal/central theta power after participants implicitly practised a finger motor sequence. Lum et al. (2022) hypothesized that the changes in theta frequency bands might be due to changes in the sensitivity of the sensory system. The sensory system might be able to optimise and therefore lessen the attentional demand with practice. Alternatively, this decrease could also be an indication that participants have created a central-symbolic representation of the stimuli in their minds (Verwey, 2023). Subsequently, the central processor could access the central symbolic representation from memory. Presumably, this enables the central processor to become more efficient as there is no need to search for a non-existent central-symbolic representation or create it anew compared to when the sequences are first learned. The central processor can then load motor representations faster into the motor buffer. Therefore, performance increases (i.e. reduced response times) while simultaneously decreasing mental load.

In line with the findings of Lum et al. (2022), the introduction of two novel sequences in block six caused a significant increase in theta activity and response times compared to the previous two blocks. Theta activity levels of block six resembled those of block one while reaction times increased (i.e. worsened) to levels between blocks two and three. A possible explanation for the increase in theta activity might be that participants themselves become aware of their worse performance (Lum et al., 2022). This increased attention to their performance might cause increased theta activity. A more plausible explanation, in line with the C-SMB, would the that participants have built central-symbolic representations of the familiar stimuli during their earlier practice. Attentional demand increases as both the perceptual processor and the central processor have to interpret the new stimuli. Comparisons between the unfamiliar and familiar central-symbolic representations might lead to the existing central-symbolic representation being adapted instead of a new one being built from scratch. Subsequently, this could explain the increase in theta activity to those of block one while response times only dropped to levels between blocks two and three.

5.3 Concatenation in Theta activity

There was no clear concatenation point in the relative theta power changes across blocks. However, all blocks displayed a theta power peak after which activity continued to decline. For blocks four and five this spike was indeed around the fourth step. The same step also coincides with the fastest average step in both, indicating some kind of connection between theta power changes and performance. A similar pattern emerged for most blocks as the fastest average step usually also had the highest relative theta power changes. This somewhat consistent pattern prompted correlation analysis between reaction speeds and theta power.

The correlation between feedback response times and theta power was significant. In other words, the higher the relative theta power change the faster the response times should be. Opposing the results found by Lum et al. (2022) who found insignificant correlations between theta power and reaction times.

Previous research has found that performance usually suffers when a concatenation-like event occurs as in the study done by Chan et al. (2023) where reaction times slowed down around the 4th input. The increase in theta power that coincides with faster response times can potentially be explained by the associative mode of the C-SMB 2.0. The associative mode takes place after a few hundred practice trials (Verwey, 2023). Once participants reached block five, they had encountered almost 200 trials. The repeated execution of centralsymbolic representations allows associations to be formed between different representations. As a result, the first few inputs of a task are enough for the motor processor to execute multiple movements while being less dependent on the central processor. The ongoing consolidation of the sequences into long-term memory might explain the still somewhat elevated theta activity even after extensive practice.

Another more speculative explanation for why theta power spikes around the fourth and fifth steps but performance does not decline might have to do with the sequences used. Each pair of sequences required the execution of a pair of earlier executed steps in reverse order for either the fourth and fifth or the fifth and sixth steps. For example, for sequence A steps one and two had to be repeated in reverse order for steps five and six (Table 1). Presumably, the motor representations are still retained in either short-term memory or at the motor level from their previous execution within the same sequence. Therefore, the central processor would not have to retrieve them from long-term memory but could load them into the motor buffer from short-term memory but in reverse order. The reversal of the previous steps could explain the increase in theta performance around the fourth step despite the sequence having been heavily practised in the past. If this were the case the motor buffer could be loaded more effectively without impacting the response speeds.

5.4 Theoretical and Practical Implications

In terms of theoretical implications, this study helps provide more insights into the relationship between performance and theta oscillations. It managed to conceptually reproduce some of the findings of past research like the works of Chan et al. and Lum 2022. This proof of concept of a whole-body motor sequence learning task provides the framework for further exploration of the connection between bodily movement and brain mechanisms. The contradicting results prompt further research into how theta activity might be used as an indicator of learning performance.

In more practical terms these results might help researchers broaden their understanding of how repeated practice affects mental load compared to novel situations. If concatenation were to be better understood this would help optimise learning performance. It could be used as a progress measure to ensure that fast or slow learners get the optimal number of repetitions. This would help identify those that need more practice or those that need more of a challenge.

5.5 Limitations and Future Recommendations

If the present study were to be replicated it could benefit from changes in terms of hardware and minor changes in its methodological approach. The dance mat participants used to respond to stimuli was unresponsive at times. This resulted in some trials being artificially prolonged or even recorded as incorrect. By the same token, this caused unnecessary frustration for some of the participants which could have disrupted the controlled environment and diverted mental attention. Other hardware elements like the backpack contraption could be improved. Participants reported that they could feel the heat from the amplifier after a few blocks. To accommodate them more this setup should be improved when it comes to airflow or comfort especially considering the length of the overall experiment (approx. 60min of wearing the backpack and dancing after setup).

In terms of methodology, the “dynamic time warping” like approach should be further validated to ensure its validity. The method we applied was similar to the one by Yamauchi et al. (2015). As a consequence, time series data was compressed down to a fixed number of observations with some data being lost in the process. This approach retains the temporal order of activity changes in the brain, but key data points could have been lost or warped in unintended ways.

Lastly, to further explore the relationship between theta power changes and motor learning future studies should implement two changes. Firstly, more information could be gained from splitting the theta oscillations into event-related synchronization and desynchronization instead of relative theta power changes. This could potentially explain the contradicting results of the present study. Secondly, to further investigate if concatenation as a concept is visible in brain oscillations longer or more complex step-sequences should be utilized. These types of step-sequences should theoretically exceed the working memory of participants and force them to develop multiple motor chunks to process the inputs.

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Appendix A

R code

https://rpubs.com/Panny/BAv0

EEG time-frequency processing

https://rpubs.com/Panny/1201635

Python script by Wiechmann Emma

https://essay.utwente.nl/87430/1/Wiechmann_BA_BMS.pdf

Appendix B

Informed Consent Form

PARTICIPANT INFORMATION SHEET

Research Project Title: Precise Individualisation of

Mental and Motor learning Enhancement (Pilot Phase)

This project has been approved by the University of Twente's Behavioral, Management and Social sciences (BMS) Ethics Committee No. 240130

Researcher Contact details:

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Invitation to participate in the study: You are invited to participate in the following pilot research study that will investigate how motor sequence learning is reflected in reaction time, brain signals and centre of mass kinematics. Participation in this study is strictly voluntary with informed consent required. You can withdraw your participation from this research study at any time without any consequence to you.

Purpose of the study: This study is designed to investigate reaction time, EEG brain signals and center of mass movement when one is learning a new motor sequence. The study will only involve coming to the laboratory for 1 x testing session to record your data during practice. This will be completed on a computer involving a step learning task while your reaction time as well as your movements are recorded using sevens motion capture sensors fixated on your legs, feet, and pelvis. In addition, you will be wearing a EEG headset that will record your non-invasive brain signals.

Eligibility to participate: To participate, you must meet the following eligibility criteria:

- You are healthy and aged between 18 and 40 years. ○ You are not currently taking any prescribed medication on a regular basis (blood thinners are ok, asthmatic medication ok if not used daily). If used regularly will require clearance their Medical Doctor prior to participation.
- You are not physically injured and are able-bodied. ○ You have not had a falling incident or heart problems within the last year. ○ You do not have any learning disabilities or diagnosed mental health issues or any neurological disorders (such as Alzheimer's, Parkinson's, Stroke, Multiple Sclerosis,
  Brain tumor, Physical Brain injuries, Seizures, or previous concussion/coma) ○ You have not previously taken part in any motor learning experiments involving the dance-step sequence learning tasks in the BMS or via SONA. ○ You can attend 1 session of data collection for up to 3 hours and a willingness to learn a dance-step and free time for between 2.5 to 3 hours to participate
- You do not mind having motion capture sensors attached to your legs, feet, and pelvis.
- You are not feeling unwell in general. ○ no previous professional training with dance, musical instruments/typing and/or gaming ○ no indications of depression or anxiety
- no indications of severe sleep problems requiring medication ○ no drug or alcohol or tobacco addictions ○ no obvious physical injuries or impairments that will affect performance on dance-step ○ must have a normal amount of mobility and physical-activity level (as assessed by IPAQ).
- Interested participants will be screened for eligibility by a researcher via phone prior to participation once more

Requirements:

Participation in the study involves attending a laboratory session <u>ONCE</u> for up to 3-hour research at the University of Twente, BMS Lab.

What is Xsens and EEG how is this data collected?

The Xsens gear is a 3D motion capture program that uses inertial sensors based on the miniature MEMS technology. Xsens inertial sensor technology will be used for orientation, velocity and positioning data. Electroencephalography (EEG) is a way of measuring electrical activity in the human brain by placing electrodes on the scalp of the head. These electrodes are non-invasive and they simply measure the summation of voltages from your scalp. No current is conducted. At each session, a cap with the electrodes will be placed on your head to measure these signals. This will involve the use of the gel substance to increase conduction.

Lab Session (~2.5 to 3 hour):

In the session, you will first be asked to provide information about your activity level and demographics such as age, education, status etc. After this, your body measurements will be taken and entered in the MVN analyze software. Following, you will be fitted with the xsens sensors. After this, you will be fitted with a cap that has the 32 x EEG sensors connected. Then, you will be asked to perform a baseline eyes orientating protocol for 5 minutes where your eyes will be open for 20 seconds and then closed for 40 seconds.

Once the equipment and you are ready, you will be asked to perform a calibration routine for the Xsens that consists of standing still, walking in a straight line, turning around and walking back. This lasts about 5 minutes. After this, you will perform a stepping task in which you train motor sequence and a testing block. Upon completion of the testing block, you will be assisted in taking the sensors off. To complete the session, you will be debriefed and thanked for your participation.

Risks and benefits: This research study does not involve any risk to your well-being beyond what would be expected from typical daily activities.

There are 4 blocks of training and 2 blocks of testing. Each block takes between 10 to 15 minutes to complete. If you feel tired, please let the researchers know if an activity is too strenuous and you require a break or if you wish to stop with the experiment. Chairs will be provided to take a seat and hold on to if needed and a safety protocol is in place.

Reporting and maintenance of data and participant information: All records containing personal information (i.e., signed written consent form) will remain confidential and no information which could lead to identification of any individual will be released unless required by law. All of the research data in this study is recorded by a unique number, meaning that your results will be non-identifiable.

There will be no way to identify your data in any communication of results. The information collected as part of the study will be retained for 10 years and stored in the principal investigator’s (Dr. Russell Chan) office (University of Twente Drienerlolaan 5, Cubicus (building no. 41), room B326, 7522 NB Enschede The Netherlands) and on secured electronic storage housed within the University of Twente, BMS Labs.

The researcher will take every care to remove responses from any identifying material as early as possible. Likewise, individuals' responses will be kept confidential by the researcher and not be identified in the reporting of the research.

Summary report of this study’s findings: When the study is published, a summary abstract of the findings will be made available to all participants. This summary can be requested and indicated to be sent via email as an electronic document upon request by the participant.

This project has been approved by the University of Twente BMS Ethics Committee. If you have any ethical concerns about the project or questions about your rights as a participant please contact:

The Secretary of this Committee, Dr. Lyan Kamphuis-Blikman tel: +31534893399; email: l.j.m.blikman@utwente.nl & ethicscommittee-bms@utwente.nl

Appendix C

Brainstorm checklist

Brainstorm checklist:

- Import raw datafile
- Brainamp add EEG positions (Colin ➐ ANT ➐ Waveguard64 (18 channels added) ○ Edit channel locations (Change EEG_NOLOC to EEG)
- *Raw file ➐ standardize ➐ re-reference electrode ("AVERAGE", "EEG")*
- Pre-process: Apply band pass filter (0.3 – 30Hz) (deselect do whole file)
- Create extended marker (s27s26) (-200ms to default value) ○ s27s26, extend, s27, s26 ○ Maximum delay: 10000ish
- Slice into blocks (training/practice based on MK markers) ○ Pipeline order matters!
- Import Pipeline for eyeblinks and heartbeats ○ Detect eyeblinks (F9, F10) (for all blocks) ○ Detect heartbeats (P9, F9, F10) (for all blocks) ○ *optionally merge blink/cardiac events into one*
- ICA component analysis (INFOMAX) ○ ICA channels: 10
    - Remove components if activity is very frontal
- If unsure check multiple different timeframes
- Slice into Block 1-6 by s27s26 markers ○ Block 1-6 pipeline and slice by s27s26
    - Import MEG/EEG: Events
    - S27s26, -200ms to 100ms
- Export preparation and Exporting to XLSX ○ Put all trials for a block into the pipeline ○ Frequency ➐ Time Frequency (Morlet wavelets)
    - Edit options
    - Change "Group in frequency bands (Hz)"
- Delete all others you don't need
    ○ Delete 6 Blocks and Redo them
    ○ Frequency for Beta ○ Export to participant folder ○ Put them in the pipeline ○ Exports as transposed.xlsx **Appendix D**

Lab protocol

A	**Preparation**
	• Open Window • Start E-prime Laptop, JoyToKey, TV, Xsens app on desktop, tablet and EEG amplifier and make sure all devices are in the same network • Open E-prime master file, EEGO software and Xsens software • Make a new participant in EEGO, insert correct ID • Welcome the participant • Greet participant in front of the room. Introduce yourself and give name of supervisor. Let them store their belongings on the table but remind them to bring their water bottle/drink. Ask them to go to the bathroom now if they need to. • Ask participant to take seat in front of the EEG setup. Let the participant read the information sheet and sign the form of informed consent. • Explain the task, form of stepping and Go/NoGo procedure. • Tell participant we will first set them up with the EEG and then the Xsens
B	**EEG Setup**
	• Tell the participant about physical contact during set-up • First measure circumference of the head on the widest part and pick the correct EEG cap • Visibly check the electrodes for dirt • Ask them to put a finger on forehead and then place the cap on them • Ask participant to close and tighten strap to fit tightly, but without discomfort • Insert the cap cable into the amplifier • Make sure no equipment is close to a table edge where it could fall • Tell the participant we will now insert conductive gel into the electrodes of the cap. In case of any discomfort thy should tell us • Check montage is CW 04875, same for amplifier, sampling rate 500Hz • Proceed with green arrow • Check notch 50Hz • Click on impedance • Fill electrodes with gel • Place tablet and amplifier in the backpack and make participant put on the backpack
C	**Xsens Setup**
	• Make a new file with participant id_X • Measure weight, height, and foot length • Tell participant where we will place the straps • Place all straps and ask for assistance for the participant if needed • Ask participant to fill out the questionnaire • Perform calibration • Drag velocity, acceleration, and position graphs into the interface
D	**Practice Block 1**

	• Start EEG recording, then Xsens then start E-prime (with correct id and session number) • Every 3-4 trials recenter position in Xsens • After block is done, stop recording on Xsens and EEG • restart E-prime • 2 min break
E	**Practice Block 2**
	• Start EEG recording, then Xsens then start E-prime (with correct id and session number) • Every 3-4 trials recenter position in Xsens • After block is done, stop recording on Xsens and EEG • restart E-prime • Questionnaire • 2 min break
F	**Practice Block 3**
	• Start EEG recording, then Xsens then start E-prime (with correct id and session number) • Every 3-4 trials recenter position in Xsens • After block is done, stop recording on Xsens and EEG • restart E-prime • 10 min break
G	**Practice Block 4**
	• Start EEG recording, then Xsens then start E-prime (with correct id and session number) • Every 3-4 trials recenter position in Xsens • After block is done, stop recording on Xsens and EEG • restart E-prime • Questionnaire • 2 min break
H	**Test Block 1**
	• Start EEG recording, then Xsens then start E-prime (with correct id and session number) • Every 3-4 trials recenter position in Xsens • After block is done, stop recording on Xsens and EEG • restart E-prime • 2 min break
I	**Test Block 2**
	• Change to next sequence in script • Start EEG recording, then Xsens then start E-prime (with correct id and session number) • Every 3-4 trials recenter position in Xsens • After block is done, stop recording on Xsens and EEG • restart E-prime • Questionnaire
J	Finish

	• Take off Backpack and carefully remove EEG cap

	• One person can start cleaning the cap • Take of Xsens sensors • Open recording in MVN Analyse reprocess on normal quality and export as MVNX file. Backup all MVN files on the hard drive. Load the behavioural data on the hard drive. • Export EEG data as CNV and EEG file and store on hard drive