A Multimodal Brain and Body Imaging Study: Examining the role of Theta Oscillations during Motor Sequence Learning

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Abstract

Extensive research has examined Motor Sequence Learning from both a behavioural and neurocognitive perspective using keypress paradigms such as the Discrete Sequence Production task. Recent development of a whole-body Dance Step Discrete Sequence Production (DS-DSP) task enabled the exploration of underlying cognitive processes in more naturalistic movement. As the theta frequency (4 - 8 Hz) is associated with memory encoding and working memory load, the current study examined theta event-related desynchronisation and synchronisation while executing a 6-step sequence according to the DS-DSP paradigm. The study was conducted with 12 participants, who were fitted with a 22-channel mobile EEG system. Concurrently, the response time (RT) of each step execution was measured, thus demonstrating the mobile brain and body imaging (MoBI) approach of this study. Results indicate a decrease in RT and relative theta power in the late stages compared to the early stages of learning. Moreover, an increase in theta power relative to the 3rd and 4th step was found in the early learning stages of learning. Usually, a spike in theta power is associated with a temporary spike in step-level RT, however this was not the case in the current study. Furthermore, the pattern of theta power observed in this study is divergent from the theta power observed in previous finger or visual sequence learning studies. This suggests that cognitive processes underlying whole-body sequential learning are different to previously investigated finger-specific or visual sequential learning. The current study postulates that an increase in step-level theta power indicates the loading of a memory chunk and suggests that the storage of memory chunks in long-term memory occurs earlier in whole-body MSL. Lastly, this research study explains possible whole-body motor mechanisms which could be responsible for the divergence between RT patterns observed in the DS-DSP compared to finger-specific or visual sequence learning tasks.

Artificial Intelligence Disclaimer:

In the development of a data cleaning and analysis script in R, OpenAI's ChatGPT (GPT-4) was used to facilitate a more efficient coding process. The use of ChatGPT for textual content was primarily to refine paragraph structure and organise thoughts without influencing the thesis's intellectual direction. Grammatical accuracy and textual clarity were enhanced through Grammarly. This approach underscores a judicious application of AI tools to augment the research process, maintaining the integrity and originality of the work.

Contents

1 Introduction

Sequential structures are fundamental to much of our goal-directed behaviour such as typing, tying shoelaces, driving a car and also more complex behaviour like playing the piano (Chan et al., 2023; Rhodes et al., 2004). Motor Sequence Learning (MSL) is known as the process of refining these skills to execute them quickly, accurately and with little cognitive effort or attention (Abrahamse et al., 2013) and is an important topic of research due to its daily relevancy. Over the years, understanding of the processes underlying skill improvement has led to well-known theoretical frameworks such as the Dual Processor Model (DPM) and the Cognitive Framework for Sequential Motor Behaviour (C-SMB) (Verwey et al., 2015, Verwey, 2001). Both aim to explain specifically the involvement of memory and encoding in the early and late stages of learning. They introduce the concept of memory chunks, which represent multiple sequential movements as one bound element, thus reducing cognitive effort and making sequence execution more efficient over time. While both frameworks have greatly elaborated on MSL, their development is largely based on keypress paradigms like the Discrete Sequence Production (DSP) task. Keypress tasks may lack the ecological validity to explain our natural daily behaviour, which involves multiple limb effectors.

For this reason, a recent study utilised a modified whole-body version of the DSP task, called the Dance Step DSP (DS-DSP) and found results challenging the typical outcomes of the keypress DSP (KP-DSP) task (Chan et al., 2022). A commonly observed outcome of the KP-DSP is the slowing of reaction time (RT) mid-sequence, which indicates an increased cognitive effort to load the next memory chunk holding further execution-related information (Verwey, 2024). This point in the sequence execution is termed the concatenation point. Interestingly, the whole-body DS-DSP task study conducted by Chan et al. (2022) did not find a typical concatenation point indicated through the slowing of RT at the $3rd$ or $4th$ sequence position. The notion indicates that (1) bodily movements might involve different cognitive processes as proposed by research based on finger-specific tasks or; (2) bodily movements involve the same cognitive processes, however the need to coordinate multiple effectors involves a form of motor execution mechanism that could plausibly explain the incongruence of the keypress and whole-body DSP findings. Effectors are bodily structures, for example a muscle, that activate after stimulation.

In addition, the concatenation in RT is related to the frontal midline theta $(4 - 8$ Hz) frequency (Chan et al., 2023; Crivelli-Decker et al., 2018), which is highly linked to memory encoding, retrieval, working memory and cognitive load (Hsieh & Ranganath, 2014; Klimesch, 1999; Xie et al., 2021). Studies examining the theta frequency in sequential

movement are usually conducted with keypress experiments, hence the current study piloted the use of a mobile 22-channel EEG system with the DS-DSP. Theta Event-Related Desynchronisation/Synchronisation (ERD/S) was used as the method of spectral decomposition to further understand the electrophysiological mechanism of memory chunk development during sequential movement execution (Pfurtscheller & Lopes da Silva, 1999). Therefore, the current study aimed to investigate how theta ERD/S transpire in a whole-body task compared to finger-specific tasks in early and late stages of learning, as well as on a steplevel basis. The following sections outline the theoretical underpinnings and neurocognitive substructure of MSL.

1.1 The (Dance-Step) Discrete Sequence Production Task

The DSP task is an experimental paradigm developed by Verwey (1999) to examine the underlying cognitive processes occurring during MSL. The implementation of a go/no-go variant by De Kleine and Van der Lubbe (2011) has further enabled the distinct separation of motor preparation and execution phases. Moreover, Chan et al. (2022) adapted the keyboardbased task into a whole-body task known as the DS-DSP task, thereby enhancing ecological validity with more naturalistic movement. The intricacies of this experimental paradigm are further elaborated on in the methods section of this study. The DS-DSP task measures the reaction time (RT) for each sequence response, which is believed to reflect the ongoing cognitive processes during motor sequence execution (Rhodes et al., 2004). Repetition and an explicit starting point per sequence help participants to eventually reproduce the entire sequence automatically after seeing the starting stimulus, essentially turning the task into a 2 choice RT task (Verwey et al., 2015). As a control condition, participants then perform an unfamiliar sequence at the end of the task.

1.2 The Cognitive Framework of Sequential Motor Behaviour (C-SMB)

The automatic execution after extensive practise is explained by Verwey (2001) in the DPM and later in the C-SMB through the development of motor chunks (Verwey et al., 2015) or abstract central-symbolic representations in short-term memory (Verwey, 2024). Due to recent refinements made by Verwey (2024) in the C-SMB 2.0, the current study will refer to the concept as memory chunks. Memory chunks encompass central-symbolic representations that can be verbal, spatial or motoric, in contrast to motor chunks which are strictly motoric. They further bind multiple sequence elements into one cohesive action and are stored in shortterm memory by the central processor through repeated practice (Verwey et al., 1996). After a chunk is stored in short-term memory, additional practise eventually encourages further storing in long-term memory (Verwey, 2024). This enables movement execution based on

activating the first chunk element from long-term memory, which triggers the loading of the whole sequence into short-term memory within one processing step (Verwey et al., 2015). Movement execution activated by the loading of the first memory chunk element is termed the chunking mode, whereas initial step-by-step execution based on loading each sequence element is termed the reaction mode (Verwey & Abrahamse, 2012). Ultimately, memory chunks integrate more effectively with the relevant effector, establishing a highly motordriven form of execution. This further reduces processing load of the central processor.

However, research indicates that short-term memory can only hold information for a chunk length containing approximately three to four actions, hence posing a limitation to the number of actions that can be bound together (Bo & Seidler, 2009). This limitation forces the development of multiple memory chunks. The transition between chunks is known as the concatenation point and is marked by an increased RT, representing increased cognitive effort due to the loading of the next chunk (Abrahamse et al., 2013). Figure 1 shows that the concatenation point in a 6-stimuli keyboard-based DSP task lies between the $3rd$ and $4th$ response.

Figure 1. Typical RT pattern observed in a KP-DSP with an increase in RT at the 4th keypress, likely caused by the loading of the next memory chunk for sequence execution. Figure from Abrahamse et al. (2013).

1.3 Recent Findings and Alternative Hypothesis on Multiple-Effector Tasks

Interestingly, the DS-DSP study conducted by Chan et al. (2022) did not find a concatenation point at the 3rd or 4th step but at the 6th step. Based on Shah et al. (2013), they suggest that the use of the lower body involved less and larger joints, which could be less taxing to coordinate and thus enhances performance. Currently, the C-SMB 2.0 does not offer any reasons as to why bodily sequence learning might differ from finger-specific learning.

Since the majority of studies examining MSL and chunking were conducted with a single movement task, Hirokane et al. (2023) conducted a complex stepping task with mice to research whole-body chunking mechanisms. Based on their results they propose the *rhythm chunking hypothesis*, which introduces the idea that chunks are majorly supported by rhythmic development. In more detail, they found that the coordination of body parts, in particular matching the movement cycle of one limb to another is done through rhythmic motor execution. Additionally, they observed that practice led to the creation of longer rhythms, including more steps within each chunk. Symbolic of a concatenation point, Hirokane et al. (2023) observed a distinct change in rhythm. While this viewpoint does not refute the concept of memory chunks, it could offer an explanation why whole-body sequence execution does not show the typical RT concatenation pattern.

One key difference between the KP-DSP and the DS-DSP is the spatial mapping of the effectors to the response location. While the KP-DSP features one-to-one mapping of each finger to a specific response key, the DS-DSP does not restrict participants. They can use both feet to respond as they wish to the four response locations on the dance mat, thus increasing the variety of execution options. Wright et al. (2019) examined the impact of using multiple effectors for a single response location. They found that when the stimulus-response mapping is spatially linked in a logical layout, the mapping time is only marginally affected by the diversity of execution options. Hence, the different mapping of effectors in the DS-DSP is likely not a reason for the divergence in the RT pattern.

Another differing physiological contribution in the DS-DSP compared to the KP-DSP is the input of the vestibular system while performing the task. In general, the vestibular system contributes to the encoding of self-motion based on head movement in space (Cullen, 2012). One study found that the deactivation of the vestibular system through injections in rats caused a decrease in the theta frequency power (Jacob et al., 2014), an important frequency related to memory encoding as discussed further in section 1.5 of this paper. Another study found that the stimulation of the vestibular system in healthy adults leads to quicker response times in a facial recognition recall task (Wilkinson et al., 2008). Since whole-body movement facilitates an activation of the vestibular system due to head movement, it could potentially explain why the RT pattern differs in the DS-DSP compared to the KP-DSP.

1.4 Neurocognitive Infrastructure of MSL

In general, brain imaging methods are used to examine the underlying neural dynamics that are needed for human cognition such as MSL (Gramann et al., 2014). The

prefrontal cortex (PFC) is associated with decision-making, working memory and attention (Miller & Cohen, 2001) and recent fMRI studies found an activation of the dorsolateral PFC when actively performing a four-key sequence task (Gonzalez & Burke, 2018; Verwey et al., 2019). Gonzalez and Burke (2018) additionally observed a stronger activation of the premotor and motor cortex (M1) during the execution of longer sequence (8) and Verwey et al. (2019) found activation over M1 in the early learning stages. Perhaps linking to a more dynamic explanation, Hikosaka et al. (2002) argue that MSL functions through an intracortical connection between the PFC and M1 as well as cortico-cortical loops within the PFC and M1 connecting to the basal ganglia and cerebellum. In more depth, the PFC seems to facilitate early MSL through spatial, effector-unspecific, highly conscious processing whereas late MSL is driven by M1 and motor-driven, effector-specific, unconscious processing (Hikosaka et al., 2002). This is similar to the line of argumentation posed by Verwey et al. (2015) in the C-SMB. They argue that the motor processor is associated with the M1 region and eventually takes over movement execution from the central processor after repeated practice. Other evidence further suggests that the involvement of the hippocampus during early learning stages facilitates better sequence reproduction the next day, stressing the importance of the hippocampus for the consolidation of sequence memory (Albouy et al., 2008). Lastly, a fMRI study investigated neurocognitive activity in unimanual and bimanual hand tasks and concluded that the bimanual task exhibited a greater neural connectivity between the prefrontal cortex (PFC) and the medial motor cortex, suggesting increased topdown processing between motor regions in multi-effector tasks (Madlon-Kay et al., 2013). This is relevant for the current research because the DS-DSP is a bimanual task since it involves both feet.

1.5 The Role of Theta Oscillations in MSL

Since short-term and working memory are so important in MSL, it is crucial to dive deeper into how information is stored and processed within it. One aspect of interest is the encoding and representation of sequential information. Neural oscillations support these processes by enabling the communication between different brain regions (Xie et al., 2021). The mid-theta frequency (4-8Hz) is of particular interest when investigating neural oscillations in memory processes since it originates from the hippocampus and medial prefrontal cortex (Hsieh & Ranganath, 2014), both areas that have previously been found to be active during MSL tasks. Theta oscillations facilitate the encoding process and promote the neural connectivity between different cortical areas (Xie et al., 2021). Additionally, higher theta power is associated with encoding new information and better memory retrieval

(Klimesch et al., 1996, as cited in Klimesch, 1999) as well as supporting high working memory load (Axmacher et al., 2010).

The power of the theta frequency has consistently shown to be higher in the early stages of learning and decrease with practise over time, which further couples with a decrease in RT (Clarke et al., 2017; Lum et al., 2023; Crivelli-Decker et al., 2018). These findings indicate that theta oscillations support early learning stages, which demand a high working memory load, and decrease as working memory load reduces through practise of the task. Regarding theta oscillations on a sequence-level basis, Crivelli-Decker et al. (2018) found an increase of theta power relative to the $4th$ position of the sequence, which was also associated with an increase in RT. These results suggest that a concatenation point in RT is linked to higher theta power, potentially caused by an increased cognitive effort needed to load the next memory chunk. The same result was found by Chan et al. (2023), who conducted the KP-DSP and found increased theta activity relative to the 4th keypress, which was also found to be a concatenation point in RT. Since the C-SMB 2.0 argues that the concatenation point denotes the loading of a memory chunk, the results of the previous studies strengthen the hypothesis that an increased theta power indicates the loading of a memory chunk.

Considering that a spike in theta power was previously observed to accompany a RT concatenation point (Chan et al., 2023; Crivelli-Decker et al., 2018), any potential increase of the theta power in the DS-DSP should align with the increase of RT. If this is the case, it would be an indication that cognitive processes underlying MSL are similar in a whole-body task compared to a finger-specific task. However, if a spike in theta power is not accompanied by a concatenation in RT, it is likely that whole-body MSL involves different cognitive processes or additional motor execution mechanisms which can bypass the slowing of RT.

To further understand the electrophysiological mechanism of memory chunk development, the current study used Event-related Desynchronization and Synchronization (ERD/S) as a tool to investigate changes in theta power during movement execution relative to a baseline period. While the traditionally known Event-Related Potential (ERP) is recognised as a course of neuronal responses caused by a specific stimulus, ERD/S originate from the source that controls the neural oscillations (Pfurtscheller & Lopes da Silva, 1999).

1.6 Expectations of the Current Study

The goal of this study is to examine the role of theta oscillations during the motor execution phase of the DS-DSP to shed further light into MSL mechanisms and the role of memory chunks. Utilising a mobile brain and body imaging (MoBI) approach through a mobile 22-channel EEG system, theta ERD/S over M1 will be analysed.

Based on the DS-DSP study conducted by Chan et al. (2022), the current research expects to (1) find no concatenation in RT at the $3rd$ or $4th$ step position. The absence of the concatenation point leads to the prediction (2) that no relative increase in theta power related to a specific step will be found. However, in line with Clarke et al. (2017) and Lum et al. (2023) this study (3) does expect to find lower theta band power in the late stages of learning compared to early stages of learning, indicating less cognitive demand needed for later sequence execution. Finally, (4) increased theta power is expected in the execution of the last stage of the DS-DSP, which involves the learning of an unfamiliar sequence. This is based on the notion that theta facilitates the encoding of new information (Klimesch et al., 1996, as cited in Klimesch, 1999) and participants likely have to invest more cognitive effort when faced with the unfamiliar sequence.

2 Method

2.1 Participants

12 participants (6 female) were recruited via convenience sampling by the researchers. Their average age was 23.3 years $(SD = 2.6)$. All of the participants were students at the University of Twente and had to comply with the following inclusion criteria: Have no history of psychological, neurological or psychiatric disorders; no problems with their sleep; no substance addictions; no physical injuries and no extensive experience with dancing or playing a musical instrument. Participants were given an informed consent form prior to commencing the experiment (Appendix A). The study has been approved by the University of Twente's Behavioural, Management and Social sciences Ethics Committee (No. 240130). Initially, 13 participants took part in this study, however one was excluded because the wrong conductive gel was used for the EEG cap electrodes. The study took between $2 - 2.5$ hours per participant, including the setup and removal of the EEG.

2.2 Materials

2.2.1 DS-DSP Task

To receive a general overview of the materials used in this study and how they were connected to each other, see Figure 2. The DS-DSP Task was programmed using the software E-Prime 3.0. A previously developed script by Chan et al. (2022) was slightly adapted and converted from E-Prime version 2.0 to 3.0. This software was recording the behavioural data such as the RT and correctness of the steps. The laptop executing the DS-DSP task through E-Prime was connected to a 77-inch LG monitor via HDMI to ensure a large-scale display of the task stimuli. A nonslip dance pad (D-Force) was connected to the laptop running the experiment via USB (see Figure 3). On the same laptop the software JoyToKey was installed,

which was responsible for mapping the input of the dance pad $(†, \rightarrow, \downarrow, \leftarrow)$ to keyboard letters ('w', 'd', 's', 'a'). This was important because the E-Prime script recognises the steps made on the dance pad as if letters on a keyboard were pressed. The dance pad was placed centred, 120cm away from the LG monitor with a viewing angle of \sim 30 \degree to the screen.

Figure 2. Overview of the experimental setup adapted from Chan et al. (2022) to include the mobile EEG system and removed the motion capture device.

Figure 3. Mobile EEG system and D-Force dance mat. EEG was recorded from channels FPz, FCz, F9, F3, F4, F10, Cz, M1, T7, C3, C4, T8, M2, P9, P7, P3 Pz, P4, P8, P10, Q1, Q2.

2.2.2 EEG

Participants wore the mobile 22-channel ANT Neuro eegoTMsports EEG (ANT Neuro b.v., Hengelo, The Netherlands). The amplifier was carried by the participant in a lightweight sports backpack alongside a Microsoft Surface Pro 2 tablet, which was recording at 500 Hz using the eegoTM software (see Figure 3). The 22 channels followed an adjusted 10-20 montage. There were no EOG channels available, hence eye movement was accounted for by the channels placed closest to the eyes, namely F9 and F10. This is further explained in the pre-processing steps. Channel CPz was used as the reference and channel FPz as the ground electrode. A specific Lab Streaming Layer (LSL) code was written to implement markers in the E-Prime stimulus presentation software. The eegoTM software supports LSL by default and the markers were received via a local network from E-Prime. An effort was made to keep impedances below 10 kΩ. This was possible most of the time, however there were rare instances during which the impedance of one electrode would remain between 10-15 kΩ after repeatedly trying to lower it. Due to time constraints and this being a pilot study the researchers decided to continue with the experiment. Moreover, the researchers observed that the impedance often decreased to well below 5 kΩ after setting up the motion capture system and explaining the task to the participants.

2.3 Procedure

Participants were instructed to wash their hair with shampoo on the day of the experiment or latest the day before. After the EEG setup was completed, they were asked to take their shoes off and begin the DS-DSP task. Figure 4 illustrates the experimental paradigm. The monitor displayed four rectangles arranged in a cross fashion on a black background, with a fixation cross in the centre that turned yellow 1000ms before the first stimulus appeared, signalling the onset of the sequence. While standing on the dance mat, participants watched a sequence of six rectangles light up in yellow in a certain sequence order for 750ms each. 1500ms after the last rectangle lit up, either the go or no-go signal was shown, symbolised by a blue or red cross respectively. Upon seeing a go signal, participants reproduced the sequence with their

Figure 4. Experimental paradigm from Chan et al. (2022)

feet on the dance mat using any strategy they preferred. If a no-go signal appeared, they were instructed to withhold any response and wait for the next sequence. In case participants responded before the go/no-go cue was shown, a message reading "Too early!" was displayed on screen. Responding after a no-go cue displayed the message reading "Error!". After each response, participants received feedback on screen indicating any mistakes or confirming a correct sequence execution with "Good!". Each participant learned two distinct sequences with each complete sequence execution counted as one trial.

The experiment was divided into six blocks of 52 sequences each, including 48 go trials and four no-go trials. The two sequences were randomly presented an equal number of times throughout a block and participants were instructed to respond as accurately and quickly. Furthermore, the first four blocks served as training, while the last two were for testing purposes. The final 6th block showed participants two completely new (unfamiliar) sequences. Participants received a 30s break halfway through each block and a summary of their mistakes and average RT per step. Due to the nature of LSL, E-Prime had to be restarted after each block and the EEG recording was stopped and restarted to ensure an easy separation of blocks in the pre-processing steps. This gave participants a 2-3-minute break in between blocks. Additionally, a 10-minute break was offered after the 3rd block. In total. participants completed 192 go trials during the training phase and 96 trials during the test phase.

2.4 Counterbalancing

As mentioned above, each participant learned two sequences throughout block 1-5 and received two unfamiliar sequences in the 6th block. To prevent foot-specific effects, a counterbalancing procedure was applied. In total, eight unique sequences were created and labelled with the letters A-H. The first participant learned sequences A+B and received sequences $C+D$ in the 6th block. The next participant learned the sequences $C+D$ and received sequences $E+F$ in their $6th$ block. This scheme was followed for all participants, ensuring that each sequence combination was learned the same number of times. View Appendix B for an overview of the counter balancing procedure and all sequences.

2.5 Data Analysis

2.5.1 Behavioural Data

Data cleaning and analysis were conducted using RStudio version 1.1.463. The dataset worked on was extracted from E-Prime 3.0 in a .xlsx format and contained all the behavioural data from all participants. The first step involved removing the first participant due to the use of the wrong conductive gel. Unnecessary variables were then eliminated, leaving the dataset

with information about the subject, block, step number and RT for each step. RT was measured from the go cue onset until a foot stepped on one of the four response options. Once a footstep was registered, the RT measurement for the next step commenced. Next, all incorrectly executed trials (sequences) were removed. Precisely, if one step within a sequence was incorrect, the entire sequence (steps 1-6) was excluded. The 12 participants executed 3456 sequences in total, of which 2867 were correct, resulting in an accuracy score of ~ 83 %. A count variable was added to indicate the number of correctly executed sequences per participant within a block. After that, the average RT per sequence was calculated by averaging the step-level RT across steps 1-6, providing an indication of the average time taken for each footstep within the sequence.

The outlier removal was performed after integrating the EEG data to this dataset to ensure complete coherence between the number of trials in the behavioural and EEG data. Sequences with an average RT greater than 1.5 times the interquartile range were considered outliers and removed, leading to the exclusion of 43 sequences across the whole dataset. In total, 98.5% data was retained for the analysis.

2.5.2 EEG

2.5.2.1 Pre-Processing

The EEG recordings were pre-processed using MATLAB Brainstorm version 24.03.2024. After importing the raw datafiles and channel positions, a bandpass filter of $0.3 -$ 30 Hz was applied. A new reference electrode was created based on the average of all electrodes. Extended markers were then designated to capture the exact timeframe during which participants responded to the sequence. This was done by extending the s27 marker (go cue) to the s26 marker ("Good!" feedback), further ensuring to retain the correctly executed trials analogous to the behavioural data. Additionally, 200ms before the go cue marker were included in the time segment to serve as a baseline for the ERD/S analysis. The recording was sliced into six blocks according to markers set by the eegoTM software, automatically created whenever the recording was started and stopped between blocks. Channels F9 and F10 were used to detect eye blinks because they were the closest to the eyes and a manual inspection of the raw data revealed blink patterns within these channels. Furthermore, the channels F9, F10 and P9 were set to detect heartbeat because heartbeat patterns could be observed in the raw data for these channels. The placement of F9 and F10 on the temples likely contributed to the visibility of the heartbeat patterns. Next, an Independent Component Analysis (ICA) using the Infomax algorithm was applied. On average, 1.7 components were removed per participant

and block. Finally, the extended marker and the additional 200ms before the go cue were segmented to individual epochs.

2.5.2.2 Dynamic Time Warping and ERD/S

A Morlet Wavelet time-frequency analysis was applied to all epochs extracting the theta (4-8 Hz) frequency. Each epoch had a different length because we extracted exactly the time it took for participants to respond (+ 200ms baseline). This approach was chosen over a fixed timeframe because behavioural data showed that trial durations varied significantly, ranging from 500ms to 6000ms. Using a fixed timeframe equal to the longest trial would make steplevel analysis impossible for shorter trials, as they would only occupy a small portion of the timeframe. Moreover, it is not possible to average the Morlet Wavelets time frequencies of all epochs due to their unequal lengths. Instead, the time frequency of each epoch for channels C3, C4 and Cz was exported as a transposed .xlsx file to continue further analysis in RStudio. The channels C3, C4 and Cz were chosen due to their position above the primary motor area (M1), which is involved in the execution of voluntary movement (Zang et al., 2003). Further, while theta power is commonly associated with frontal areas, Lum et al. (2023) also observed changed in the theta frequency power over central electrode sites.

Following Yamauchi et al. (2015), we utilised a method mimicking Dynamic Time Warping (DTW) to standardise epoch lengths whilst retaining step level data. DTW is a technique which enables the alignment of two sequences holding different speeds or time lengths, making it possible to align the steps of two different sequences (Müller, 2007). Figure 5 provides a visual representation of this method.

Figure 5*.* Diagram Visualizing DTW. From Information Retrieval for Music and Motion (p.70), by M. Müller, 2007, Springer. Copyright 2007 by Springer-VerlagBerlinHeidelberg. Permission to reuse obtained under license number 5803001301940.

Classic DTW algorithms merely allow the comparison of two sequences at a time and furthermore result in the warping of datapoints, causing compression or stretching of data (Yamauchi et al., 2015). However, alternative methods for standardising epoch lengths exist. Yamauchi et al. (2015) proposed reducing time series data to a fixed number of datapoints by averaging many small fragments within the sequence. In our study, the number of datapoints per epoch ranged from approximately 500 - 3000, based on epoch length ranging up to 6 seconds with a recording frequency of 500Hz. We decided to reduce all epochs to 300 datapoints. This number was chosen because our epochs were longer than those in the referenced study, which used 100 and 200 datapoints. Figure 6 visualises the process of averaging an entire sequence into 300 datapoints. The process involves placing 300 equally spaced 'bins' throughout all datapoints and calculating the average within each bin, resulting in all epochs containing 300 datapoints of raw theta frequency power. Subsequently, six larger bins were placed over the 300 to obtain an average raw theta frequency estimate per step.

Figure 6. The DTW process.

After applying this method to each epoch, the last step was to calculate the ERD/S percentage in reference to the baseline of -200ms before the go cue. The following formula was used and based on research conducted by Chan et al. (2023), with Powertimepoint representing the raw theta frequency average per step, previously calculated:

ERD/S $(\%)$ = (Powertimepoint - Powerbaseline) / Powerbaseline x 100

This resulted in a dataset containing the theta ERD/S percentage per step for every participant and block. Finally, this data was merged with the behavioural dataset, completely aligning each step, to continue modelling and post-hoc analyses.

2.5.3 Statistical Modelling and Post-Hoc Analyses

After successfully cleaning, preparing and merging the behavioural data and EEG recordings, linear mixed effects regression models (LMER) were created using the lme4 package (version 1.1-35.3) with an α level set to 0.05. To examine learning over time, a model was developed with *RT* as the dependent variable and *block* (1-6) as the independent predictor variable. *Participants* (1-12) were included as a random effect to control for

individual differences. Subsequently, a repeated measures ANOVA was conducted to assess the effect of *block* on *RT*. To determine if a concatenation in *RT* occurs, the independent variable *step* (1-6) was added as an interaction with *block* in the model. Another ANOVA was conducted to examine the effect of *block*, *step* and *block*step* on RT. The models developed to analyse the behavioural data thus spell out as follows:

- Learning over time: $RT \sim Block + (1)Subject$
- Concatenation analysis: $RT \sim Block*Step + (1)Subject$

To analyse theta ERD/S it was first necessary to decide whether to use the measurements from channels C3 and C4 or Cz for further continuation. Since previous studies found that especially the midline theta power is associated with cognitive demand and changes over the course of learning (Clarke et al., 2017; Eisma et al., 2021), the data recorded at Cz was eventually chosen to proceed with. Additionally, the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC) were calculated for the models with each channel respectively. Usually, the models with the lowest AIC and BIC are selected because they indicate the best fit (Vrieze, 2012). However, Chan et al. (2023) also conducted learning curve analyses with the DS-DSP and found high variability between the learning of individuals, suggesting that theta models with the highest AIC and BIC, reflecting more unique information, might be preferable. In this study, the model with the highest AIC and BIC was Cz.

In the next step, a model was made with theta *ERD/S* as the dependent variable and *block (1-6)* as the independent variable to assess *ERD/S* throughout learning, including *participants* as a random effect factor. An ANOVA was conducted to test the effect of *block* on theta *ERD/S*. To analyse theta *ERD/S* on a step-level basis, the independent variable *step (1-6)* was added as an interaction with *block* to the model. An ANOVA was applied to test the significance of *block*, *step* and *block*step* on theta *ERD/S*. The models developed to analyse the theta frequency thus spell out as follows:

- Theta over the course of learning: $ERD/S \sim Block + (1)Subject$
- Theta on a step-level: $ERD/S \sim Block*Step + (1)Subject$

Linking back to this study's hypotheses, analysing the relative change in theta power across all blocks gives an indication of how theta power changes over the course of learning. The step-level model gives more detailed results about changes in theta power within sequence execution and the existence of memory chunks. Post-hoc tests using estimated marginal means were run on all models with the emmeans package under version 1.10.1 to contrast the predictor variables. The full R script used to clean and arrange the data can be found via a link to RPubs in Appendix C.

3 Results

3.1 Behavioural

The detailed output of all models, ANOVA and post-hoc contrasting analyses reported in this section can be viewed through the link provided in Appendix C. The residuals of the LMER model analysing the effect of block over RT were plotted using a histogram and Q-Q plot to check the distribution of RT. RT is roughly normally distributed with a slight skew to the right.

An ANOVA was conducted to examine the effect of block on RT using a LMER model, which revealed a significant effect, $\chi^2(5) = 1842.2$, $p < .001$. A plot visualising the average RT over all blocks can be seen in Figure 7. It is apparent that the average RT decreases until the $5th$ block, with a steep decrease until the $3rd$ block, followed by a slight decrease until the $5th$ block. The 6th block contained the unfamiliar sequence and depicts an increased RT compared to blocks $3 - 5$.

Figure 7. The y-axis depicts the mean sequence RT, and the x-axis depicts the block. The error bars represent an 83% confidence interval. Learning over time is indicated by a gradual reduction in RT until the $5th$ block. An increase in RT is observed in the $6th$ block where participants executed the unfamiliar sequence.

Concatenation in RT was analysed using a LMER model with block and step as fixed effects and subject as a random effect. An ANOVA revealed a significant effect of the block*step interaction, $F(25, 16932) = 2.63$, $p < .001$, indicating that the effect of step on RT

varies across blocks. Further, a significant main effect of step $F(5, 16932) = 1856.74, p < .00$, and block were found, $F(5, 16932) = 121.11$, $p < .001$. Figure 8 visualises the concatenation plot of all blocks.

A post-hoc pairwise comparison using estimated marginal means contrasting each step within the blocks revealed a significant difference of RT between step 4 and step 5 in block 3, -35.15 (SE = 13.3), $z = -2.64$, $p = .0083$, and block 4, -38.17 (SE = 13.3), $z = -2.867$, $p =$.0476. However, in those cases step 4 is on average faster than step 5. Moreover, step 3 was found to be significantly slower, 40.38 (SE = 13.3), $z = 3.039$, $p = .0286$, than step 4 in block 3. Figure 8 visualises the RT for each step across all blocks. It is clear that a classic concatenation pattern, indicated by an increase of RT in the $3rd$ or $4th$ position, cannot be observed.

Figure 8. Visualisation of RT per step across all blocks. The red rectangles highlight a significant reduction in RT at the $4th$ step in blocks 3 and 4.

3.2 Relative Theta Power

To examine changes in theta power over the course of learning, an ANOVA was conducted on a LMER modelling theta ERD/S across all blocks. This revealed a significant main effect, $F(5, 16932) = 14.014$, $p < .001$, indicating that theta ERD/S varies over the course of learning. Figure 9 shows theta ERD/S for all blocks. The relative theta power is significantly lower in block 4, 280.45 (SE = 48.8), $z = 5.74$, $p < .0001$ and block 5, 283.04 $(SE = 48)$, $z = 5.89$, $p < .0001$, compared to block 3. The unfamiliar sequence block 6 is not significantly different from block 1, 2 and 3.

Figure 9. The y-axis depicts the mean relative sequence theta power, and the x-axis depicts the block. The error bars represent \pm one SE away from the mean. Lower theta power in late stages of learning (blocks 4 and 5) compared to early stages of learning (blocks 1-3) can be observed. Furthermore, block 6 shows similar theta power as the early learning stage.

Concatenation in theta ERD/S was analysed using a LMER model with a block*step fixed effect interaction and participants as a random factor. Subsequent ANOVA revealed a significant main effect of block $F(5, 16932) = 14.2018$, $p < .001$, a significant main effect of step, $F(5, 16932) = 34.5014$, $p < .001$, and a significant interaction of block*step, $F(25, 16932)$ 16932) = 2.1225, *p* < .001.

Post-hoc contrasts revealed a significant difference of theta ERD/S between step 3 and step 6, 562.78 (SE = 115), $z = 4.876$, $p < .0001$, step 4 and step 6, 609.85, (SE = 115), $z =$ 5.284, *p* < .0001, in block 2. This significant difference remains in block 3 while additionally step 3 and step 5, 446.20 (SE = 117), $z = 3.811$, $p = .0019$, step 4 and step 5, 381.12 (SE = 117), $z = 3.256$, $p = .0144$, show a significant difference in block 3. No steps show a significant difference of theta ERD/S in blocks 4 and 5, while block 6 shows the same stepcontrasts as block 2. Figure 10 shows the changes in relative theta power per block on a steplevel.

Figure 10. Relative theta power changes on step-level. The red rectangles indicate a significant increase in theta power at step 3 and 4 in blocks 2, 3 and 6.

4 Discussion

The primary aim of this study was to examine the role of theta oscillations during the motor execution phase of the DS-DSP to shed further light on whole-body MSL and the mechanisms of motor memory encoding compared to finger-specific MSL. The predictions for the current study were outlined as follows: (1) No concatenation in RT around the $3rd$ or $4th$ step was expected. (2) No relative theta power increase on a step-level was expected because this is usually accompanied by a concatenation in RT. (3) It was anticipated to find a decrease in theta power in the late stages of learning compared to the early stages. Lastly, (4) an increase in theta power was expected to occur in the last block because participants were learning the unfamiliar sequence.

As anticipated, the current study (1) did not observe a concatenation point in RT around the $3rd$ or $4th$ step position, aligning with the findings of Chan et al. (2022). On the contrary, a reverse concatenation point emerged, marked by the fastest RT observed at the $4th$ step in blocks 3 and 4. This effect is explained in more detail in section 4.1. Against expectations, (2) a significant increase in theta power relative to the $3rd$ and $4th$ step in blocks 2, 3 and 6 was observed. The increase in theta power relative to the 4th step falls in line with the findings of Chan et al. (2023) and Crivelli-Decker et al. (2018). However, the theta increase in the current study occurs during early learning stages (blocks 2, 3) and during the execution of the unfamiliar sequence (block 6), which is arguably representable of early learning (Verwey et al., 2019). Additionally, (3) a decrease in relative theta power over the

course of learning was found, indicated by a significantly lower theta power in blocks 4 and 5 compared to block 3, thus in line with the findings of multiple previous studies (Clarke et al., 2017; Lum et al., 2023; Crivelli-Decker et al., 2018). Lastly, (4) as expected, an increase in theta power was observed in the unfamiliar sequence block (6).

4.1 Learning over Time and Step-Level based on Response Time

The behavioural results show that participants' RT progressively decreased until the 5th block. This is in line with results found by other sequence learning studies, such as the implicit learning experiment conducted by Lum et al. (2023) and the DS-DSP task conducted by Chan et al. (2022). An increase in RT was observed in the $6th$ block when participants were executing the unfamiliar sequence. The steepest decrease in RT occurred between the 1st and the 2nd block, suggesting that most of the learning and familiarisation with the experimental setup, i.e. adjusting to the responsiveness of the dance mat, occurred early in the task.

Regarding step-level performance, the current study did not find the classic RT concatenation point around the $3rd$ or $4th$ position of the sequence and is thus in line with the results found by Chan et al. (2022) in their DS-DSP experiment. On the contrary, the current study observed that the $4th$ step was significantly faster than the $3rd$ and $5th$ step in blocks 3 and 4. The occurring effect can thus be termed as a reverse concatenation point. It is possible that participants were speeding through the sequence execution before slowing down for the last two steps. A reverse concatenation point is not described by Abrahamse et al. (2013) or Verwey et al. (2015) and the absence of an increase in RT would point to the lack of loading a memory chunk according to the C-SMB. However, the very fast response in the 4th step could be an indication that participants develop a rhythm when executing the sequence. As Hirokane et al. (2023) argue, they found a change in rhythm to be symbolic of the start of a different chunk. It is therefore possible that participants developed one rhythm for executing the first four steps and then execute the remaining two steps in its own rhythm to optimise their performance. Hence, the behavioural findings show that whole-body sequential learning involves different or adjusted motor mechanisms compared to finger-specific tasks. A closer look at the relative changes in theta power is needed to further comprehend the role of memory chunks involved in whole-body sequence execution. First, the relative changes in theta power in early and late learning stages are described.

4.2 Relative Theta Power of Learning Over Time

The current study found lower relative theta power in the late stages of learning in comparison to the early stages of learning. This is indicated by a significant reduction of theta ERD/S in blocks 4 and 5 compared to block 3. These results are in line with previous studies

(Clarke et al., 2017; Crivelli-Decker et al., 2018; Lum et al., 2023). As increased theta band activity is associated with the encoding of new information and working memory load (Axmacher et al., 2010; Klimesch et al., 1996, as cited in Klimesch, 1999), it can be deducted that participants invested higher cognitive efforts until block 3 to retrieve the relevant sequence information and execute the movements. Blocks 4 and 5 showed a significant decrease in theta power compared to block 3, signalling a reduction of working memory load as the sequences are practised extensively. Moreover, increased theta power was found in block 6 when participants were executing the unfamiliar sequence, which is in line with the results of Lum et al. (2023). Their study found increased theta power in the last block containing a random sequence. This finding indicates that enhanced cognitive efforts were needed to learn a new set of movements and supports the notion that theta oscillations are involved in encoding new information.

4.3 Relative Theta Power on Step-Level

Despite the absence of a concatenation in RT, the current study found support for the development of memory chunks based on the increase of theta power relative to the $3rd$ and $4th$ step in blocks 2, 3 and 6. The increase relative to the $4th$ step aligns with findings from Chan et al. (2023) and Crivelli-Decker et al. (2018), although the current study found the spike in theta power in the early and not the late stages of learning. Referring back to the C-SMB 2.0, Verwey (2024) argues that the separation of sequence elements can already occur after 20 repetitions and generally develops into fully formed memory chunks and central-symbolic response representations after approximately 100 repetitions. Considering that increased theta power indicates high working memory load (Klimesch et al., 1996, as cited in Klimesch, 1999), it is likely that the spike in theta power mid-sequence in block 2, 3 and 6 facilitates the memory encoding process through the loading of a memory chunk. Keeping in mind that one block is comprised of 42 sequence trials, seeing memory chunks form within the second block, marking 84 executed trials in total, is reasonable.

According to the C-SMB 2.0, initial execution occurs in the reaction mode, wherein participants execute the movement step-by-step through loading each sequence execution element individually. During this process central-symbolic representations of the sequence and response are stored in short-term memory. After repeated practise those representations become easily available in the chunking mode by loading merely the first sequence element. Looking at the theta power spike in block 2 and 3 it becomes apparent that participants have entered the chunking mode and load a memory chunk around the $3rd$ or $4th$ step position. While this process shows the development of automaticity in movement execution (decrease

in RT), it does not mean that cognitive efforts are reduced immediately, as suggested by Verwey (2024) and Abrahamse et al. (2013). As theta power remains relatively high in blocks 2 and 3 compared to late stages of learning, the central processor is still majorly involved in loading and activating the memory chunk. However, the C-SMB 2.0 also suggests that eventually memory chunks can be stored in long-term memory. The general decrease of theta power in blocks 4 and 5 and lack of power spikes could indicate that this is indeed the case. From this point on, movement execution occurs through triggering the first sequence element from long-term memory, allowing the rest of the sequence information to be activated in short-term memory within one processing step (Verwey et al., 2015). This allows for the least amount of effort required by the central processor while the motor processor can take over sequence execution.

While the general cognitive processes outlined in the C-SMB 2.0 correspond to the pattern of theta power found in the current study, the spikes in theta power relative to the 4th position clearly occurred in the early stages of learning. This stands in contrast to Crivelli-Decker et al. (2018) , who found an increase in theta power relative to the 4th position in their object recollection task in the late stages of learning. Furthermore, the current findings stand opposed to the KP-DSP study conducted by Chan et al. (2023), who found a spike in theta power around the 4th keypress from block 2 to block 5, meaning in the early and late stages of learning. This indicates that whole-body MSL unfolds differently from finger-specific or visual sequence learning.

The increase of theta power at the $4th$ position in late stages of learning found by Crivelli-Decker et al. (2018) implies that the formation of memory chunks takes longer in visual sequence learning tasks, perhaps because the central processor has to build centralsymbolic representations of various complex objects such as a truck, elephant or avocado. This can arguably take more time and repetitions than building memory chunks holding sequence execution information based on the yellow rectangle stimuli indicating spatial mapping of effectors in the DS-DSP. Looking at the KP-DSP study conducted by Chan et al. (2023), the theta power increase at the $4th$ keypress already occurred in block 2, which corroborates with the current study, however it could be observed until block 5. This suggests that keypress based MSL also facilitates the formation of memory chunks, but the cognitive load of the central processor loading the memory chunk does not reduce with practise the same way that it does in whole-body MSL. The remaining theta power spike in block 5 of the KP-DSP study might demonstrate that the memory chunk is still stored and activated by the central processor in short-term memory and not yet in long-term memory. Consequently, the

motor processor has not yet taken off a substantial amount of cognitive load from the central processor. The following sections dive into mechanisms which potentially offer an explanation why whole-body sequential learning shows a different pattern in RT and theta power than visual or finger-specific learning.

4.4 Motor Mechanism Difference Between the KP-DSP and DS-DSP

As outlined in section 4.1, one possible explanation for the lack of a concatenation in RT could come from the rhythm chunking hypothesis posed by Hirokane et al. (2023). Our brain appears to consolidate the memory chunk for further sequence execution while the body is able to bypass the slowing in RT, caused by a rhythmic control of limb coordination. While Verwey and Dronkert (1996) have previously disregarded a rhythmic development in sequential execution, they utilised a keyboard-based task to examine its existence. In the current DS-DSP study, step execution got progressively faster until the 4th step, followed by a slower execution of steps five and six. While this study did not explicitly define a rhythm, it is clear that the first four steps were executed in a progressively faster pace and the last two steps were executed in a slower pace, hence developing a pattern. The change in both paces marks a switch in rhythm, which Hirokane et al. (2023) associates with the start of a new chunk.

The absence of a concatenation point in RT could further be facilitated through the major activation of the vestibular system occurring in whole-body movement compared to performing a sedentary sequence learning task. A previous visual recall study found that the stimulation of the vestibular system leads to decreased response times in the recollection phase (Wilkinson et al., 2008), hence the activation of the vestibular system could explain the fast RT times in the DS-DSP. Moreover, Jacob et al. (2014) outline that the deactivation of the vestibular system causes a decrease in the theta frequency power. This implies that an activation of the vestibular system could enhance the theta frequency power, thus improving memory encoding process and facilitating faster RT.

This study hypothesised that the lack of a theta power spike in the late stages of learning could indicate that the memory chunk is already stored in long-term memory and that this process did not yet occur in the sequence learning studies conducted by Crivelli-Decker et al. (2018) and Chan et al. (2023). Birdsell (2023) carried out a vocabulary learning task during which one half of the participants learned words whilst sitting in a chair and the other half learned words while walking on a treadmill. The results showed no significant effect of exercise on short-term memory, however a mild to moderate positive effect of walking was found on the retention of the words in long-term memory. This suggests that because wholebody MSL involves a lot of movement, it facilitates better storage of information in long-term memory than keypress MSL, which is done in a sedentary position. Thus, explaining why the spike in theta power was not present in the late stages of learning in the current study.

Another viewpoint outlined in the introduction is that the use of multiple effectors increases top-down processing to guide the selection of response action. Madlon-Kay et al. (2013) found that bimanual tasks facilitate higher neural connectivity between the PFC and the medial motor cortex compared to unilateral tasks, which were only associated with the activation of effector-specific areas. While the DS-DSP involves the use two feet, the increased theta power found over the first three blocks indicates more bottom-up processing than top-down. Hence, it is likely not the case that the bimanual aspect of the DS-DSP facilitated a faster response action based on top-down processing.

4.5 Limitations and Future Recommendations

It should be noted that this study served as a pilot to analyse the DS-DSP with a mobile EEG system, hence any limitations can be viewed as an opportunity to improve the experimental paradigm.

Participants were asked to fill out a post-questionnaire where they could indicate remarks about the experiment. Four out of 12 participants indicated that the dance mat was not registering some of their footsteps. Participants indicated that this was occasionally frustrating. Two participants further indicated that their feet were too large for the size of the dance mat and that this factor led to errors at times. The foot size was recorded for the purpose of a whole-body motion capture, which was a separate aspect of this research. An analysis of the relationship between foot size, body hight and RT or accuracy could clarify whether foot size significantly influences performance.

Given the association of the PFC with memory encoding and various studies pointing to the notion that early learning facilitates the activation in the PFC while later learning facilitates more activation over M1 (Gonzalez & Burke, 2018; Hikosake et al., 2002), a future study could additionally analyse theta power over the FCz electrode since this position captures the prefrontal activity. A comparison between theta activity over M1 and FCz could potentially explore even more intricacies within the cognitive mechanisms underlying wholebody MSL.

Another potential for future DS-DSP studies lies in the exploration of introducing longer sequences. As Abrahamse et al. (2013) note, practice can lead to the formation of longer chunks and the development of longer rhythms (Hirokane et al., 2023). It would be interesting to examine whether learning longer sequences would eventually show an RT

concatenation point and if so, examine whether it is accompanied by an increase in theta power.

5 Conclusion

The aim of this study was to examine whole-body MSL with the DS-DSP and specifically investigate changes in relative theta power during the movement execution phase to disentangle underlying motor memory encoding processes. As a result, this study found that whole-body MSL shows increased theta power relative to the $3rd$ and $4th$ steps during early learning stages, despite the absence of a concatenation point in RT. Due to the association of increased theta power with high cognitive demand and working memory load, this study suggests that memory chunks are consolidated in bodily movement, although not observed through the slowing in RT. The study further suggests that the storing of memory chunks in long-term memory occurs faster in whole-body MSL than in visual or fingerspecific sequence learning. This is indicated by the lack of a spike in theta power in the late stages of learning, suggesting that the motor processor has taken over most of the cognitive load from the central processor. Furthermore, a general decrease in theta power over the course of learning was observed, adding support to similar research findings that associate high theta power with the encoding of new information and increased working memory load. The lack of an increase in RT in the DS-DSP could be explained by the development of a rhythm while executing the sequence. It was observed that participants RT was progressively faster until the $4th$ step, after which the remaining two steps were executed slower.

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7 Appendix A

Supervisor Contact details:

Victoria Lakomski Behavioral, Management & Social Sciences Email: xxxx

Dr. Russell Chan (Ph.D) Behavioral, Management & Social Sciences Email: xxxx

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:

- o You are healthy and aged between 18 and 40 years.
- o 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.
- o You are not physically injured and are able-bodied.
- o You have not had a falling incident or heart problems within the last year.
- o 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)
- o You have not previously taken part in any motor learning experiments involving the dance-step sequence learning tasks in the BMS or via SONA.
- o 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
- o You do not mind having motion capture sensors attached to your legs, feet, and pelvis.
- o You are not feeling unwell in general.
- o no previous professional training with dance, musical instruments/typing and/or gaming
- o no indications of depression or anxiety
- o no indications of severe sleep problems requiring medication
- o no drug or alcohol or tobacco addictions
- o no obvious physical injuries or impairments that will affect performance on dancestep
- o must have a normal amount of mobility and physical-activity level (as assessed by IPAQ).
- o Interested participants will be screened for eligibility by a researcher via phone prior

to participation once more.

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Requirements:

Participation in the study involves attending a laboratory session ONCE 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:

8 Appendix B

Sequences and Counterbalancing Procedure

9 Appendix C

R script containing:

- Behavioural data cleaning
- EEG data preparation
- Dynamic Time Warping method
- ERD/S Calculation
- Statistical Models
- Post-Hoc

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