EEG correlates of motor skill learning: an independent component approach

Yvonne Greeuw[1,2*]

Abstract
Motor learning is a vital ability of the human brain in which multiple cortical areas like the primary motor cortex are involved. One way to investigate the fast motor learning brain dynamics is with use of electroencephalography (EEG). This non-invasive and mobile technique records continuous electrical activity on the brain cortex with a high temporal resolution. Although there has been considerable research into motor learning in humans, the mechanisms behind movement acquisition and execution are still largely unknown. A better understanding of motor learning is relevant to treatment and training in neurorehabilitation and sports medicine. The aim of this study was to provide insight into the neurophysiological mechanisms behind motor skill learning compared to motor execution. Motor *skill* learning is defined as the acquisition of a complex movement sequence by improving accuracy without comprising on speed. To investigate the neurophysiological mechanisms behind motor skill learning, 128-channel EEG was recorded in 20 young, right-handed, healthy participants while performing a motor skill learning task and a motor execution task. With use of adaptive mixture independent component analysis (AMICA), time-frequency analysis and equivalent dipole fitting source localisation, functional neurophysiological correlates of motor skill learning were investigated. Due to an excessive amount of electrical bridged electrodes, 12/20 participants were excluded from further analyses. In the 8 remaining participants, a cluster of independent components of electrical activity was located on the left primary motor cortex. In the motor learning task, lower β-frequency power was found in these components compared to the control task. This suggest that motor execution could be distinguished from motor learning on EEG by means of β activity in the left primary motor cortex. The results of this study can be used for future research on motor skill recovery in rehabilitation, motor learning in sports medicine and research on interventions to enhance motor learning, like non-invasive electrical brain stimulation.

Keywords
Motor skill learning — EEG — Independent component analysis (ICA)

[1] *Department of Rehabilitation, Erasmus Medical Center, Rotterdam, the Netherlands*
[2] *Department of Neuromechanics and Motor Control, Delft University of Technology, Delft, the Netherlands*
*__Corresponding author__: Student number: 4446534

Contents

Introduction

Motor learning is relevant to everyone, from young to old. Whether you play tennis, brush your teeth or work on the computer, you learn or improve motor skills. In the literature, the term motor learning is used for a variety of learning paradigms involving movement. Examples are: learning to improve reaction time, learning a finger tapping arrangement, or adjusting movements to external perturbations [1]. The learned information about movement planning and execution is stored in our brain [2].

Rehabilitation physicians and sports doctors continuously aim for finding the best ways of restoring or improving motor skills by using motor learning strategies. A big challenge is posed by the individual differences in motor learning capacity. Diversity in brain function and structure could explain some of the individual differences in motor learning [3]. However, most of the variance in motor learning between subjects is still poorly understood, which limits current efforts to improve motor learning. Interventions aiming on enhancement of motor learning, like non-invasive electrical stimulation of the brain, are popular research subjects nowadays [4], [5],[6]. The effects of electrical stimulation are inconsistent and occasionally debated [7],[8]. It is important to fully understand

the working mechanisms behind motor learning in the brain to investigate if and how these interventions could work [9].

One way to investigate the brain during motor learning is with use of electroencephalography (EEG) [10],[11],[12]. This non-invasive, mobile and relatively affordable technique records continuous electrical activity on the brain cortex with a high temporal resolution, which is beneficial in case of very fast cortical motor events [13]. Cortical structures, of which activity can be measured on EEG, play a major role in motor *skill* learning specifically [14]. In the literature, motor skill learning is defined as the acquisition of a complex movement sequence by reducing movement variability and improving accuracy without comprising on speed [15],[14]. In this study, motor skill learning is defined as an increase in accuracy during a serial isometric pinch task.

In EEG research, the primary motor cortex, premotor cortex and supplementary motor areas have found to be involved in motor learning [16],[17]. Studies on this topic report modulations in α and β-activity (7.5-15 and 15-30 Hz, respectively) before and during motor events [18],[19],[20],[21],[22]. The timing, source and direction of these EEG modulations are inconsistent in these studies. These inconsistencies confirm that the neurophysiology behind motor learning and performance is still not completely understood [23].

From an earlier conducted literature review, the phenomena most often and consistently linked to motor learning were alterations in event-related β-desynchronisation [10],[19], [24],[12],[25]. Desynchronisation describes the decrease of power in given frequency bands [26]. However, it is unclear if this activity can be related to motor learning, motor execution or motor repetition.

The purpose of the present study was to distinguish neurophysiological correlates of motor skill learning from motor execution on EEG. To test for motor execution, a control task was designed. It was hypothesised that accuracy increase would be bigger in the motor learning task than in the control task. During both tasks, 128-channel EEG was acquired. To be able to draw physiologically plausible conclusions, independent components of electrical activity were studied [27],[28]. It was investigated whether independent components in the primary motor cortex, premotor cortex and supplementary motor areas could be found consistently. Our hypothesis was that event-related β-desynchronisation for these independent components is stronger during the motor learning task, compared to the control task.

Methods

Participants

Twenty healthy, right-handed participants, aged between 18-35 years were recruited and gave written informed consent. Exclusion criteria were: known neurological/psychological disorders, pregnancy, metal parts/implants in the brain, use of neuromodulatory medication or drugs or received brain stimulation in the last month. All procedures were approved by the Ethics Committee of the Erasmus Medical Center in Rotterdam.

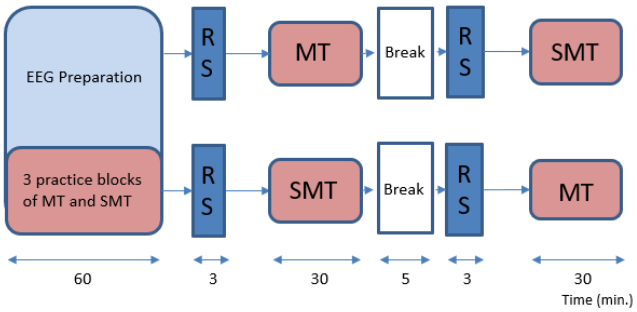

Figure 1. Study design. All participants started with EEG preparation and simultaneously practised each of the two tasks. Participants were randomly assigned to the first or second row and started with the motor learning task or control task, respectively. RS = resting-state EEG, MT = motor learning task and SMT = simple motor task (control task)

Study design

The experiment took place in one session where participants sat in a chair facing a computer screen on a table. In Figure 1 the study design is schematically presented. Participants started with the motor learning task (MT) or control task (SMT). The order of the two tasks was randomised among the participants to be able to correct for possible transfer and fatigue effects. During preparation of the 128- electrodes EEG cap, the participants performed 3 practice blocks of each of the two tasks to make sure the tasks were performed in a right manner. Before every motor task, 3 minutes of eyes-open, resting-state (RS) EEG was acquired. Then, the control task or motor learning task was performed. Another 3 minutes of eyes-open, resting-state EEG was acquired and the other motor task was performed consequently. In between the two tasks, the participants had a short break and were offered something to eat and drink while the EEG cap remained on the head. In Appendix B, a checklist of the complete protocol can be found.

Motor tasks

To test for motor skill learning a serial, visually guided isometric pinch task (SVIPT) was used. This task was used in other studies as a motor skill learning task due to the slow, approximately linear learning behaviour participants show for a long period of practice [6], [29]. To be able to exclude the effects of motor execution, a similar task without indicated sequence was designed. In Figure 2, the SVIPT (right) and control task (left) are depicted. During both tasks, participants move the black rectangle cursor on a screen to the right by pinching a force transducer. The pinching was done five times, in a specific sequence in case of the motor learning task, at time points with 750 ms interval. For the motor learning task, the force/displacement relation was logarithmic and can be described by the following formula:

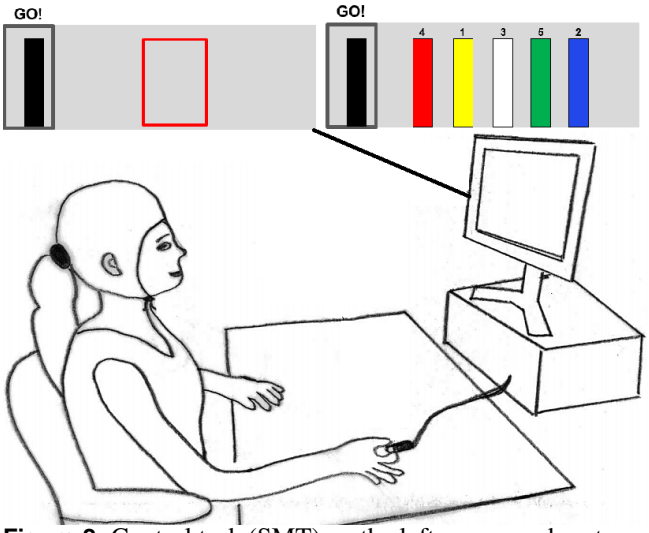

Figure 2. Control task (SMT) on the left screen and motor learning task (MT) on the right. The participant moved the black rectangle cursor to the coloured targets in the above indicated sequence by pinching the force transducer five times with the right thumb and index finger. The harder the participant pinched, the more the cursor moved to the right. In the control task (left), the red target had to be reached five times in one trial, so there was no sequence. Applied force and cursor location data was collected and EEG was recorded during both tasks.

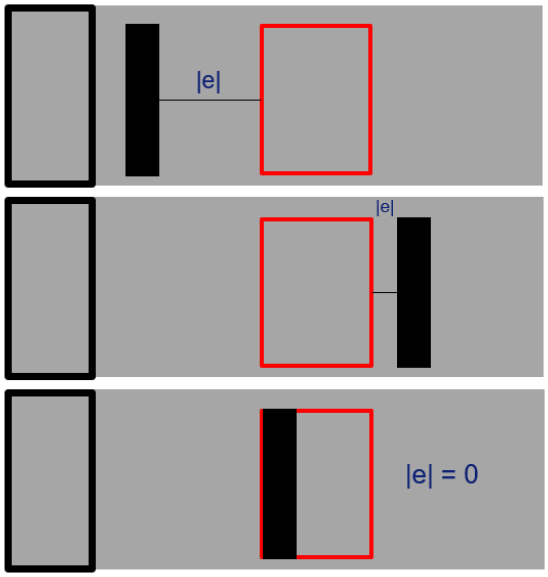

Figure 3. The absolute error in pixels ($|e|$) between the cursor (black rectangle) and the closest border of the target block (red rectangle) was considered as a measure of accuracy. If the midpoint of the cursor was inside the target, the absolute error was set at 0.

$y = 260.577log(25x + 0.215443) - 400$, where y is the position in pixels and x is the applied force as a percentage of the participant's measured max force. The farthest target was set 45% of the participant's maximum pinch force. For the control task, the force/displacement relation was linear, as described by the following formula: $y = 191.1680x$. The average force required in the control task was the same as the average force required for the motor learning task, to equalise the effort for both tasks. A new feature to the tasks was auditory guidance, the participant knew when to press the force transducer by listening to metronome ticks with a 750 ms interval (100 bpm). The start of the trial was indicated with three metronome ticks and a visual 'GO!' signal above the cursor at the third tick. This made the behaviour of all participants during all blocks standardised in terms of speed. One trial consisted of five times pinching. Participants performed 20 blocks of 10 trials for both tasks. A pinch was judged as correct, if the cursor block was inside of the target block in a time window of -100 to 100 ms around the metronome tick. An extra margin of 25 pixels (0.5 of the cursor width) was given on both sides of the target, so if the cursor was half way in the target block, the pinch was judged as correct. After each block, participants received visual feedback on the percentage of correct pinches per target block. The control task was designed to be considerably easier. The participants did not need to follow a sequence and the target block was bigger in this condition. After three practice blocks, participants showed a nearly constant value of the percentage of correct trials in the control task. The instructions that every participant read before the experiment, can be found in Appendix B.

Behavioural data analysis

Motor skill learning can be assessed as a practice-induced change in accuracy without compromising on speed [15],[14]. In our tasks, the speed of movement was indicated by a metronome, so this parameter was approximately constant for both conditions. The primary learning parameter was therefore defined as an increase in accuracy of movement. Accuracy[1] is a qualitative term to describe whether there is an agreement between a measurement and it's target value and can be viewed as the proximity of a measurement to a true value [31], [30]. To assess accuracy in our study, the absolute errors between the target blocks and peak cursor location points around the metronome ticks were calculated. A window of -100 ms to 100 ms around the metronome ticks was evaluated. From the cursor location points in that window, the maximum value was taken as peak cursor location of the participants. This cursor location point is also taken into account during the motor task evaluation, so the participants receive feedback on the same measure. The accuracy definition followed in this study is depicted in Figure 3. If the peak location point was located inside of the target block, the absolute error was set at 0. If the peak location point was located outside of the target blocks, the error was defined as the absolute dis-

[1] Accuracy is often confused with precision, which is the repeatability of a measurement [30]. Precision does not describe the proximity to a true or target value.

tance between this peak location and the nearest border of the target block. The absolute error was averaged over all targets, and over 10 trials per block. The more accurate people behave, the lower the absolute error. The motor learning parameter was transformed into a learning accuracy index, which was calculated as the difference in accuracy between the first 50 and the last 50 trials.

EEG

Data acquisition

Data was acquired with a 128-electrode EEG cap and a TMSi Refa amplifier system with a sampling frequency of 2048 Hz. The electrodes were localised following the international 10-20 system [32]. Peri-ocular EEG and electromyography (EMG) of the right first dorsal interosseous muscle were recorded and also amplified with the TMSi Refa system. The EMG electrodes were referenced in a bipolar configuration. The EEG electrodes and peri-ocular EEG were referenced to a common reference. All data was imported and analysed using Matlab R2016b with Signal Processing Toolbox [33], the EEGlab Toolbox [34] and the Fieldtrip Toolbox [35].

Data preprocessing

First, low-frequent drift was removed with a zero-phase high-pass FIR filter (Hamming window) with cutoff frequency (-6dB) at 0.5 Hz. To remove line noise, notch filters were used with cutoff frequencies at [49 51], [99 101], and [149 151] Hz. Noisy, high frequencies were removed with a low-pass filter with a cutoff frequency at 225 Hz. The filter order was estimated in the default mode of the *eegfiltnew* function of EEGLAB. The data was resampled to 1024 Hz because of working memory limitations. Visually detected bad and flat channels were excluded. The presence of connections between electrodes due to leakage of electrolyte (bridges) was checked with use of the *eBridge* function [36]. Data sets with more than 40 bridged electrodes or more than 25 bridge pairs were excluded for further analysis. More information about bridges can be found in Appendix A. Then, the resting state-, motor task and control task data were merged into one file and saved with the 1024-Hz sampling frequency for future use.

The moment of movement onset ('GO!' signal) is saved as an event in the EEG data. Non-overlapping epochs of -2 to 5 seconds were created around these events. Five pinches last for 3750 ms, so a period before and after the trial was included in these epochs. Bad epochs were rejected automatically using the *autorej* function in EEGlab which detects extremely large potential fluctuations with a probability threshold of 6 standard deviations and ensures a maximum of 5% epoch rejection [34]. After this, the data was referenced to the common average of all channels, with exception of the EMG channel. The merged files were resampled to 256 Hz for faster independent component analysis (ICA). Reason for using ICA is that EEG electrodes record a mixture of electrical and artefactual activity. A source separation tool like ICA disentangles these signal mixtures. It does so by organising all the EEG signals into components with similar information. Hence, it reduces the mutual information in the total data set. The AMICA algorithm, which uses an adaptive mixture of ICA's, was applied as a source separation tool in this study [37]. This algorithm produces the largest mutual information reduction and the most near-dipolar components compared to other source separation algorithms [38]. In EEGLAB, AMICA was called with the *amica15* function. The output independent components were ordered in decreasing order of EEG variance accounted for. Therefore, only the first 60 components were included for further analysis. A more comprehensive description of the EEG pre-processing and the used m.files can be found Appendix A and C.

Data analysis

The AMICA sphere and weights matrix were projected back onto the original 1024 Hz merged data sets including motor task and control task measurements. These data sets were divided into epochs of -2 to 5 seconds around the task events. Bad epochs were rejected using the *autorej* function. After that, the data is visually inspected to remove possible remaining bad epochs. Sources of independent component electrical activity were estimated with use of a spherical four-shell head model as volume conductor [39]. These sources of electrical activity can be described by single equivalent dipoles [38]. Equivalent dipoles were fitted using the DIPFIT2.x plugin of EEGLab [34]. When the activity of a component's source was projected back to the scalp, the mismatch between the scalp projection and the model pole projection is named the residual variance. Components described by an equivalent dipole with a residual variance $< 15\%$ were included for further research [40]. Components belonging to out-of-brain dipoles were excluded.

All included independent components of all participants were clustered into 12 clusters based on their fitted dipole location with the k-means clustering algorithm. The number of clusters was chosen so that every participant would contribute at least one component to every cluster. Equivalent dipole location outliers (3 SD) were not included in the clusters. In our right-handed task, only left-hemisphere equivalent dipole clusters were of interest. After evaluation of these clusters, the ones with a centroid located around left the primary motor cortex, supplementary motor cortex and premotor cortex were analysed further. Only one component per subject, with the closest Montreal Neurological Institute (MNI) coordinates to one of the motor areas, was included in order to perform fair statistics.

Event-related desynchronisations (more generally, event-related spectral perturbations (ERSP)) were calculated for the remaining clustered components and compared between the two different conditions within participants[26]. The *newtimef* function in EEGlab is used to calculate ERSP with use of a Morlet Wavelet time-frequency decomposition in the frequency range of 2-45 Hz. The Morlet Wavelet transform operates by computing the similarity over time between the input signal and Gaussian-windowed complex sinusoidals [41]. This similarity is computed with a convolution in the time

domain, or more efficiently, multiplication in the frequency domain, like done in our study. The number of cycles of the used sinusoidal is a parameter that determines the time-frequency resolution of a Morlet Wavelet transform. The more cycles, the higher the frequency resolution but the lower the time resolution. The number of cycles of the used Morlet Wavelets started at 3 and increased to 13.5 for the highest frequencies. A shortening factor of 0.8 was added, which determines linear shortening of the time window used for increasing frequencies. In a pure Fourier transform this factor would be 0, so the time window stays the same for every frequency. This would result in many cycles for the higher frequencies and therefore a lower time resolution. In the case of a factor 1, a pure wavelet would be implemented, but here the frequency resolution at higher frequencies would become less. A factor of 0.8 is an intermediate option decided upon to retain a good time-frequency resolution. For the comparison of the two conditions, motor learning task (MT) and control task (SMT), the data was divided by a common baseline. As the baseline period, a period of 2000 ms to 1000 ms before the event (t=0), where EEG activity was approximately similar in both conditions, was taken. This research was preregistered at the Open Science Framework and can be found at `https://osf.io/ufsyd/`.

Statistical analysis

The difference in accuracy index between conditions was tested with a non-parametric Wilcoxon signed rank test. The difference in β-ERSP between conditions was tested at every single time/frequency point with a paired t-test. The significant p-value threshold was set at 0.05. The used m.files can be found in Appendix C.

Results

Due to the excessive amount of bridged electrodes, 8/20 participants could be included for further analysis. Characteristics of all included participants are shown in Table 1.

Characteristic	Included for final analysis
M/F [n]	3/5
Age [years], mean (SD)	24.1 (±3.0)

Table 1. Participant characteristics *(n=8)*. M = Male, F = Female, SD = Standard Deviation.

Behavioural results

The development of the absolute error averaged over the included participants per block is shown in Figure 4. Individual differences were indicated with error bars, representing the spread (SD) between participants. Especially during the first block, the individual differences were big for the motor learning condition. It can be observed that the absolute error decreased in the motor learning condition, while for the control condition, the absolute error remained approximately constant.

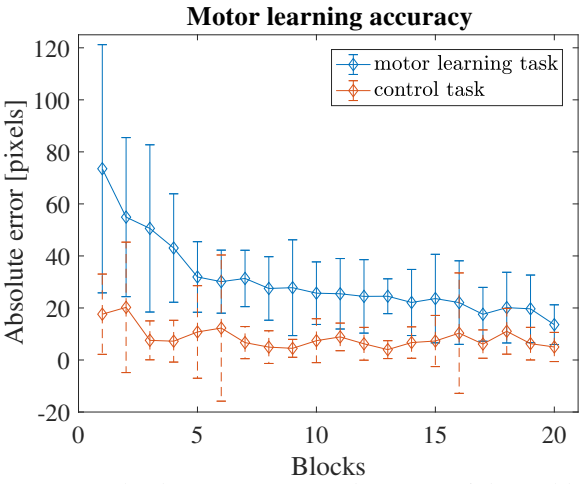

Figure 4. Absolute error averaged over 10 trials per block and over 8 included participants. The bars indicate the standard deviation between participants in a block.

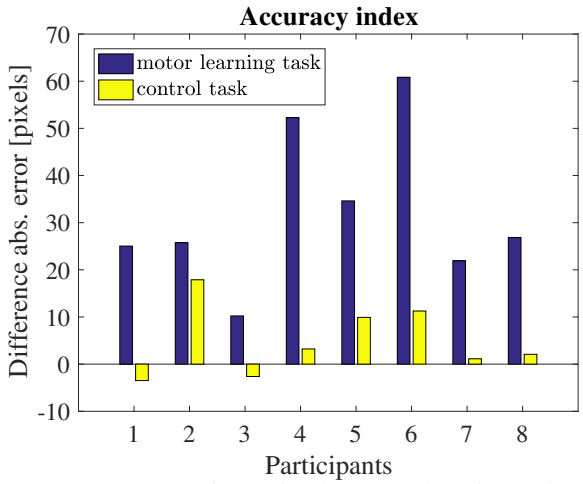

Figure 5. Accuracy index for the motor learning task and control task per participant. The bars indicate the difference in mean absolute error [pixels] between the first and last five blocks.

The graph with behavioural data of all 20 participants can be found in Appendix A and showed a similar trend. In Figure 5 the accuracy indices for both conditions are shown for the included participants. The accuracy index represents the mean absolute error decrease from the first five blocks to the last five. All included participants had a higher accuracy index for the motor learning task than for the control task.

EEG results

After AMICA source separation and removal of components with equivalent dipoles that where outside of the head or with <15% residual variance, 192 independent components remained. These components of all participants together were k-means clustered into 12 clusters based on dipole location. The scalp maps of averaged electrical activity of the clustered components is shown in Figure 6. Five clus-

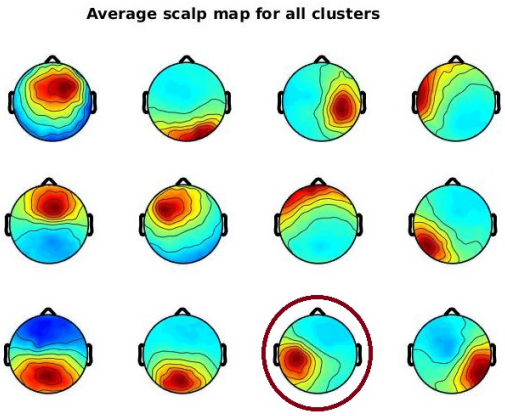

Figure 6. Average scalp map of electrical activity for all 12 clustered components (top view). The colours represent scalp potential differences proportional to μV. The colour scale is from blue to red (low to high potential). The encircled scalp map represents the cluster located on the left primary motor cortex.

ters were located on the left hemisphere as shown in Figure 7 on the left. In the right image, the blue cluster with a centroid (red dot) at MNI = [-43 -18 45], Talairach = [-42 -16 42] (`https://bioimagesuiteweb.github.io/webapp/mni2tal.html`) is depicted. This corresponds to the left cerebrum, frontal lobe, primary motor cortex, Brodmann area 4. The blue cluster encompassed 8 equivalent dipoles, one for each included participant. No clusters located on other motor areas were found.

In Figure 8 the ERSPs averaged over the cluster on the primary motor cortex are plotted . In the upper figure the motor learning task is depicted with corresponding mean location trace in pixels underneath. The middle figure represents the control task, with corresponding location trace. In the lower figure, the areas where the difference between the conditions is statistically significant ($p<0.05$) are shown. Other non-significantly different areas are coloured green in this figure. The zero time point indicates the trial onset, at the 'GO!' signal. Frequencies from 2 to 45 Hz are plotted on the y-axis and the total trial duration (3750 ms) including a period of 1000 ms pre-trial is shown. The location trace shows pinch activity with 750-ms intervals around the five metronome ticks per trial, starting at 750 ms. For both conditions, an α- and β power (7.5-15 and 15-30 Hz, respectively) suppression relative to baseline occurs just around and after the trial onset. During the pinches, which can be observed in the location traces, the β-power decreased in the motor learning task and control task. A decrease in α-power was found constantly during the total trial in both conditions.

Statistical results

The difference in accuracy index between the control task and motor learning task was significant on group level ($p = 0.0078$). Before trial onset and around 1000 and 1800 ms after trial onset, a statistically significant difference ($p < 0.05$) in low-frequent EEG δ power (2-3 Hz) was detected. Also, just after 1000 ms, a burst of significant θ power (4-7.5 Hz) was observed. Furthermore, from 1000 ms till 3900 ms after trial onset, statistically significant differences in power in the β- and lower γ (30-100 Hz)-frequencies were found between the two tasks.

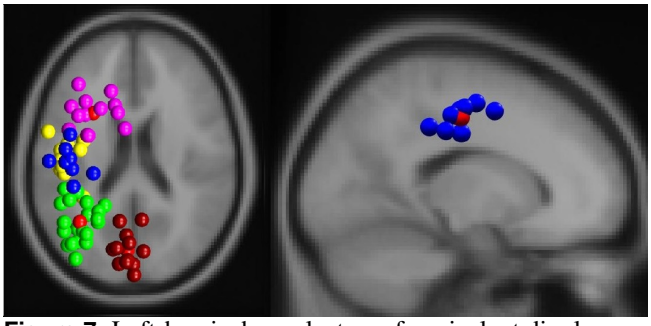

Figure 7. Left-hemisphere clusters of equivalent dipoles, obtained by the k-means clustering algorithm are depicted on the left (transversal view). Each cluster has its own colour, centroids are coloured red. The cluster with its centroid located at the primary motor cortex is shown on the right. One dipole represents one clustered component per participant (sagittal view).

Discussion & Conclusion

The present study investigated motor skill learning related changes in brain activity for a motor learning task compared to a control task. To test this, EEG data and behavioural data were collected during a visually- and auditory-guided sequential isometric pinch task and a control condition. In order to connect the behavioural motor learning principles to EEG, we expected to find independent components of electrical activity on the primary motor cortex, supplementary motor cortex and premotor cortex by means of ICA. Our study revealed differences in oscillatory β activity on the left primary motor cortex between motor learning and motor execution.

On behavioural level participants increased their accuracy more in the motor skill learning task than in the control task. This supports the hypothesis that motor skill learning, defined as an accuracy increase, is bigger in the motor learning task compared to the control task. From time-frequency analysis of components clustered on the primary motor cortex, an event-related decrease in α and β frequencies right after the onset of the trials was found. This phenomenon is often called event-related desynchronisation, which insinuates that the power decrease is caused by a decrease in synchrony of underlying neuronal populations [26]. We will use the more neutral term event-related spectral perturbation, or ERSP. Other studies that investigated movement-related EEG phenomena also report modulations in α- and β-ERSP before and during motor learning tasks [10],[26],[22]. Our study revealed significant

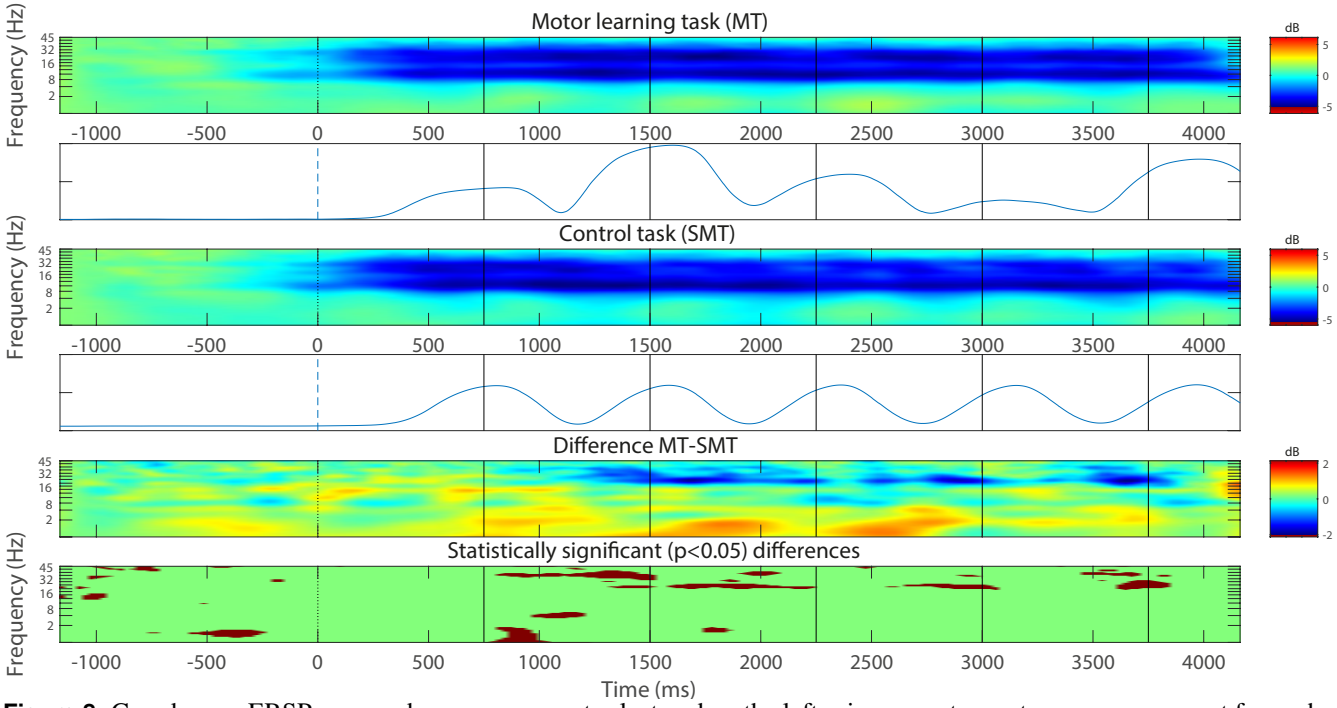

Figure 8. Grand mean ERSP averaged over components clustered on the left primary motor cortex, one component for each participant. The first two figures represent the time-frequency plot of the motor learning task (MT) with corresponding mean location trace underneath. The third and fourth figure represent the time-frequency behaviour during the control task (SMT) with corresponding mean location trace below. At time point zero, the trial starts with a 'GO!'signal. The metronome ticks are indicated with black vertical lines. The colours in the plot indicate dB normalised power for specific time points and frequencies, compared to a common baseline period for both conditions (-2000 to -1000 ms). In the fifth figure, the ERSP of the control task was subtracted from the motor learning task. In the lower figure, only the areas where the differences between both conditions were statistically significant ($p<0.05$) are coloured red.

differences in β ERSPs during a motor skill learning task, compared to a motor execution task. The decrease in β power, relatively to baseline, is stronger in the motor learning task. The difference in β ERSP seems to occur in multiple 'bursts', which could be related to the 750 ms pinch interval. With use of the Morlet Wavelet transform, the time resolution is decreased, so the metronome ticks can not be directly coupled to the ERSP data. The differences in β power are not likely to be caused by differences in required pinch force, because in that case, we would expect the differences to occur mainly around the second and fifth pinch. Therefore, the detected differences in β power could be interpreted as the electrophysiological differences between motor skill learning and motor execution in the brain.

In earlier conducted research, motor learning was associated with a decrease in brain activity after training [18],[42]. This phenomenon is often described as 'neural efficiency'. Neural efficiency describes that skilled people need less brain activity to perform the same actions and thus need less power in movement-related brain areas and frequencies [10]. We suggest that neural efficiency could be seen as a form of habituation. Habituation is a kind of learning that occurs naturally when people are exposed to repeated stimuli [43]. This would explain the found decrease of brain activity with solely movement repetition in earlier conducted research [44]. It could be that the control task is so easy that habituation already took place after three practice blocks. This is supported by a visually observed decrease in β-ERSP between the first three blocks of the control task, compared to the last three blocks. In the motor learning task, the participant needs more attention and effort, which could lead to higher β-ERSP. When the motor learning task would last longer or take more sessions and people become an expert, this would imply that the β-ERSP would be similar to a habituated task.

The reason that the ICA's did not result in a consistent component decomposition was found to be bridge forming between electrodes. This finding was supported by the relation between the detected amount of bridges and the number of reliable ICA components (see Table 3 and Figure 16, Appendix A). It has been reported before that bridges distort the corresponding EEG topography [45]. Possibly, many researchers are unaware of the risk of bridge forming or do not take this into account during analysis of EEG data [36]. In the 8 remaining participants, a cluster of components located on the left primary motor cortex was found. In an extensive MRI motor learning meta-analysis and review, the left primary motor cortex was indicated as an important right-hand motor learning area [46]. Unlike in functional imaging studies, separate

components on the premotor cortex and supplementary motor area could not be differentiated in our study [47]. A reason for this could be field spread of electrical activity, which makes it hard to separate cortical components that are located close to each other with EEG [48]. Studies that do describe EEG analysis of these motor areas, used single channel data instead of component data for their analyses [11], [21], [12]. Because of field spread through the skull and brain tissue, it could be questioned if all motor learning areas can be separated with EEG recordings only.

The question if EEG time-frequency parameters like β ERSP can be causally linked to neurophysiological parameters like motor learning is another interesting discussion. It deals with the manifest variable, EEG data in this case, and the latent variable, which is the underlying activity of neurons that cause certain behaviour [49]. It is impossible to measure the latent variable directly, so coupling to known neurophysiological mechanisms of oscillations need to be made carefully. To be able to draw more physiologically plausible conclusions, this study investigated the oscillatory behaviour of independent components [28]. For the detection of causal relations between motor learning and EEG, more research is needed. Many studies use different EEG paradigms, data preprocessing techniques and analysis methods which can influence the results considerably [49]. In this research, we aimed for using as mainly automated preprocessing- and analysis procedures in order to make the results reproducible.

Limitations

The design of the motor tasks with five pinches per trial, visual and auditory feedback posed a challenge for EEG data analysis. Motor learning processes could overlap during the pinches and the combination of sensory and motor information that arise around the same time points during trials, could be hard to distinguish on EEG. Also, the difference in accuracy index between both conditions was not very distinct within all participants. This could be caused by the feedback that participants received in the control task. The feedback could have directed the participants to use other movement strategies. They could, for example, pinch the force transducer longer around the moment of the metronome tick. This would make it possible to still improve accuracy in the control task. Another limitation of this study was that with equivalent dipole fitting on standardised head models, only a rough estimation of the source of activity can be made [39]. Due to the inverse problem, the exact location of the source of electrical activity was unknown.

Recommendations for future research

More plausible conclusions about the causality between β oscillations and motor learning can be drawn in studies where brain oscillations are modulated, for example with transcranial Alternating or Direct Current Stimulation (tACS and tDCS). From such research, it is to be expected that an effect of electrical stimulation on motor learning would occur via β-power modulation. This effect is indeed found in earlier research on α and β stimulation with tDCS and tACS[50]. Source separation tools like ICA could also bring us a step further into understanding the neurophysiological principles in the brain [28]. It is often advised to use as many channels as possible for the best ICA results. In our experience, however, a big number of channels also poses a big risk of forming bridges between electrodes. We found that 64 electrodes also led to good source separation of (sensori-)motor components, as confirmed by earlier research[51]. We would therefore recommend to consider the risk of bridges when deciding whether or not to use more than 64 electrodes in future research. Solving the inverse problem of source localisation remains a challenge in EEG research. A study that combines the spatial resolution of MRI and the temporal resolution of EEG may provide more insight on the neurophysiology behind motor learning.

The results of the present study bring us a small step closer to the fundamental understanding of motor skill learning on a neurophysiological level. It could be used for future research on motor skill recovery in rehabilitation, motor learning in sports medicine and research on the potential benefits of electrical stimulation on motor learning. Furthermore, with upcoming brain-computer interfaces, the results can be useful for neurofeedback during specialised motor exercises. With direct feedback on brain activity, motor training could become more personalised and effective in the future.

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Appendix

A. Comprehensive Methods

A.1 Motor tasks

The motor learning task was designed in Labview by Zeb Jonker, a PhD student at Erasmus MC. The motor learning task already existed and was based on other serial visually guided isometric pinch tasks [6], [29]. The control task was designed to control for motor execution only, so no motor learning was expected here. As described before, motor learning was defined as an increase in accuracy. In the control task, the target block was made very wide and it was at the same location for all five pinches. This was in contrast to the target blocks in the motor learning task, where the target blocks were at different locations and needed to be reached in a specific sequence. The average force needed to reach the block in the control task, was the same as the average force needed to reach the five targets in the motor learning task. In Figure 9 the location trace averaged over 200 trials during both tasks is shown. The participants were randomly assigned

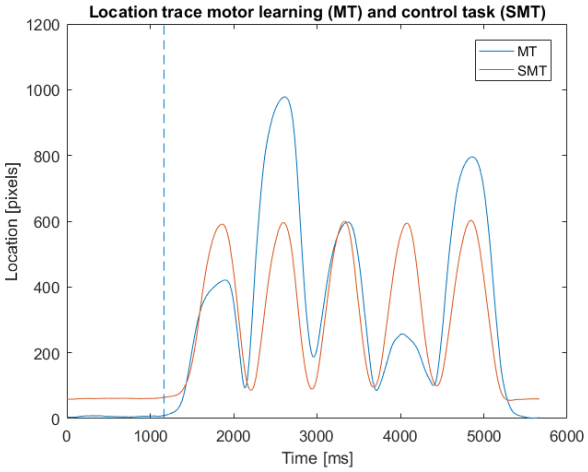


Figure 9. Location trace averaged over 200 trials of the control task (red) and motor learning task (blue).

to either a group that performed the motor learning task first, or second. In Table 2, this is shown in a table.

B.3 Randomisation table motor tasks

The performance data was exported in txt. files. To be able to analyse the results of this data, Matlab and the m.files Cleandata.m, Importtrial.m and Analysis_motor_task.m were used (see Appendix C). Importtrial.m was needed to convert the punctuation of the exported files to Matlab language. Then, the data was organised in a tracemat while time shifts due to missing trials were removed. This resulted in a matrix with steps, applied force and location of the cursor per participants and condition.

There are multiple options to define motor learning parameters. Accuracy and precision are used the most in descriptions of motor improvement. In our motor skill learning task, precision increase is a logical consequence of an increase

MT - SMT	SMT - MT
110	101
104	102
107	103
115	105
106	108
114	111
112	113
109	116
117	119
118	120

Table 2. Subject numbers and their assignment to one of the two groups that indicate the sequence of motor tasks. MT = motor learning task, SMT = simple motor task (control).

in accuracy. It is impossible to be close to the target blocks every trial without being precise. During the control task, the target is bigger and therefore, this is not necessarily the case. The accuracy could be assessed for the total movement during a trial, so while reaching the target blocks five times or around the target point. However, studies on motor learning stated that the main improvement occurs in proximity of the target point, so the movement around those points should be assessed [52]. In Figure 9 and an example of the location trace averaged over 200 trials can be seen for the motor learning task and control task. The time points 750, 1500, 2250, 3000 and 3750 indicate the moments where the tick of the metronome occurs. At that moment, for the motor learning task, the cursor is supposed to be at 400, 1000, 600, 200 and 800 pixels, respectively. In the control task, the cursor should be at 600 pixels at all five moments. To investigate the motor performance it can be chosen to analyse the movement of the cursor around the target locations or around the metronome ticks. Because of the changing strategy that some participants apply and the sometimes very off-target location of the own peaks, it is decided not to analyse the movement around the target locations. Reason for changing strategy is the feedback that participants receive, they see the percentage of correct hits per target block. A hit is considered 'correct' if the participant moves the cursor between the margins around the target within the window of the metronome tick. Another possibility is to analyse the location of the cursor at the exact moment of the metronome tick or in a window around this tick. The variability between locations of peaks and locations at the metronome tick can be big. One reason is that with different strategies, the same results can be achieved. To get the cursor at the right moment at the right time, one can overshoot or reach the target slowly, or stay longer at the target location, for example. Hence, the metronome locations are not necessarily a good indication of motor performance. The location of the cursor around the metronome ticks, as shown in the right figure, was therefore chosen to analyse. There was still a lot of variation in this measure, so a window of 200 ms around the metronome ticks was evaluated. From the location points in that window, the maximum value was taken as aim location of the participants. This window was also taken into account during the motor task evaluation, so the participants received feedback on the same measure. In Figure 10 the window borders and target borders are shown for the averaged location trace of the control task.

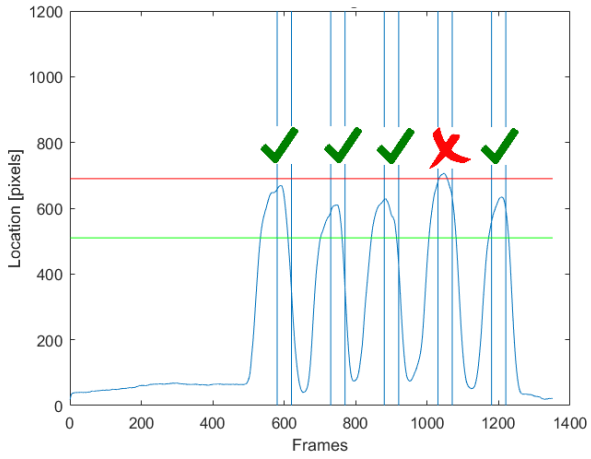


Figure 10. Example of evaluation of the control task pinches (SMT) for location traces of one trial. One frame is 5ms. The window around the metronome ticks are indicated with blue vertical lines. The borders of the target block are depicted as the green and red line.

To assess accuracy, the absolute errors between the target blocks and the aforementioned peak points around the metronome ticks are calculated [31]. The difference between the motor learning task and control task posed a challenge for this measure. It was not possible to calculate the difference between the middle of the targets for both tasks because the target block in the simple motor task is bigger. It wax chosen to take the whole block into account, rather than the middle of the target blocks as a target, because the participants received feedback on the amount of correct hits where the total width of the block (+ 25 pixel margin) was considered as correct. Therefore, if the cursor was located inside of the target block, the absolute error was set at 0. If the cursor was located outside of the target blocks, the error was defined as the absolute distance between the single location of the cursor and the nearest border of the target block. The absolute error was averaged over all targets, because it does not matter for the performance if someone performs better around the first target or the last. The lower this measure, the more accurate participants behave. Precision describes how constant the behaviour of participants is. It is calculated by taking the same location peaks in the (-100 ms to 100 ms)-window around the metronome ticks. Then, the standard deviation of these peaks per individual target over all trials is considered. The lower this standard deviation, the higher the precision. The precision learning index for both conditions is shown in Figure 13. The index is plotted on the vertical axis against all 20 participants on the

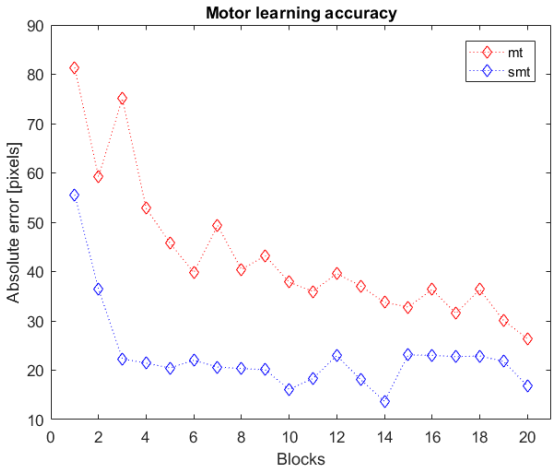


Figure 11. Accuracy averaged over all 20 participants

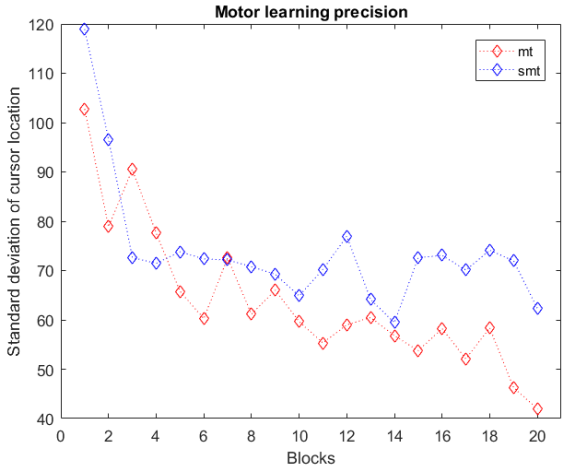


Figure 12. Precision (SD [pixels] over cursor location points), averaged over all 20 participants

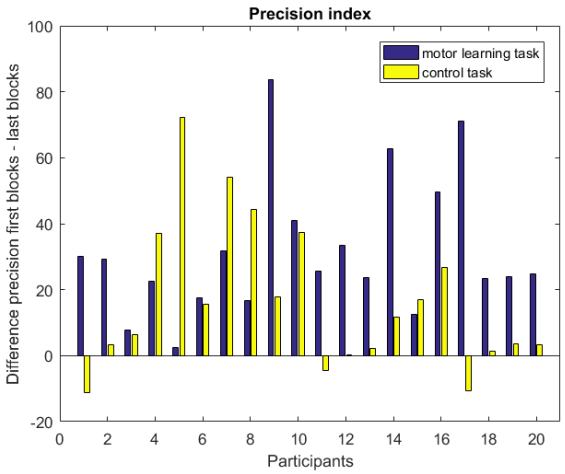


Figure 13. Precision index for the motor learning task and control task for every participant. The bars indicate the difference between the first and last five blocks in precision.

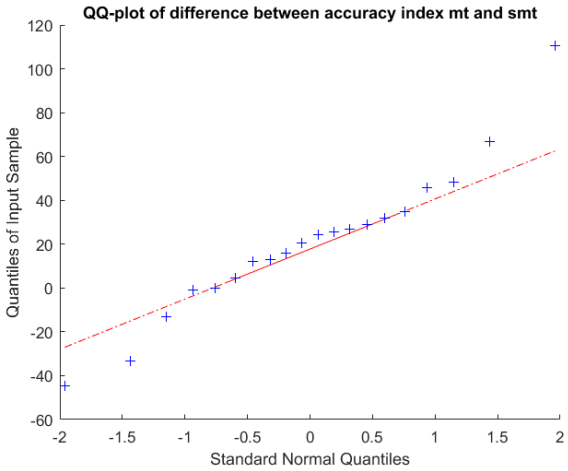


Figure 14. Qqplot of accuracy index differences

horizontal axis. Most participants (17 out of 20) increase their precision for both motor tasks. This means that the standard deviation of the cursor location around the target decreases. In 16 out of 20 participants, the precision increase is higher in the motor learning task than in the control task. The difference between the mean precision and accuracy during the first five blocks compared to the last five are defined as motor skill learning indices. This difference is independent of the initial motor performance of participants and is therefore a good indicator of individual motor skill learning. The motor learning indices are compared between the two tasks and the difference within participants is tested for significance with a paired t-test [53]. It is expected that for both tasks the precision increases, but for the motor skill learning task, the accuracy increases more than in the control task. The used m.file 'Analysis_motor_task.m' can be found in Appendix C. In Figure 11 and 12 the accuracy and precision were averaged over all participants. It can be seen that on average, people increase in precision and accuracy with practice. Some individual participants, however, do not improve on both measures and some deteriorate. The differences in indices are not normally distributed, as can be checked by the qqplots as in Figure 14.

Therefore, the non-parametric Wilcoxon signed rank test is used. This resulted in a significant difference for both the accuracy and precision index ($p = 0.0124$ and $p = 0.0438$, respectively).

A.2 EEG analysis

After loading the data into EEGlab, the raw data was filtered with a FIR filter. Zero-phase filters like this can be used for EEG- coherence and –synchrony measures, because it retains phase information [49]. Reasons for applying the notch filter at 50 Hz and harmonics is the line noise that was apparent in many trials. This can be demonstrated by plotting a spectral plot of data, where a peak at 50 Hz and harmonics can be observed. Due to the high pass filter at 1 Hz, the EEG data did not have to be demeaned to remove DC offset. This is

tested by taking the mean of the data for an individual channel and compare this to demeaned data, both values were close to zero. The channels were visually inspected for rejection for the resting-state and EEG data around the motor events. In Figure 15 an example of bad channel removal by visual inspection is shown, noisy channels were inspected and removed. Channels that showed flat lines were also removed. To be able to see where unexpected, strange data comes from,

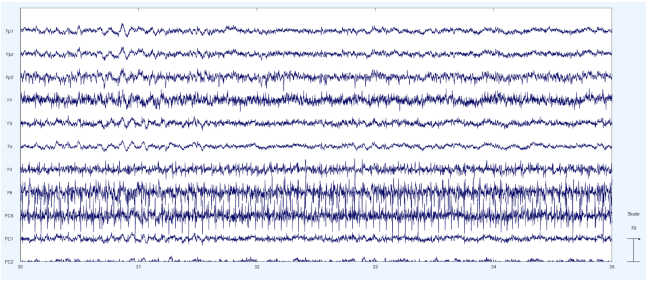


Figure 15. Example of visual inspection of channels. Channel potential in μV is plotted on the y-axis and time in seconds on the x-axis. For this dataset, the output of the first 10 channels are plotted for 5 seconds. Channel F8 and FC5 can be considered as bad channels.

screenshots of the impedances before and after the measurements were made. In this way, it is still possible to check parts of the measurements after acquiring the data. During the first measurements particularly, it was tedious to get the required low impedances ($< 5k\Omega$) for all 128 channels. Because of this, too much gel was used, which resulted in many bridges between the electrodes in 12 out of the 20 participants. Another possible explanation for the high amount of bridges was the high temperature in the measurement room, which could lead to excessive sweating. We think that the sweat could have made the electrode gel more viscous so it leaks easily to the other electrodes. The bridged channels were detected by using the *eBridge* function [36]. This function only behaved consistently when the data was high-, low-pass and notch filtered as described in the preprocessing section. Table 3 represents the number of bridged channels and corresponding number of fitted dipoles with less than 15% RV. The lower the RV, the higher the 'dipolarity' of components. More independent linear EEG decompositions lead to more near-dipolar components [38]. Pairs indicate the amount of connections between different bridged electrodes. It could be that one bridged channel connects to multiple others, then the amount of pairs is higher than the amount of bridged electrodes. It was not possible to detect the bridges by simply calculating the rank of the data matrix in Matlab, because this computation does not work well on EEG data for unknown reasons. This is confirmed by the observation that the rank of the data stays the same after average referencing, which should reduce the rank by one.

In Figure 16 a scatter plot between the number of bridged electrodes and the number of fitted dipoles with less than 15% RV is shown. In the first attempt to make the ICA decompositions better, all bad channels and bridged channels were removed. After that, we spherically interpolated all removed channels before performing ICA. The rationale behind this was the chance that the removed channels could be very asymmetrical resulting in biased components towards certain regions. The ICA did not result in better independent components after interpolation, however. We concluded that it depends on the location and the amount of removed channels if interpolation is useful and logical to use, but in general, more included channels give better ICA decompositions. In Figure 17 an example of the resulting channels in a participant with many bridges is shown, it is clear that it is not sensible to interpolated between the remaining electrodes here. The

Table 3. Relation between EEG bridges and fitted dipoles

Participant	Bridged channels (Pairs)	Dipoles <15% RV
101	12 (9)	57
102	84 (453)	23
103	65 (1032)	23
104	63 (236)	32
105	92 (498)	11
106	90 (307)	29
107	21 (16)	56
108	43 (63)	35
109	74 (72)	31
110	49 (42)	23
111	32 (29)	40
112	37 (28)	59
113	74 (132)	33
114	36 (22)	45
115	23 (13)	52
116	47 (34)	38
117	51 (66)	37
118	27 (22)	45
119	26 (16)	44
120	54 (35)	62

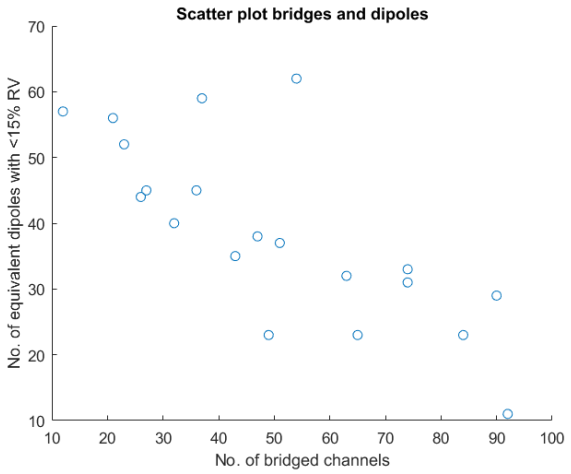


Figure 16. Scatter plot of bridged channels and fitted dipoles with <15% RV.

autorej function of EEGlab was used to further clean the data, which detects extremely large potential fluctuations with a probability threshold of 6 standard deviations with a maximum of 5% epoch rejection [34]. Examples of horizontal eye artefacts (a), eye blink artefacts (b) and muscle artefacts (c) that can be typically found in EEG measurements are shown in Figure 18. Before the ICA decomposition, the artefacts like in Figure 18 do not have to be removed manually, because they are recognised as individual components. Only the *autoreject* function is therefore used to reject non-stationary artefacts which are harder to separate for ICA.

Independent Component Analysis

ICA is one of the many blind source separation algorithms used in signal processing. A source separation tool like ICA could provide more insights in functional reorganisation of cortical networks mediating motor skill learning [55]. The Infomax algorithm for ICA is used frequently in EEG research as it is able to maximise independent transferred information while it does not need any information about the input distributions. Also, Infomax is applicable to nonlinear networks [56]. Before performing ICA, it was expected to find some prominent ocular and cortical motor components in the EEG data, due to the nature of our designed motor tasks. In Figure 19 an example of a clear ocular component is depicted on top and a cortical component is shown underneath. Another method to confirm these observations, is by plotting the power spectra and time domain data of the components as can be seen in the same figure. Notice that in this plot, the 50-Hz line has not been removed yet. From the event-related potential image on the upper right, a clear difference between the eye movements in the control task and motor learning task can be seen. This participant started with the control task, this was very simple so one could gaze at the screen during the first half of the trials. In the motor learning task, the cursor need to be followed closely, which shows in this component.

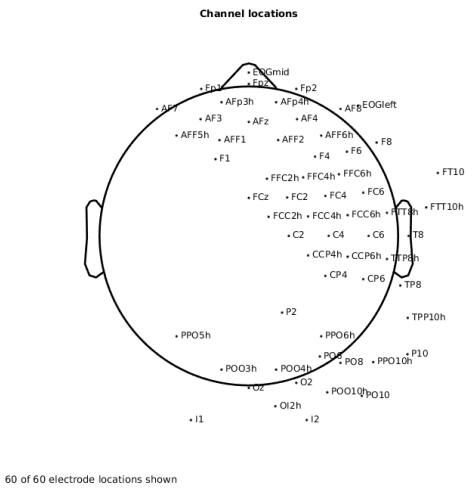


Figure 17. Scalp plot with removed bridged channels

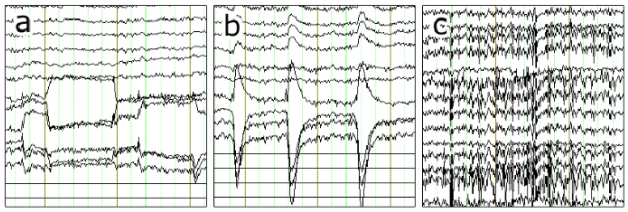


Figure 18. Horizontal eye movement (a), eye blink (b) and muscle (c) artefacts as typically seen on EEG. The upper channels show normal low amplitude oscillations. Retrieved from: Oude Bos, D. P. (2008). "Automated Artefact Detection in BrainStream A Evaluation of An Online Eye and Muscle Artefact Detection Method" [54]

Cortical components can be recognised by the typical EEG α-peak, while ocular components show more power at lower frequencies and typically have a declining curve. In Figure 20, an example of a cortical EEG component is shown. The rounder the scalp map projection, the better the component is separated from other activity. During the preprocessing steps

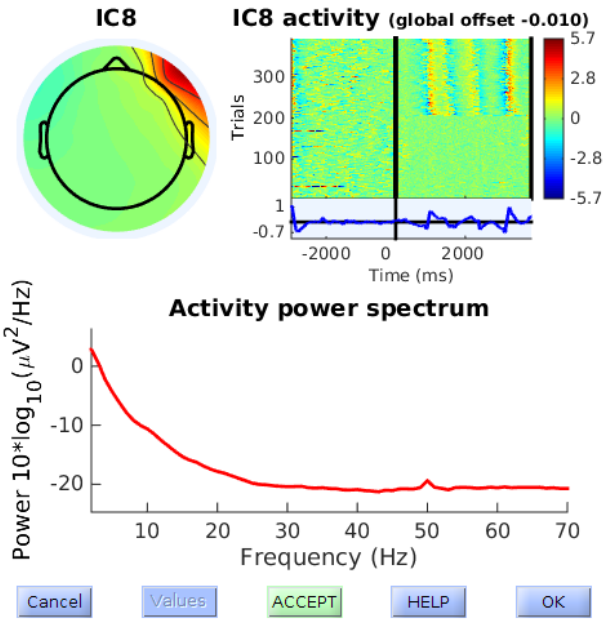


Figure 19. Horizontal eye component, the upper left figure shows the scalp map projection of the component. The upper right figure shows event-related potentials over all trials and averaged over all trials (blue line). The typical eye activity power spectrum is shown in the lower figure.

and examination of the data, the ICA decompositions did not work as expected. For some participants it worked really well, but for others, many different and elongated decompositions did not result in consistent and clear components. We tried many things to solve the 'bad' ICA decompositions, where most solutions involved going through the preprocessing steps again and again. We performed many ICA's with help of

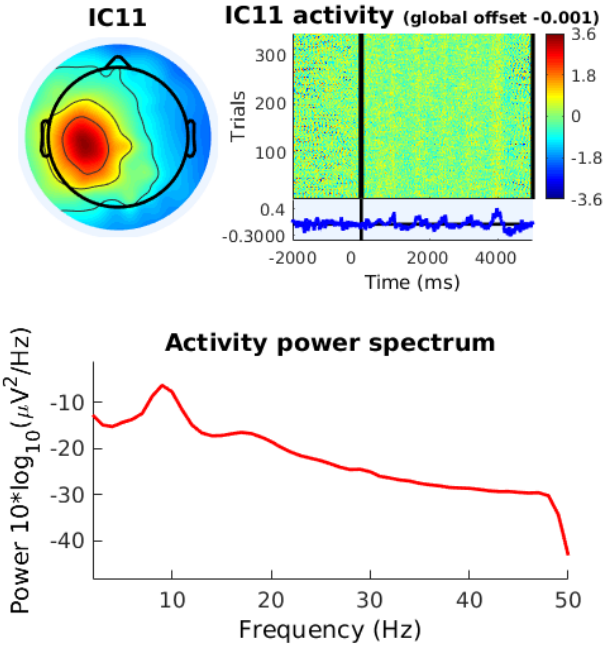


Figure 20. EEG component, the upper left figure shows the scalp map projection of the component. The upper right figure shows event-related potentials over all trials and averaged over all trials (blue line). The typical EEG spectrum is shown in the lower figure.

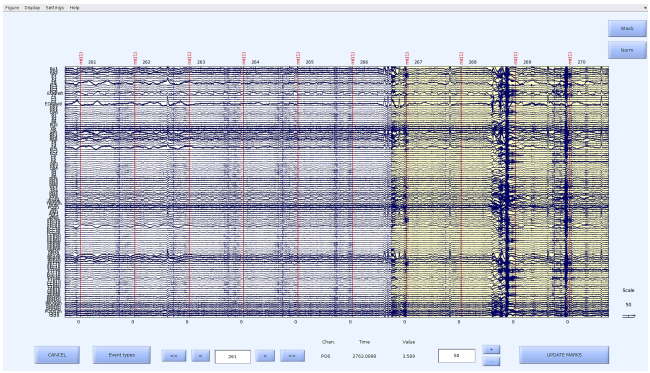


Figure 21. Print screen of manual rejection of bad trials. Potentials per electrode are plotted on the vertical axis against the time. The red lines indicate the event 'mt', a motor learning task event. The orange coloured epochs are manually rejected.

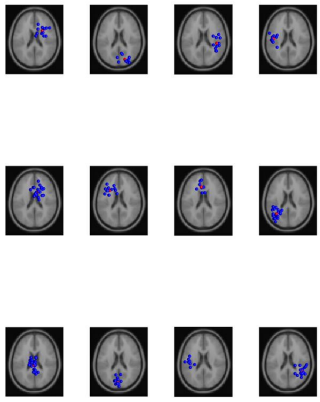


Figure 22. Equivalent dipoles for all clusters

the quick cudaICA algorithm that uses a graphics processing unit [57]. The cudaICA function uses the Infomax algorithm to find independent sources with maximal temporally independent information [56]. We tried using stricter ICA stop criteria, different filters, strong or mild channel- and epoch rejection, and so on but nothing delivered better results. Also, different ICA algorithms were tested, where different forms of the Infomax algorithm (*runica* function in EEGLAB and faster versions like *binica* were used frequently. After many attempts of the fast cudaICA, we concluded that it is best to keep more channels for ICA decomposition, even if they appear noisy. Also, we concluded that the reason for the bad ICA decompositions had to be the number of bridged electrodes for some participants. We decided to include 8/20 participants that showed reasonable scalp map plots of the components after cudaICA decomposition. An alternative algorithm we tested was the adaptive mixture of independent component analyser (AMICA) decomposition, which produces the largest mutual information reduction and the most near-dipolar components [38]. This algorithm can accommodate non-stationary environments and arbitrary source densities, which arise in EEG data [37]. The AMICA algorithm gave the best results in terms of distinguishable components on the plotted scalp maps after ICA. Therefore, we chose to use this algorithm as the final source separation method. This is also tested as the most efficient source separation algorithm, which is another plus [38]. In EEGLAB, AMICA can be called with the *amica15* function. The output components are ordered in decreasing order of EEG variance accounted for by each component. For this reason, only the first 60 components were inlcuded for further evaluation. The AMICA weights and sphere matrix were projected back onto the original merged data sets. Bad trials still needed to be removed in these sets so after epoching (-2 to 5 seconds around an event), a semi-automatic rejection followed. The automatic part was with use of the *autoreject* function as described before and after that, the trials were visually inspected and manually rejected. In Figure 21 an example of trials that were removed manually are marked in orange. The event ('mt') is the motor learning task event.

Source localisation After source separation with ICA, it is know which sources of activity are independent, but we do not know the location of these sources. To do so, different source localisation techniques could be applied. A common one is the translation of electrical activity to an equivalent dipole source. Equivalent dipoles of the found independent components were fitted in a standard spherical head model

using the DIPFIT2.x plugin of EEGLAB [34]. The rationale behind this is that every independent component can be traced back to a dipolar source [38]. A residual variance of 15% is an acceptable threshold for estimation of the reliability of independent components [58]. Only dipoles that were located within the head and with a residual variance of less than 15% were included for further analysis. The remaining components were included in an EEGLab STUDY design. This design automatically removes components that are described by out of head dipoles. The included independent components were statistically clustered (k-means algorithm) in 12 clusters based on their 3-D dipole locations. In Figure 22 the resulting clusters are shown. The first cluster consists of dipole location outliers of $\pm$ 3 SD's. The fourth, eighth and eleventh cluster looked promising. After comparison of cluster centroid MNI and Talairach coordinates, it was concluded that the third cluster on the third row (no. 11) most likely consisted of left primary motor components. The seventh cluster could be of

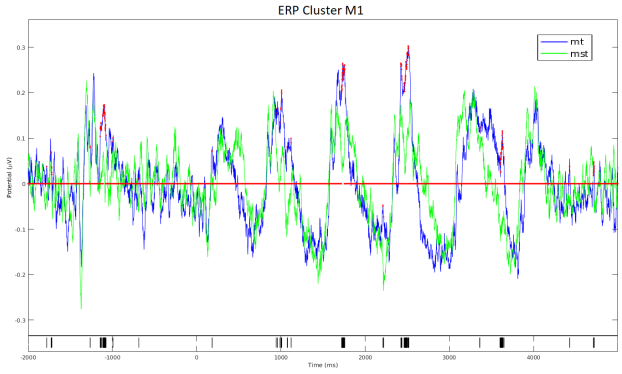


Figure 23. Grand mean ERPs for the left primary motor cluster averaged over all trials for the motor learning task (MT) and control task (SMT). Significant different parts are marked red in the graph and with black blocks underneath the graph.

auditory origin. The ERPs of this primary motor cortex cluster, with statistically significant differences ($p<0.05$) between conditions marked red, is shown in Figure 23. Event-related spectral perturbations that have been linked to general motor behaviour are so-called event-related desynchronisations (ERD). This event-related attenuation of brain signals is calculated with the following formula: $(A-R)/R*100$, where A is the power in the defined frequency band and R is the power in the preceding reference or baseline period [26]. To localise frequency band-specific activity in time, a Morlet Wavelet convolution can be performed with using wavelets of different frequencies. The *newtimef* function in EEGlab uses Morlet Wavelets to calculate Event-Related Spectral Perturbations. As a baseline period, the period of 2000 ms to 1000 ms before the event, were the ERP's of both conditions were more or less alike, is chosen. In this manner, the readiness potential which usually occurs around -400 ms at M1, was not included in the baseline [59]. The sound of the metronome start around -1500 ms, but it is chosen to take this into account as a baseline because this sound is also apparent during the tasks. For the comparison of the two motor tasks, a common baseline is chosen. For future research, it would be useful to see if resting state data of every participant could be better as a baseline period.

From the behavioural data, it could be seen that some participants still improve their accuracy in the control task, especially during the first blocks. To check if the found 'motor learning' differences also occur in the last trials compared to the first, the ERSP difference plots for these trials are plotted in Figure 24.

A.4 tDCS and TMS

I spent the first three months of my graduation on the extensive research of Joris van der Cruijsen and Zeb Jonker considering electrical and magnetic stimulation methods of the brain. We learned to work with Transcranial Magnetic Stimulation (TMS) and transcranial Direct Current Stimulation (tDCS). It is assumed that tDCS increases cortical excitability, as is recently confirmed by research on a new motor network tDCS configuration and transcranial magnetic stimulation (TMS) [60]. TMS can be used to asses the cortical excitability with use of motor evoked potentials. My interests were if tDCS improved motor learning, if this could be reduced to EEG parameters and if the measured excitability was related to this. We tested if new stimulation electrodes that could be attached to an EEG cap worked during several pilot experiments. During these experiments, the stimulation electrodes, made from Ag/Cl did not work as expected. The stimulation protocols could not be finished due to impedance problems. We tested if we could improve the impedances by scratching the scalp more, using more gel, using other stimulation protocols and so on, but nothing worked. Then, I performed an experiment to test if the stimulation protocol would work with the lowest impedance possible, by putting the two electrodes in a jar with conductive gel. Still, it was not possible to finish the complete protocol due to high impedance warnings in this 'ideal impedance' setting. From this experiment our suspicion arose about an electrochemical reaction that took place at the stimulation sides. We tested this by switching the anodal and cathodal electrodes and noticed that the stimulation protocol could last a bit longer then. Another clue that an electrochemical reaction took place, was precipitated material on the forehead of a pilot subject after repeated stimulation. We proposed that the electrodes should be made of material that was suitable for electrical stimulation, like platinum. The delivery time of these electrodes was 3 months, so we decided to change my research to more fundamental EEG motor learning research.

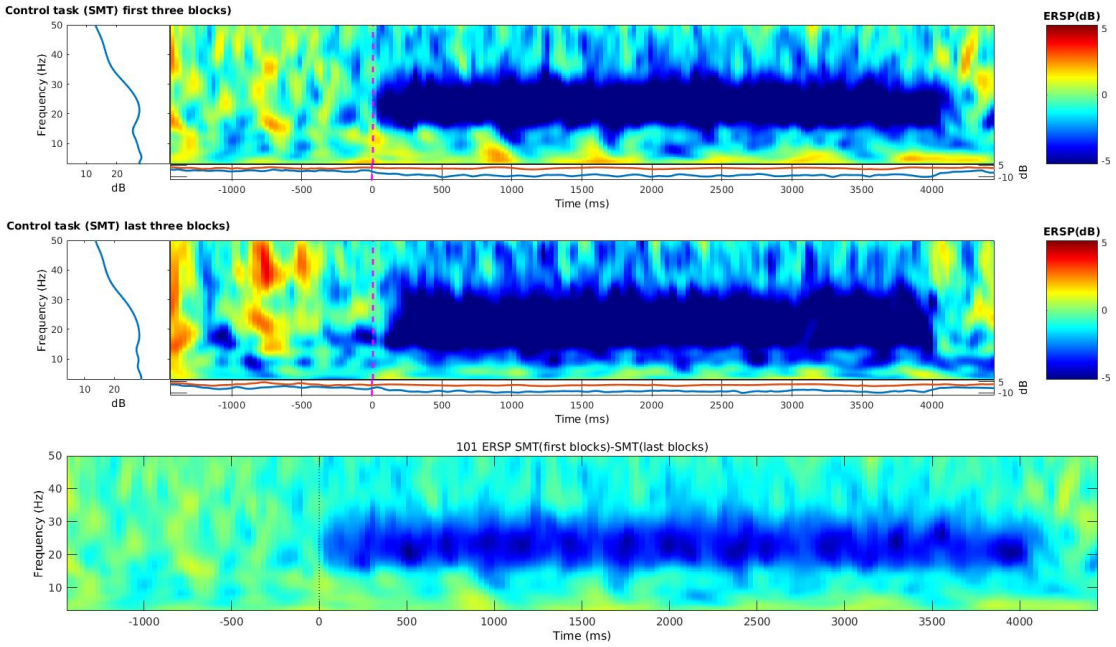


Figure 24. ERSP plots for the simple motor task. Averaged over the first three (upper figure) and last three (middle figure) blocks. The colours in the plot indicate dB normalised power for specific time points and frequencies, compared to a baseline period of -2000 to -1000 ms. The averaged power spectrum of the baseline is shown in the two most upper left plots. The envelopes of ERSP are depicted underneath the first two plots. The lower figure depicts the differences between the first two plots.

B.1 Preregistration

Study Information

1. Title : MotorEEG

2. Authors : Joris van der Cruijsen, Yvonne Greeuw

3. Research Questions

   Though there is evidence to suggest that human motor performance on EEG is related to modulation of the β-frequencies in relevant cortical motor areas, this phenomenon has never been compared between motor execution and motor learning. Therefore, we will measure high-density EEG during a motor skill learning task and compare this to a motor control task to see if motor learning and performance can be distinguished on EEG.

   The relation between pre-trial EEG and the motor learning/motor action conditions. Are there clear differences in cortical activity between the two conditions, i.e. what brain activity is related to motor learning specifically?

4. Hypotheses

   The expected relevant cortical motor components are located on the primary motor cortex, premotor cortex and supplementary motor areas.

   It is expected that the increase in accuracy and precision in the motor learning task is bigger than in the control task.

   If motor learning can be distinguished from motor performance, then event-related β-desynchronisation (so lower β power) in contralateral cortical motor components will be stronger for the motor learning task, compared to the control task.

   In the preparatory phase, stronger α and β event-related desynchronisation occurs in the motor learning condition, compared to normal motor action, at the contralateral motor cortex and premotor cortex.

Sampling Plan

5. Existing data

   Preregistration (selection of description that best describes your situation)

   Registration prior to creation of data

   Registration prior to any human observation of the data

   Registration prior to accessing the data

   Registration prior to analysis of the data

B.1 Preregistration

Registration following analysis of the data

5.1.1. Registration prior to analysis of the data: *As of the date of submission, the data exist and you have accessed it, though no analysis has been conducted related to the research plan (including calculation of summary statistics). A common situation for this scenario when a large dataset exists that is used for many different studies over time, or when a data set is randomly split into a sample for exploratory analyses, and the other section of data is reserved for later confirmatory data analysis.*

6. Explanation of existing data

After collection of the data, the first steps were preprocessing of the very large datasets. Because of limited access to a fast computer with GPU, this step takes a long time. Clear independent components, which should result from the last preprocessing step, are not found yet, so we have not been able to statistically test our hypotheses by analysis of differences between conditions in the components.

7. Data collection procedures.

Twenty healthy, right-handed participants, aged between 18-35 years were recruited and gave written informed consent. Participants with known neurological disorders were not included in the study. Subjects were recruited via advertisement at the Erasmus MC in Rotterdam and the Technical University of Delft and were offered 25 euros payment for participation in the 2,5-hour experiment.

8. Sample size

20 participants.

9. Sample size rationale

In similar studies, 12-20 participants are included so this is taken as a guideline. Due to limited time and the time-consuming 128-channel EEG cap gelling, the sample size of 20 people is chosen in this study.

10. Stopping rule

The data collection is terminated after reaching of the required 20 subjects.

Variables

In this section you can describe all variables (both manipulated and measured variables) that will later be used in your confirmatory analysis plan. In your analysis plan, you will have the opportunity to describe how each variable will be used. If you have variables which you are measuring for exploratory analyses, you are not required to list them, though you are permitted to do so.

B.1 Preregistration

11. Manipulated variables

    The manipulated variable is the location of the targets in the motor task, where to the subject needs to move a cursor on a screen by pinching a force transducer. In the motor learning task, the location of the targets are at five different places and need to be reached in a specific sequence. In the control task, only one target is apparent which is placed in the middle of the aforementioned five targets, making the task considerably easier.

12. Measured variables

    Measured motor performance variables are: the location of the cursor [pixels] during 200 trials of each motor task, number of ‘correct hits’ in the motor tasks. Measured brain variables are: potential differences [μV] at the 128 scalp electrodes during both motor tasks and during two times 3 minutes of resting state before and between the tasks.

13. Indices

    The accuracy index is averaged over all five targets and 10 trials per block. The precision index is calculated as the standard deviation of the location around the metronome ticks per target over 10 trials.
    The potential differences of the scalp electrodes are processed following a pre-processing pipeline, as described in the analysis section. The outcome variable of the analysis will be averaged across all included trials per condition, per subject.

Design Plan

In this section, you will be asked to describe the overall design of your study. Remember that this research plan is designed to register a single study, so if you have multiple experimental designs, please complete a separate preregistration.

14. Study type

    Experiment - A researcher randomly assigns treatments to study subjects, this includes field or lab experiments. This is also known as an intervention experiment and includes randomized controlled trials.
    Observational Study - Data is collected from study subjects that are not randomly assigned to a treatment. This includes surveys, ñnatural experiments,î and regression discontinuity designs.
    Meta-Analysis - A systematic review of published studies.
    Other - please explain.

15. Blinding

B.1 Preregistration

Blinding describes who is aware of the experimental manipulations within a study. Mark all that apply.

15.1.1. No blinding is involved in this study.

15.1.2. For studies that involve human subjects, they will not know the treatment group to which they have been assigned.

15.1.3. Personnel who interact directly with the study subjects (either human or non-human subjects) will not be aware of the assigned treatments.

15.1.4. Personnel who analyze the data collected from the study are not aware of the treatment applied to any given group.

16. Study design

The study is conducted following a within-subject design, so all subjects perform the two motor tasks, where the order of the tasks is randomised.

17. Randomization

We applied permuted randomization of the subjects, we divided them into two equally-sized groups of 10 persons. One group started with the control task and performed the motor learning task after that and the other group conducted the tasks the other way around. Assignment to one of the two groups is done with use of the randperm function in Matlab.

Analysis Plan

You may describe one or more confirmatory analysis in this preregistration. Please remember that all analyses specified below must be reported in the final article, and any additional analyses must be noted as exploratory or hypothesis generating.

A confirmatory analysis plan must state up front which variables are predictors (independent) and which are the outcomes (dependent), otherwise it is an exploratory analysis. You are allowed to describe any exploratory work here, but a clear confirmatory analysis is required.

18. Statistical models

Within subjects, the difference in learning index for both conditions will be tested with a paired t-test. The mean event-related α- and β power over all trials will be tested within subjects, between the two conditions with a paired t-test as well.

19. Transformations

The motor learning parameters are transformed into a learning indices, which are calculated as the difference in accuracy and precision of the last 50 trials and the first 50 trials. Accuracy is defined by the absolute minimal difference between peak cursor location at the window of 200 ms width around the metronome tick and the target block. If the cursor location is inside of the target, the accuracy is 0. This measure is averaged per target over all trials in a block.

B.1 Preregistration

Precision of movement is defined as the standard deviation of the peak cursor location at the same window around the metronome tick, per target, per block.
The EEG data is preprocessed in the following manner: all data was recorded with a sample frequency of 2048 Hz and imported and analysed using Matlab R2016B and the Signal Processing Toolbox (The MathWorks, Inc., 2016) and the EEGlab Toolbox (Delorme & Makeig, 2004). First, the raw data was inspected after removal of low frequent drift with a 1- Hz high pass FIR filter and line noise with a notch filter at 50 Hz and harmonics. Noisy, high frequencies were removed with a 200-Hz low-pass FIR filter. Visually detected bad and flat channels were excluded from the start. After this, the data was referenced to the common average of all channels, with exception of the EOG and EMG channels. Consequently an Independent Component Analysis (ICA) is performed with use of the Infomax and AMICA algorithms, provided by the EEGlab toolbox (Delorme & Makeig, 2004). Obtained components that have a clear scalp maps around the primary motor cortex, premotor cortex or anterior cingulate cortex and which can be described by a dipole with <15% residual variance, will be included for further analysis.
To investigate the difference between the conditions, the data will be analysed in time-frequency domain with use of Morlet wavelet transforms. Event-related changes in α- and β-power will be calculated for the aforementioned motor components.

20. Follow-up analyses

In a follow-up analysis, the influence of the sequence of the motor tasks will be analysed with use of a simple t-test to compare between subjects in the two different randomised groups.

21. Inference criteria

To draw conclusions from the results, p-values below 0.05 will be considered significant. Since a specific direction of results is expected for the behavioral motor learning parameter, one-tailed tests will be used.
For the pre-trial and event-related changes in power, a decrease is expected, so one-tailed test will be applicable to the EEG data as well.

22. Data exclusion

Following the earlier described EEG preprocessing steps, bad channels and abnormal trials will be removed from the data before analysis takes place. Bad channel rejection is described in the appendix and a standardised script will be used to reject the abnormal trials. If, due to technical reasons such as excessive line noise or bridged channels, the data was is not suitable for analysis, it will be rejected.

23. Missing data

B.1 Preregistration

Incomplete or missing data will not be included in the analysis. It could still be used for exploratory questions and to help prevent technical problems in further EEG experiments.

24. Exploratory analysis (optional)

    The influence of pre-trial brain state, measured on EEG, is found to be correlated with motor learning. Our question is if connectivity between the earlier found relevant motor areas is a predictor of motor skill learning.

Script (Optional)

The purpose of a fully commented analysis script is to unambiguously provide the responses to all of the questions raised in the analysis section. This step is not common, but we encourage you to try creating an analysis script, refine it using a modeled dataset, and use it in place of your written analysis plan.

25. Analysis scripts (Optional)

    (Optional) Upload an analysis script with clear comments. This optional step is helpful in order to create a process that is completely transparent and increase the likelihood that your analysis can be replicated. We recommend that you run the code on a simulated dataset in order to check that it will run without errors.

Other

26. Other (Optional)

    If there is any additional information that you feel needs to be included in your preregistration, please enter it here.

MotorEEG Erasmus MC

B.2 **Checklist EEG meting**
Version 1.4 5/5/2018

IDi
Task: MT/SMT SMT/MT
Date:

Items	✔	Notes:
Airco at comfortable temperature		
Set the tables in measurement setting		
Turn on computers, stimulation and recording programs		
Turn on EEG amplifier, connect cables to computer, trigger cable (3thd on the right) and electrodes		
Lay these within reach: - 2 syringes filled with gel - Paper towels - Alcohol pads - Nupreb Scrub - EEG cap S/M (<57 cm) or M/L - 5 electrodes - Measuring tape		

When subject arrives

Start time:		
Welcome, information		
Ask if the subject needs to go to the toilet before application of electrodes		
Declaration of consent signed by subject (first visit)		
Turn off all the phones in the room		

EEG & EOG & EMG

Clean the right mastoid and attach ground electrode of Refa		
Control if cap fits check location of Cz		nasion – inion: cm left ear – right ear: cm
Inform subject about gelling		
Gel electrodes and check impedances (< 15 kΩ)		
Clean skin next to the right and in between the eyes and attach the (green) EOG electrodes (13,19)		
Clean fdi muscle of the right thumb and the index finger tendon		
Attach the EMG electrodes on the fdi (red) and tendon (black)		
During EEG gelling: measure max force and three practice blocks of SMT (first) and MT (last), independent of sequence		Let subjects read instructions
Check trigger during motor task practice		
Save impedances (pre-measurement)		

MotorEEG Erasmus MC

Checklist EEG meting
Version 1.4 5/5/2018

B.2

ID:	
Task: MT/SMT	SMT/MT
Date:	

Check EEG and EMG signals on the screen and show subject the influence of muscle contractions and eye movements		
Record resting state EEG for 3 minutes, save as condition: rs		

Motor task

Start Labview Maxforce		
Move screen further away		
Pinch three times and press calculate if they are similar, save maxforce_subject		
Open Labview and load max force, type correct filenames below task, trial and block.txt		Draw 5 (MT) or Yvonnebreedblokje (SMT) (depends on sequence!)
Check settings: bpm (750 ms) and 20 trials, 10 blocks, click run		
Change filename and start EEG recording: mt (or smt)		
Let subject perform 20 blocks of the first task		
Stop EEG measurement, give subject something to drink and eat		
Perform resting state EEG measurement for 3 minutes		
Open Labview and load max force, type correct filenames below ,task, trial and block.txt		
Change filename and start EEG recording: mt (or smt)		
Let subject perform 20 blocks of the second task		
Save impedances (post-measurement)		DONE! ☺

End time:

After measurements

Write down email of subject for financial compensation		
Shut down all computers, turn of amplifier and clean table		
Clean the EEG cap with toothbrush		
Replace tables etc. back to original position		
Check if there are enough supplies for next time		

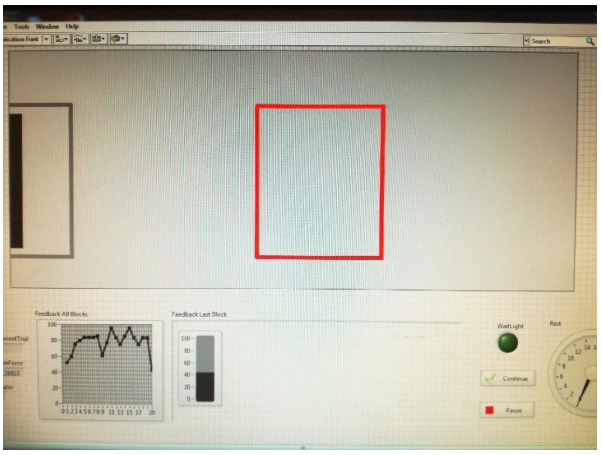
B.3

Motor task instructions

Please read the instructions carefully and ask questions if anything is unclear. You will have three practice sessions per task, in which the investigator will give feedback.

One-Block Task:

In this task, you will move a black rectangle from the left home position on the screen, to a red target block in the middle of the screen, see Figure 1. You can move the black rectangle by pinching the force transducer using thumb and index finger. The amount of pinch force is related to the movement of the black rectangle, the harder you pinch, the more the block moves to the right.



After three beeps of the metronome, you can see a GO signal above the home block and it is your turn to pinch. Each time the metronome ticks from then, which will be 5 times per trial, you need to pinch the force transducer. It is your goal to get the black rectangle inside the red block at each beep of the metronome. Between the beeps, you have to let loose of the force transducer and let the black rectangle return to it's home position. The time between the 5 beeps is constant. After 10 trials (of each 5 beeps), you will have the opportunity to take 15 seconds rest.

Figure 1: One-Block Task

Five-Block Task:

This task has the same design of the beeps of the metronome. The big difference this time is the fact there are 5 target blocks, which have to be reached with the black rectangle in a specific sequence, from 1 to 5, see Figure 2.

There will be three beeps and a GO signal and from then you need to move the black rectangle to the first block at the tick of the metronome, then back to the home position, then at the second block at the second tick of the metronome and back to the home position, then to the third, etc. Make sure the black rectangle is inside of the target block at the moment of the metronome beep! The time between the 5 beeps is constant. After 10 trials (of each 5 beeps), you will have the opportunity to take 15 seconds rest.

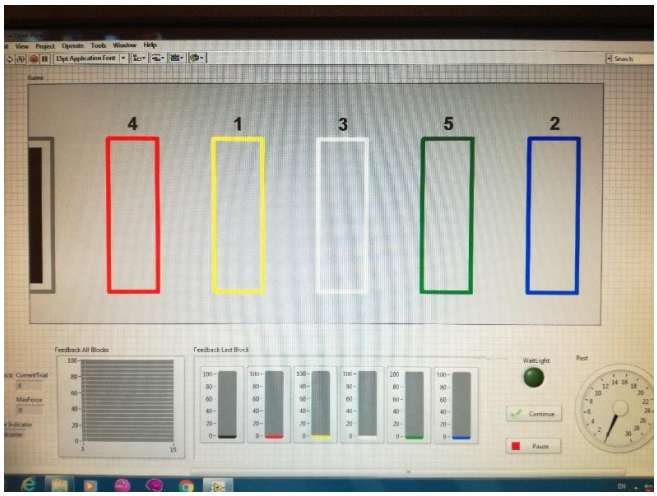


Figure 2 Five-Block Task