EFFECTS OF DIFFERENT INHIBITORY NON-INVASIVE BRAIN STIMULATION PROTOCOLS ON PERFORMANCE IN A MOTOR SEQUENCE LEARNING TASK

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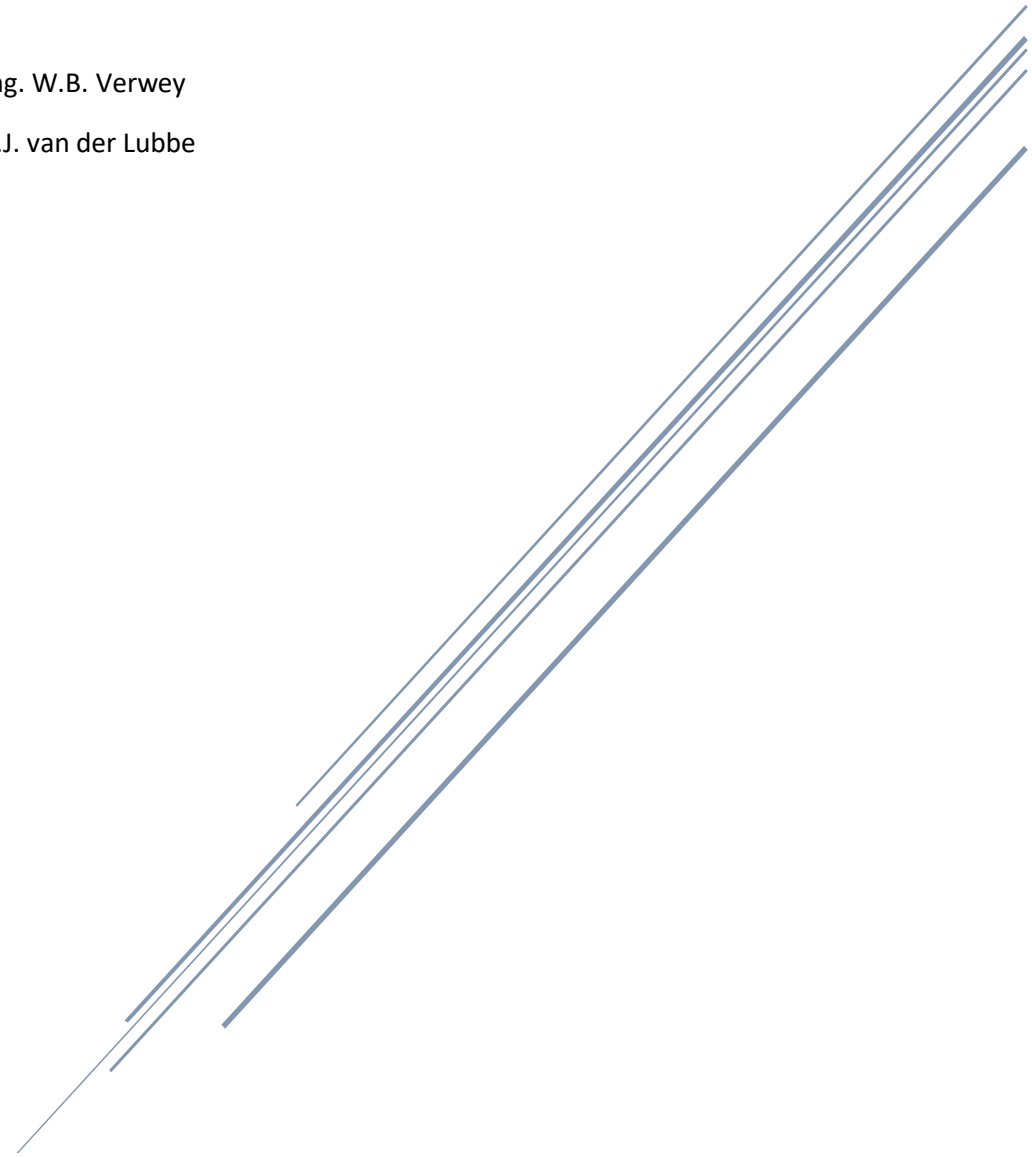

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LEIBNIZ RESEARCH CENTRE FOR WORKING ENVIRONMENT AND HUMAN FACTORS

Contents

Abstract

Non-invasive brain stimulation technologies (NIBS) are often used to study the functionality of the human motor system. For the aforementioned purpose two protocols are commonly used, namely continuous theta burst stimulation (cTBS) and conventional repetitive transcranial magnetic stimulation (rTMS). The literature shows mixed effects for the inhibitory effects on cortical activity of these different stimulation protocols. The goal of this study was to compare the effects of the two protocols in the same experimental context when the supplementary motor area (SMA) is targeted by the stimulation. In the present study a mixed, single-blinded, sham-controlled research design was used. The experiment took place on two consecutive days. As a behavioral experimental paradigm, the discrete sequence production task (DSP-task) was employed. Based on performance measures during task execution within and between the different experimental groups, the comparison of the protocols was conducted. The results showed inhibitory effects of both stimulation protocols when the stimulation was applied on day one. No effect of the stimulation was revealed when the stimulation was applied on day two. Nevertheless, it was shown that the conventional rTMS protocol had stronger inhibitory effects compared to the cTBS protocol.

Introduction

Transcranial magnetic stimulation (TMS) is a non-invasive brain stimulation technique which is commonly used to study the physiology of the human neural system (Jung et al., 2020; Verwey et al., 2002). Furthermore, TMS is used to modulate cortical activity in a faciliatory or an inhibitory manner (Weisz et al., 2012; Huang et al., 2005). A valuable methodology for the aforementioned purposes is repetitive transcranial magnetic stimulation (rTMS), as this procedure can change and modulate cerebral brain activity that leads to short-term changes of cortical excitability during (online-rTMS) and after the stimulation (offline rTMS; Hoogendam et al., 2010). During the application of rTMS, a series of magnetic pulses is given to a specific cortical region which induces changes of neural activity. Besides the findings that rTMS changes cortical activity, mixed results were reported regarding the inhibitory or faciliatory effects of the stimulation caused by different rTMS protocols (Weisz et al., 2012; Fitzgerald et al., 2006; Huang et al. 2005).

Two particular protocols often produced inhibitory effects on cortical excitability, namely 1 Hz rTMS, and 50 Hz continuous theta burst stimulation (cTBS; He et al., 2020; Strzalkowski et al. 2019; Casula et al., 2014; Benali et al., 2011; Dafotakis et al., 2008; Zafar et al., 2008; Huang et al. 2005; Nakamura et al., 1997). 1 Hz rTMS is applied for a fixed time interval, often 20 min, with evenly distributed magnetic pulses throughout the predefined stimulation interval before task performance (offline rTMS) (Takeuchi et al., 2008). Instead, the cTBS stimulation protocol is continuously applied in 5 Hz bursts (three 50 Hz pulses per burst) for an overall application time of 40 seconds (Huang et al., 2005). Whereas the 1 Hz rTMS protocol described above showed rather consistent inhibitory effects on cortical activity in clinical and non-clinical settings (e.g., Casula et al., 2014, Verwey et al., 2002), results from studies utilizing the 50 Hz cTBS protocol revealed mixed results regarding its inhibitory effects (e.g., Hamada et al., 2012; Huang et al. 2005). Thus, a comparison of the 1 Hz rTMS and 50 Hz cTBS protocol in the same experimental design is important to further investigate the proposed inhibitory effects of both protocols. Regardless of the mixed effects produced by the discussed stimulation protocols, it has been argued that both protocols create the described aftereffects of the stimulation through depression of synaptic activity during and after the stimulation process (Di Lazzaro et al., 2011). Besides the two discussed protocols, other protocols can induce similar effects (Kakuda et al., 2011; Fitzgerald et al., 2006). These protocols vary regarding their parametrization (e.g., time of online rTMS, frequency of pulses or intensity of stimulation; de Jesus et al., 2014; Lang et al., 2006). In addition, the reported

aftereffects of rTMS vary among studies. According to Klomjai et al. (2015) a possible explanation for these differences are non-standardized parameters of the stimulation process like shape and orientation of the coil, use of navigated brain stimulation and varying parameters of stimulation protocols. Other studies stress the importance of inter-individual differences of cortical physiology and thus varying effectiveness of non-invasive brain stimulation techniques as a possible explanation for the mixed research results (Latorre et al., 2019, Maeda et al., 2000a).

One particular type of research focused on the functionality of pre-motor areas utilized the described offline inhibitory 1 Hz rTMS protocol to investigate the functions of the supplementary motor area (SMAproper and preSMA) in the development of sequential motor skills. Studies by Ruitenberg et al. (2014) and Verwey et al. (2002) implied that the SMA is involved in executing the individual responses in a familiar motor sequence while the preSMA is engaged in initiating segments of these motor sequences. Evidence for this notion came from experiments employing the discrete sequence production (DSP) task. In the classical version of the task, participants execute a series of key presses (typically 6 or 7) in reaction to key-specific stimuli (Fig.1).

Based on the reaction times captured during well-learned motor sequence execution a classification of execution phases was proposed. The first phase (T_1, Fig. 1) is the initiation phase. It is hypothesized that, apart from the time uncertainty at the start, the longer reaction time to the first stimulus of the sequence is associated also with the retrieval of a pre-learned sequence from long-term memory (LTM). The fast execution of subsequent key presses during the execution phase indicates that a sequence representation instead of single key presses is retrieved from LTM at the initiation phase. Further observations imply that longer sequences (> 4 key presses) are subdivided into segments in that reaction time during sequence execution was decelerated halfway through a longer sequence at T_4 (i.e., the concatenation point; see. Fig. 1). The concatenation point is thought of as an indicator for the retrieval of a new segment representation from LTM leading to increased reaction times at T_4. It was shown that motor segments, also called motor chunks, are used as a cognitive unit for easier sequence execution.

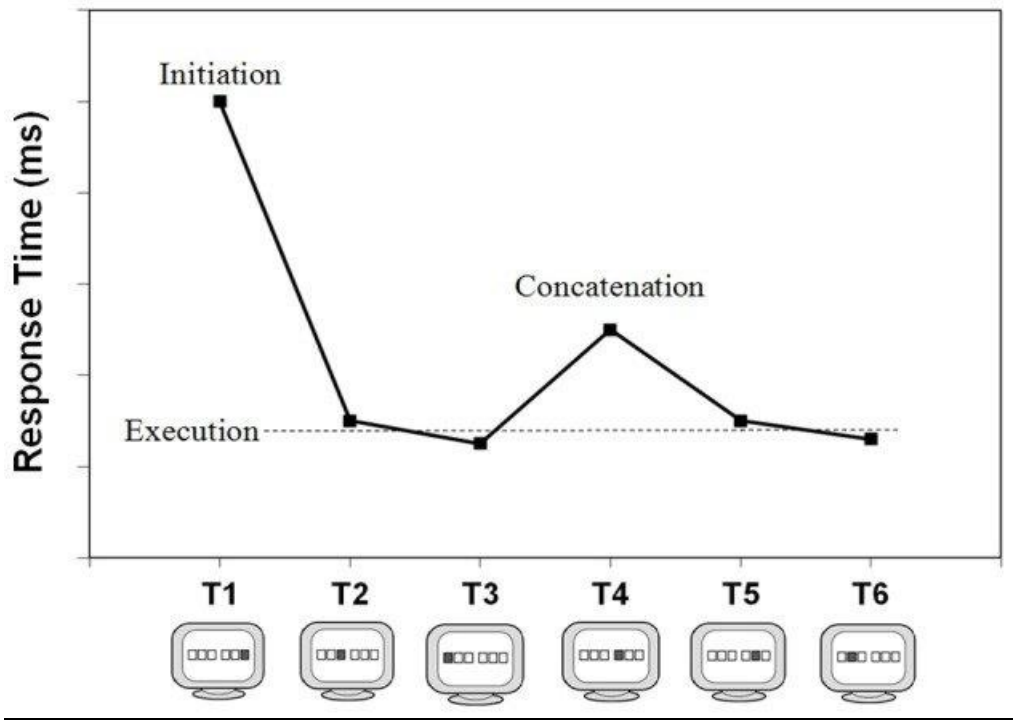

Figure 1: Executing a 6-key sequence and its typical reaction time pattern. It involves the processing phases initiation the slow response observed at T1, concatenation (the slow response observed at T4), and (mere) execution. Please note that with smaller sequence lengths (<5 keypresses) the relatively slow response time halfway through (concatenation) is not typically observed. Retrieved from Abrahamse et al., 2013.

To investigate the role of the SMA during motor sequence execution Verwey et al. (2002) and Ruitenberg et al. (2014) utilized the aforementioned 1 Hz rTMS protocol to reduce excitability of the SMA and preSMA, respectively. The results by Ruitenberg et al., (2014) and Verwey et al., (2002) showed that the inhibitory stimulation of preSMA led to longer reaction times during the initiation and concatenation, implying a functional role of preSMA during the retrieval and initiation of new motor sequences or motor chunks. A possible explanation for the results obtained by Ruitenberg et al., (2014) and Verwey et al., (2002) was presented by Jaffard et al. (2008) and Sumner et al. (2007), who proposed the involvement of preSMA during proactive and reactive motor inhibition mechanisms. Reactive motor inhibition is always guided by an external stimulus that cues subsequent motor actions (e.g., the initiation stimulus of the DSP-task), whereas proactive motor inhibition mechanisms are guided by internal preparatory often subconscious cognitive processes (e.g., an endogenous cue that initiates the second segment in a motor sequence during DSP task conduction). Thus, a suppression of preSMA by means of non-invasive brain stimulation techniques might lead to increased motor inhibition of proactive and reactive cognitive mechanisms. This increased motor inhibition could lead to the increased reaction times observed during the initiation and concatenation of a motor sequence in the DSP-task consequently. In comparison, the results

by Ruitenberg et al., (2014) and Verwey et al., (2002) showed an overall deceleration of reaction times throughout the execution of the motor sequence, implying a higher involvement of SMAproper during overall motor sequence execution than during the initiation and concatenation of a sequence.

An unpublished follow up study (ten Kate, 2018) tried to replicate the results of Ruitenberg et al. (2014) and Verwey et al., (2002) utilizing the described inhibitory 50 Hz cTBS protocol to stimulate SMAproper and preSMA and found no inhibitory effect on initiation and overall execution of well-learned key pressing sequences during DSP-task execution (ten Kate, 2018). This raises some concern on the possibility to utilize 50 Hz cTBS to affect the SMA.

Anatomy and functional role of the supplementary motor area

The SMA is located in the dorsomedial frontal cortex (Nachev, Kennard, & Husain, 2008). Anatomical studies have shown that the SMA can be subdivided into the presupplementary motor area (preSMA) and the SMAproper (Nakamura, Sakai, & Hikosaka, 1998). The two sub-areas are located adjacently and have a functional relationship (Nachev et al., 2007). The preSMA has been argued to be associated with the initiation and organizing of movement sequences whereas the SMAproper would be associated with the execution of these sequences (Shimizu et al., 2019; Ruitenberg et al., 2014; Verwey et al., 2002). Evidence for this function of the preSMA comes from a study by Nakamura, Sakai and Hikosaka (1998). These researchers monitored neural activity of the preSMA during acquisition of a motor sequence learning task. They reported that before the actual execution of a motor sequence the activity in the preSMA increased significantly even when sequence execution was highly practiced. These results support the assumption that the preSMA is involved in the initiation of a motor sequence by retrieving and organizing a sequence before its execution.

Evidence for the involvement of SMAproper during the execution instead of the preparation of a motor sequences, was obtained by neuroimaging studies. A study by Shima and Tanji (2000) investigated neuronal activity of the SMA in monkeys. The study showed that 64% of the motor related neurons in the SMA were active during motor execution, whereas only 6% of the motor related neurons in the preSMA were active in the same time interval. Further evidence that supports the notion of the SMA as a functional unit for motor execution comes from an animal study using muscimol, a GABA agonist, to inhibit activity of the supplementary motor area. One of these studies involved injecting muscimol unilaterally into the preSMA. The injection induced inactivation of the preSMA and led to a significant

decrease of the ability to learn new motor sequences but the execution of overlearned motor sequence was unaffected. However, a bilateral injection of muscimol into either the preSMA or SMAproper lead to impaired execution of well-learned movement sequences too, supporting the notion of SMA being involved especially in the execution of well-practiced motor sequences (Nachev, Kennard, & Husain, 2008).

Besides evidence from animal experiments, further evidence for the functionality of the SMA was presented by studies employing human subjects. One of these studies was conducted by Wymbs and Grafton (2013). These authors investigated the function of SMA during motor sequence performance. The study used paired-pulse TMS over the SMA to investigate the role of SMA during motor performance of moderately and extensively practiced motor sequences. The results showed that disruption of SMA (a distinction between preSMA and SMAproper was not made) activity led to higher error rates during initiation of motor sequences and also to longer reaction times during sequence execution. This effect was present regardless of amount of practice (low, moderately, extensively). These results reinforce the notion of SMA involvement during sequence learning, production and performance and support the results reported by Verwey et al., (2002).

Further evidence for involvement of SMA during the execution of a DSP task was obtained by Verwey et al. (2019). The authors monitored neuronal activity utilizing functional magnetic resonance imaging (fMRI) during the execution of a DSP task. Furthermore, the authors monitored brain activity throughout the different practice phases of motor sequence learning and performance proposed by the cognitive framework for sequential motor behavior (C-SMB). The results reported by Verwey et al. (2019) showed that SMA was active during all phases of motor sequence learning and execution, but that different parts of the SMA are involved in reacting to individual stimuli and in executing (new and practiced) sequences.

The C-SMB proposes the involvement of two functional processing units during motor sequence execution, a central processor and a motor processor. These processors are involved to a varying extend during different phases of learning and execution of motor sequences. The C-SMB proposes three different modes of motor sequence learning and execution. (1) a reaction mode in which the central processor translates each key specific stimulus into the associated motor response, (2) a central-symbolic mode where the central processor retrieves motor responses from verbal and/or spatial representations of the sequence (e.g., a verbal representation of one´s PIN) and translates these representations into motor responses, and after extended practice (3) the chunking mode where motor sequence-specific representations are formed and executed by the motor processor. So, after extended practice

the central processor becomes less involved during the chunking mode and the motor processor more (Verwey et al., 2019; Verwey et al., 2015).

Research questions

Given the findings discussed above the present study compared the effects of 1 Hz rTMS and 50 Hz cTBS protocols to stimulate the SMA before DSP task performance. We investigated this with the same DSP-task as used by Verwey et al. (2002). This task involves responding to a single sequence-specific stimulus. Not displaying key-specific stimuli was expected to increase the role of the SMA while executing familiar keying sequences.

Research suggests an inhibitory role of both stimulation protocols (Ruitenberg et al., 2014; Huang, 2005; Verwey, 2002). The present study can help to increase experimental efficiency and reduce discomfort of the participants in future studies because the cTBS protocol lasts for only about 3 minutes, whereas rTMS protocols take 20 up to even 45 minutes (Chung, Hoy, & Fitzgerald, 2015). Similar reaction time effects were expected of both interventions. Specifically, it was expected that stimulation of the SMA with rTMS and cTBS would both cause delayed responding in all phases (initiation, execution and concatenation) of motor sequence execution.

As the intensity of aftereffects due to rTMS and cTBS have been found to vary over time (Weisz et al., 2012; Maeda et al., 2000a; Verwey et al., 2002), the aftereffects were examined at three different intervals after stimulation (0-20-40 minutes). These time intervals were chosen based on the results obtained by Verwey et al., (2002) who found different aftereffects, 0 and 20/25 minutes after stimulation. As the effect was not significant after 20 and 25 minutes the present study involved additional testing after 40 minutes to investigate more prolonged effects too. We were interested in whether the 50 Hz cTBS and the 1 Hz rTMS protocol would induce different inhibitory effects after 0, 20 and 40 min.

Methods

Participants

The sample comprised 32 participants (male = 12, female =24) in the age range of 18 to 34 years (M = 25.0, SD = 3.6). The participants were recruited via social media advertisements and were monetarily compensated for taking part in the study or awarded with study credits. In the case of an early withdrawal from the study, the participants were paid proportionally. All participants were right-handed, had normal or corrected to normal visual acuity and were all (fluent in) German. An internal and neurological medical examination revealed a good physical and mental health condition for all participants. The medical examinations included a pre-screening questionnaire (see Appendix 1), control of blood pressure and neurological examination tools to assess coordination, vision, sensory and motor skills (for a complete list see Appendix 2).

The exclusion criteria were in accordance with the general TMS safety guidelines (Rossi et al., 2009). These guidelines indicate exclusion of participants diagnosed with chronic or residual neurological diseases, epilepsy (or prior evidence of epileptic seizure), skull fractures or brain tissue lesions, intracerebral ischemia or bleeding and local or global aphasia. Furthermore, participants with implanted pacemakers or deep brain stimulation were excluded from the study. Finally, alcohol and drug addiction (including nicotine) and the intake of drugs affecting the central nervous system were exclusion criteria.

The study was approved by the ethics committee of IfADo, the Leibniz Research Centre for Working Environment and Human Factors in Dortmund, Germany (proposal number 172). The research conformed the Declaration of Helsinki guidelines. Written informed consent was obtained from all participants.

Design

In the present study a mixed, single-blinded, sham-controlled research design was used to assess effects of stimulation on reaction time (RT) and error rate. One participant group received a 20 minutes 1 Hz rTMS intervention and the other group a 50 Hz cTBS intervention for 40 seconds. Subjects in both groups received a real and a sham stimulation. The sham and real stimulation sessions took place on consecutive days with 24 hours between session interval and their order was counterbalanced across the participants. All participants

were randomly assigned to one of the groups and were kept blind to the stimulation (cTBS/rTMS) and the stimulation order conditions. Every participant performed a baseline block before the intervention and experimental blocks directly after the intervention, 20 minutes and 40 minutes after the intervention.

Apparatus

Stimulus presentation and response registration were controlled by E-prime© 2.0 experimental software package that was installed on a computer running Windows 7. All background applications which could have an effect on the delay rates during response registration were disabled. The computer was disconnected form the internet. The keying sequences were pressed on a standard QWERTZ-keyboard with a fast PS2 connection. The stimuli were presented on an Iiyama HM703UT tube screen with a screen diagonal of 43 centimeter.

rTMS and cTBS were delivered using a Mag & Moore PowerMAG Clinical pp TMS device with a 70 mm figure-of-eight coil. During the 1 Hz rTMS and 50 Hz cTBS interventions the coil was statically placed on the participants' head using a Mag & Moore coil holder. The head of the participant was separately fixated utilizing a vacuum pillow (see Fig. 2).

Figure 2: Setup of the coil holder fixating the stimulation coil on the participants' head. The head of the participant was separately fixated utilizing a (blue) vacuum pillow.

Behavioral Task

As the experimental paradigm a discrete sequence production (DSP) task was used. The same version was used as in Verwey et al. (2002). It involved participants pressing the entire sequence of six keys in response to a single sequence-specific stimulus (one of the following letters: O,X,E,D,G,I,L,M). The participants practiced two letter sequences at home, one 1x6 sequence and one 2x3 sequence. The 1x6 sequences involved bcvnvc, nvbcbv, cbnvnb and vncbcn. The 2x3 sequences involved ncbncb, cvncvn, vbcvbc and bnvbnv. Additionally, each sequence was preceded by a sequence-specific stimulus (for a list of stimuli per sequence, see Appendix 5).

In the test phases, the participants pressed the practiced keying sequences on a keyboard in response to the sequence-specific stimulus. After sequence completion or an error, the next sequence-specific stimulus was presented. Each trial consisted of full sequence performance and trials were separated by a 1500ms interval. The participants were urged to stay below an eight percent error rate during each practice and experimental block. No participant exceeded the eight percent error rate. All sequences were executed with the left hand.

TMS procedure

For rTMS and cTBS the center of the figure-of-eight coil was positioned 3 cm anterior to Cz, according to the international 10-20 system of electrode placement. The junction area of the coil was positioned laterally to the sagittal midline of the participants' head. A recent guideline for psychiatric treatment with TMS suggests coil placement 3 cm anterior to Cz for focal stimulation of SMA-proper (Lefaucheur et al., 2020). In comparison to Verwey (2002) who used FCz (10% of the distance between inion and nasion i.e. 4 cm anterior to Cz) as a reference for TMS coil position for SMA-proper stimulation, the present study adhered to the suggestions of the most recent guidelines published by Lefaucheur et al. (2020). For the sham condition, a sham coil was used which produced the same sound but no magnetic pulse. The stimulation intensity for cTBS was defined as 80% (Huang, 2005) of the individual's active motor threshold (AMT), whereas the stimulation intensity for rTMS was defined as 90% (Ziemann et al., 1998) of the individual's resting motor threshold (RMT).

Baseline measurement: In order to determine RMT and AMT, the cortical "motor hotspot" of the musculus abductor digiti minimi (ADM) was determined for each participant

using electromyography (Sohn et al., 2004; De Gennaro et al., 2003). Bipolar electrodes were attached to the tendon of the right little finger and ADM. Subsequently the location of the left motor cortex was searched in steps of 1 centimeter starting at Cz until a reliable muscle response was recorded by the EMG. For this exploration, the figure-of-eight TMS coil was used while producing single pulses in five second intervals. When the "motor spot" was found the intensity of the magnetic pulse of the TMS coil was adjusted until the recorded amplitude of the muscle evoked potential (MEP) was approximately 1µV. Then 25 pulses were recorded and the amplitudes of the MEP's were averaged. This procedure was repeated twice. Only if the averaged amplitude of the recorded MEP´s was between 0.85 µV and 1.15 µV with a standard deviation of at least half of the mean for all recordings, the motor hotspot location was regarded reliable.

When the motor hotspot was determined, the RMT or AMT (based on the condition) was measured. For RMT determination the TMS Motor Threshold Assessment Tool (MTAT 2.0, http://www.clinicalresearcher.org/software.htm) was used. The software proposes various TMS-pulse intensities to apply on the motor hotspot at the participants' head. The software measures the intensities of the EMG responses to the given pulses and estimates the 95%-confidence interval for the resting motor threshold based on the collected data. For determining the AMT, the participant was instructed to press the little finger of the right hand onto the tabletop with maximum force. The force amplitudes of these actions were visible on a screen for both the researcher and the participant. Subsequently, the participant was instructed to press the little finger with 20% of the average maximum force onto the tabletop as indicated on the screen. When the pressing force was reached TMS pulses were applied over the predetermined motor hotspot. The active motor threshold was reached when 3 out of the 6 administered pulses led to a muscular reaction which was defined as amplitudes of the MEP´s higher than the 20% maximum force described before.

cTBS protocol: cTBS was executed utilizing stimuli bursts (three stimuli per burst at 50 Hz, 20ms inter-stimuli interval) with an inter-burst interval of 5 Hz resulting in a total of 5 bursts of 3 stimuli per second. This protocol was executed for 40 seconds and resulted in a total of 600 pulses throughout the entire experiment (see Fig. 3).

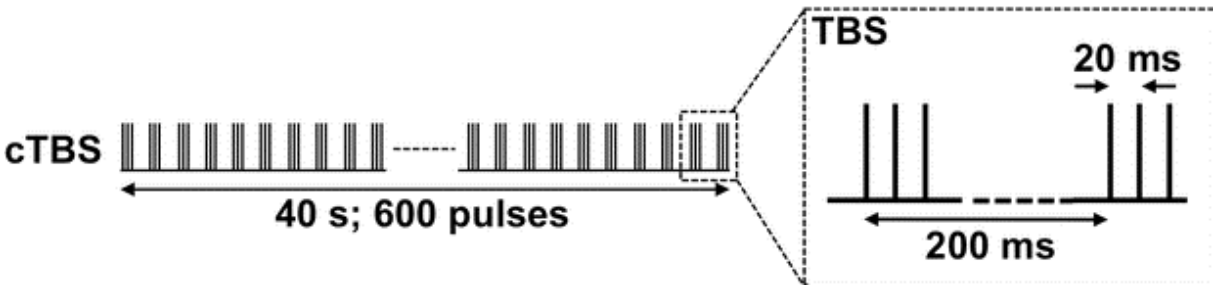

Figure 3: Graphical representation of the course of the continuous theta burst (cTBS) protocol showing the length (ms) of the inter-burst interval (5 Hz; 200ms) and intra- burst stimuli (50Hz; 20ms) The 40 seconds application of the protocol led to 600 pulses in total. Retrieved from Wu et al. (2018).

rTMS protocol: The frequency used for the rTMS protocol was 1 Hz. 1 Hz rTMS was administered for 20 minutes and resulted in 1200 pulses throughout the experiment.

Experimental Procedure

Prior to the first experimental session, two series of six letters (one 1x6 and one 2x3) and the sequence-specific stimuli were given to the participants. The participants were instructed, before they would come to the institute, to learn two letter series consisting of the stimulus letter (taken from the set OEGLXDIM) followed by the 6 letters indicating the response sequence (including bcvnvc, ncbncb, nvbcbv, cvncvn, cbnvnb, vbcvbc, vncbcn, bnvbnv). As the participants arrived at the institute an oral explanation of the study procedure was presented. After the oral explanation, they received a written description of the course of events in the study and signed the informed consent. Subsequently, every participant was medically examined to ensure an appropriate health condition. After they filled out the questionnaire, the participants were instructed to verbally reproduce the learned sequences that were given to them before the start of the experiment. If they were not able to reproduce the sequences verbally four times without an error, they received 15 additional minutes for learning and were retested.

The next step was the identification of the individual stimulation intensity as described before. The participants were seated in a chair designed for TMS application and their head was fixated. When the motor hotspot was found, it was marked on the participants' head. Subsequently the ATM was determined for participants in the cTBS condition whereas for participants in the rTMS condition the RMT was determined. Subsequently, the location of the SMA was determined by identifying Cz and marking the spot of SMA three centimeter anterior to Cz with a waterproof marker. After this, the participants were seated in front of the experimental setup. Next, the task instructions were given, and the participants started

practicing the sequences by pressing the keys in response to the sequence-specific stimulus. The little, ring, middle and index finger of the left hand represented the keys c, v, b and n, respectively. Participants practiced each of the two sequences 210 times in randomized order (cf. Verwey et al., 2002). The practice trials were divided in three practice blocks containing 140 trials. Each block was interrupted by a break of 5 minutes, while there also was a 20-s break in the middle of each block.

When participants finished the practice blocks, the first experimental block was carried out. The experimental block contained 20 trials per sequence in a randomized order (40 trials in total). The procedure of reacting to the sequence-specific stimulus by pressing the corresponding keying sequence was identical for the experimental as for the practice blocks. The first block served as baseline for the subsequent experimental blocks. After assessing the baseline, stimulation took place. Before actual stimulation the head of the participant was fixated again. For the stimulation, the coil holder was set up and the coil was fixated over the SMA. The stimulation was administered in front of the experimental setup in order to avoid the participant from moving after stimulation. Next the cTBS/sham or rTMS/sham protocol was applied based on the participants' assigned experimental and stimulation condition. The order of stimulation and sham-stimulation was counterbalanced across days and groups (cTBS vs. rTMS).

Directly after the stimulation, the second experimental block was executed by the participant while the subsequent third and fourth experimental blocks were executed 20 and 40 minutes after the end of the TMS intervention. During the execution of all blocks the light in the room was dimmed.

After the experimental blocks on Day 1 had been finished the participants were thanked for their cooperation and were instructed about the next experimental session. This instruction included to desist from washing their hair to preserve the marked "motor hotspot" and the mark for the location of SMA on the participants head in order to use the same locations in the next experimental session. At the beginning of Day 2, a new AMT or RMT was determined using the preserved mark of the "motor hotspot" on the participants' head. The procedure was identical to the procedure on Day 1.

After determining the RMT or AMT, the first test block was executed to assess a new baseline performance. Subsequently, the stimulation protocols as described for Day 1 were applied over the SMA. The next step was the execution of the remaining three experimental blocks.

After completion of the experimental blocks the participants executed an awareness test on the computer. The awareness test assessed whether the participants could explicitly recall the learned sequence when the sequence is presented in a temporal and spatial order. The test was performed on the same experimental setup as the experimental paradigm. During the awareness test the keyboard was covered to ensure that the participants rely on memory recall instead of recognition. (for procedure overview see Fig. 4). Finally, the participants were paid and thanked for their participation. All experimental procedures took place under Covid-19 related safety measures.

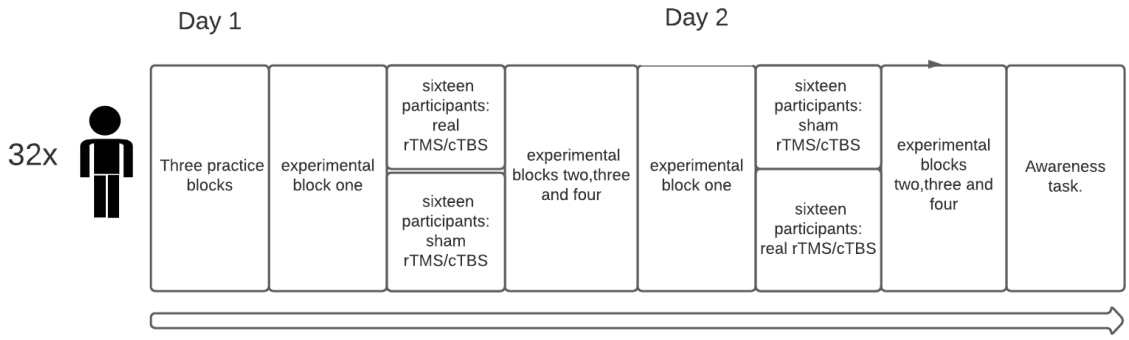

Figure 4: Procedure of the experiment excluding preparatory steps (sequence learning, medical examination, determination of AMT and RMT). 32 subjects participated in the experiment. Three practice blocks were executed and included 140 sequences per block. Experimental block one was used as baseline performance measurement. Sixteen participants received real 1Hz repetitive transcranial magnetic stimulation (rTMS) or 50 Hz continuous theta burst stimulation (cTBS) and 16 participants received sham rTMS or cTBS on day one. The participants who received real rTMS or cTBS on day one, received sham rTMS or cTBS on day two. The participants who received sham rTMS or cTBS on day one, received real rTMS or cTBS on day two. All experimental blocks included 40 sequences per block and were executed at different timepoints (zero,20 and 40 minutes after the application of the real or sham stimulation).

Awareness task

After the end of the last experimental block, the participants performed an awareness task on the computer that included two tests. Furthermore, five questions regarding their used cognitive strategies during the conductance of the awareness task and their past experience with similar tasks were included. During both tests, participants clicked with the mouse six successive element-specific squares on the display in the order they thought they had pressed keys. During the spatial awareness test, the mentioned elements were displayed as four-square placeholders lined up next to each other's, like in the practice and experimental blocks. The participants were asked to click the two sequences they had learned and executed throughout the experiment in the same succession with the computer mouse. Each placeholder

represented one key (c,v,b,n). During the verbal awareness test, four placeholders were displayed at the top, left, bottom and right across the screen in a rhombus shape. Each placeholder contained one of the letters of the two sequences (c, v ,b , n). The participants were asked to click the placeholder based on the succession of the two learned sequences.

Data Processing:

The mean reaction time (RT) for every participant, and every sequence and key press was calculated for each practice and experimental block. RT was defined as the time interval between sequence-specific stimulus presentation and the initiation (first key press) of the associated sequence. Further RT´s were defined as the intervals between successive key presses throughout the sequence. Sequences containing an error (an error leads to abortion of the key pressing sequence) were excluded from the analysis process. No outliers were detected by means of boxplot visualization (see Appendix 3) and no further data was excluded from the data set.

A mixed ANOVA was used on the proportions of correctly performed sequences as an estimate for differences of error rates. An arcsine transformation reduced skewness of the data and meeting the assumptions of the ANOVA test. All reported pairwise comparisons were tested by means of paired samples t-tests with Bonferroni correction. No outliers were detected by the means of boxplot visualization (see Appendix 4). Thus, no outliers were removed. Data preparation and cleaning was done using E-Prime 2.0-DataAid, R and Microsoft Excel. Greenhouse-Geisser corrections are reported when sphericity assumptions were violated.

Results

No adverse events occurred during the application of the different non-invasive brain stimulation procedures. The stimulation procedures were well tolerated by the participants.

Practice phase

Reaction Times

A mixed ANOVA on RTs was carried out with Group (2: rTMS vs. cTBS) and Stimulation Order (2: first day sham stimulation and second day real stimulation (SR group)

vs. first day real stimulation and second day sham stimulation (RS group)) as between-subject variables and Block (3), Sequence Structure (2: 1x6 vs. 2x3), and Key (6) as within-subject variables. None of the Group effects showed significant results. For Block a significant main effect was detected, $F(1.23, 34.52) = 177.35$, $p < 0.01$, $\eta_p^2 = 0.86$, implying faster RTs across successive blocks (Block 1: M = 435ms, SE = 18ms, Block 2: M = 325ms, SE = 14ms, Block 3: M = 305ms, SE = 12ms). A Sequence Structure main effect showed that the 1x6 sequence was executed slower than the 2x3 sequence (405ms vs. 308ms), $F(1,28) = 41.82$, $p < 0.01$, $\eta_p^2 = 0.60$. Furthermore, a significant interaction of Block and Sequence Structure was revealed, $F(1.28, 35.74) = 12.06$, $p < 0.01$, $\eta_p^2 = 0.30$, which showed faster execution of the 2x3 sequence by Block compared to the 1x6 sequence (Block 1: M = 72ms, SE = 11ms, Block 2: M = 37ms, SE = 6ms, Block 3: M = 37ms, SE = 7ms).

In addition, a significant Key main effect was found, $F(1.56, 43.56) = 209.74$, $p < 0.01$, $\eta_p^2 = 0.88$, showing increased reaction times at Response 1 (R_1) to R_2 (M = 549ms, SE = 36ms, $p < 0.01$) and R_3 to R_4 (M = 88ms, SE = 16ms, $p < 0.01$) implying longer reaction times during the initiation and the proposed concatenation point during sequence execution.

Accuracy

Error rates were investigated utilizing a mixed ANOVA with Group (rTMS vs. cTBS) and Stimulation Order as between-subject factors and the within-subject factors Block (3), Sequence Structure (1x6 vs. 2x3) and Key (6). The ANOVA was performed on the arcsine transformed error proportions of performed sequences.

No significant main effects were found for the between subject factors Group and Stimulation Order and the associated interactions, implying no performance differences between experimental groups.

A significant main effect was found for Block, $F(1.55,43,43) = 5.03$, $p = 0.01$, $\eta_p^2 = 0.15$, indicating an increasing error rate with practice. Furthermore, a main effect was found for Sequence Structure, $F(1,28) = 11.81$, $p = 0.02$, $\eta_p^2 = 0.30$, showing a higher error rate for the 1x6 sequence compared to the 2x3 sequence (1x6: 1.23%, 2x3: 0.48%). Also, the interaction of Sequence Structure and Block showed higher error rates within the 1x6 sequence compared to the 2x3 sequence regardless of Block, $F(1.51, 42.28) = 23.58$, $p < 0.01$.

A significant main effect of Key indicated differences in error rates based on the executed key press, $F(3.61, 101.18) = 14.18$, $p < 0.01$, $\eta_p^2 = 0.34$. Pairwise comparisons showed an increased error rate comparing R_1 to R_2 implying a higher error rate at the initiation

point (R_1: M = 2.23%, SE =0.13%: R_2: M = 1.1%, SE = 0.11%, $p < 0.01$). At last, the interaction of Key and Sequence Structure revealed significant differences of error rates based on the key press and the executed sequence structure, $F(3.79,106.23) = 2.79$, $p = 0.03$, $\eta_p^2 = 0.34$. Pairwise comparisons revealed higher error rates for both sequence structures at R_1 (2x3, R_1: M = 2.17%, SE = 0.30%, R_2: M = 0.72%, SE = 0.10%, $p = 0.03$; 1x6, R_1: M = 2.24%, SE = 0.32%, R_2: M = 1.30%, SE = 0.20%, $p < 0.01$). This indicates that during the practice phase, the participants made more errors during the initiation of a sequence compared to the subsequent key presses. Besides this similarity, a difference was revealed, namely an increased error rate during the execution of the 1x6 sequence at R_5 compared to the 2x3 sequence (2x3, R_4: 1.10%, SE = 0.18%; R_5: M = 0.80%, SE = 0.13%, $p = 1.00$; 1x6, R_4: M = 1.10%, SE = 0.13%; R_5: M = 2.40%, SE = 0.33%, $p = 0.01$), implying a higher error rate during the performance of the 1x6 sequence after the concatenation point at R_4.

Test phase

Reaction Times

A mixed ANOVA on RTs with Group (rTMS vs. cTBS) and Stimulation Order (2: first day sham stimulation and second day real stimulation (SR group) vs. first day real stimulation and second day sham stimulation (RS group)) as a between-subject variable was conducted. As within-subject variables, Stimulation (2: Real vs. Sham), Sequence-structure (2: 1x6 and 2x3), Delay (4: Baseline, 0, 20, and 40 min) and Key (6) were included.

The visual inspection of the data suggests an inhibitory effect on task performance for both stimulation protocols for all delays after the application of the stimulation (see Fig. 5). However, the interaction of Group, Stimulation and Delay revealed that the suggested inhibitory effects were not statistically significant, $F(2.50,70.02) = 1.04$, $p = 0.37$, $\eta_p^2 = 0.04$.

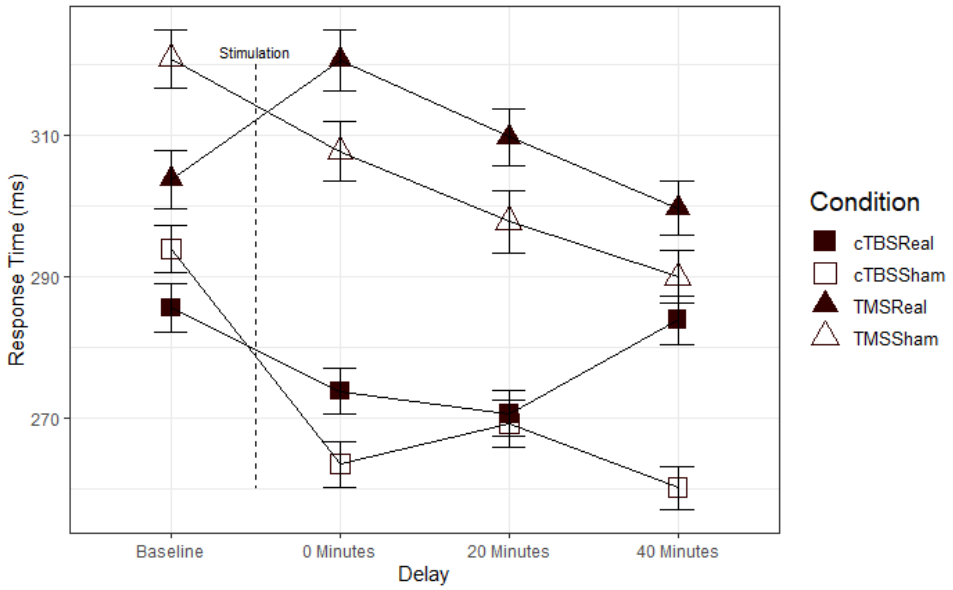

Figure 5: The Figure above shows the overall effects of the stimulation without the distinction between days Averaged response times per block and condition are presented in milliseconds (ms). The vertical line after Baseline represents the intervention. The error bars show the standard errors of the means.

Besides the reported non-significant effects, a significant main effect of Stimulation Order revealed a generally faster task performance of the group that received the real stimulation on day two and the sham stimulation on day one (SR group) compared to the group that received the real stimulation on the first day and the sham stimulation on the second day (RS group), $F(1,28) = 5.35$, $p = 0.03$, $\eta_p^2 = 0.16$ (SR: $M = 264$ms, RS: $M = 318$ms). The interaction of Stimulation Order and Stimulation revealed significantly slower reaction times, when the real stimulation was applied on the first day compared to the sham stimulation on the second day, $F(1,28) = 27.68$, $p < 0.01$. This effect of the stimulation was absent when the real stimulation was applied on day two (see Fig. 6). This result shows that the effectiveness of the stimulation was dependent on the day of its application.

Additionally, the interaction of Stimulation Order, Stimulation and Delay indicated slower performance based on the day of the stimulation application and the delays of task performance, $F(2.50,70.02) = 7.27$, $p < 0.01$, $\eta_p^2 = 0.21$. This effect was present regardless of the applied stimulation protocol, $F(2.50,70.02) = 0.22$, $p = 0.88$, $\eta_p^2 = 0.01$. A pairwise comparison for the interaction of Stimulation Order, Stimulation and Delay compared by day of the received stimulation showed that the task was performed significantly slower after the real stimulation on day one compared to the sham stimulation on day two (0 min: $M = 27$ms, $SE = 9$ms, $p = 0.04$, 20 min: $M = 35{,}41$ms, $SE = 10$ms, $p = 0.01$, 40 min: $M = 46$ms, $SE = 11$ms, $p = 0.01$). However, no significant effect of the stimulation was revealed when the real stimulation was applied on day two and the sham stimulation on day one. The interaction of

Stimulation Order, Group, Stimulation and Delay revealed no significant effect showing that the effects of stimulation were independent of the applied stimulation protocol. These results support the assumption of inhibitory effects of both protocols (see Fig. 6). However, the visual inspection revealed different inhibitory effects, namely that the cTBS protocol produced the strongest inhibitory aftereffects after 40 minutes whereas the rTMS protocol produced the strongest aftereffects directly after the stimulation. (see Fig. 6). To summarize, these results imply that the day of the stimulation is a critical factor for the effectiveness of both protocols.

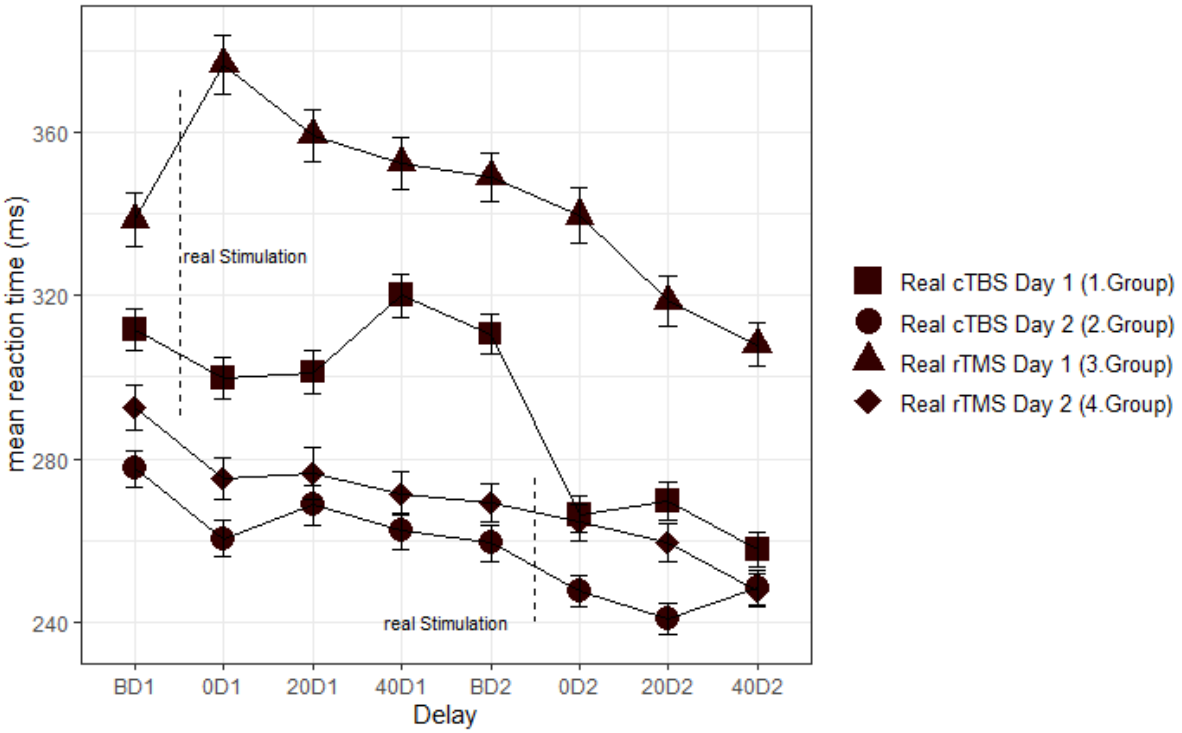

Figure 6: Effects of the stimulation based on the day the stimulation was received. Averaged response times are presented in milliseconds (ms)The error bars represent the standard error of the mean. The abbreviations on the x-axis indicate the Delay (BL: B;0 Minutes: 0; 20 Minutes: 20, 40 Minutes:40) and the day of the testing (Day 1: D1, Day 2: D2). The vertical dotted lines show the timepoint of real stimulation application (real rTMS and real cTBS).

Besides the effects of Stimulation, a main Sequence Structure effect showed a higher mean RT in the 1x6 Sequence (296ms) than in the 2x3 Sequence (274ms), $F(1,28) = 25.77$, $p < 0.01$, $\eta_p^2 = 0.48$, implying a faster execution of the 2x3 sequence compared to the 1x6 sequence. Furthermore, the interaction of Sequence Structure, Stimulation and Stimulation Order indicated performance differences between the sequence structures based on the stimulation and the day the real stimulation was received., $F(1,28) = 18.28$, $p < 0.01$, $\eta_p^2 = 0.40$. Pairwise comparison showed significantly slower performance during the execution of the 1x6 sequence in the RS group ($M = 31$ms, $SE = 10$ms, $p < 0.01$) when the real stimulation was applied, compared to the sham stimulation where no significant performance difference

between the two sequences was observed (M = 12ms, SE = 10ms, p = 0.25). For the SR group, a generally slower execution of the 1x6 sequence was observed regardless of the application of the real or sham stimulation (real: M = 36ms, SE = 10ms, $p < 0.01$; sham: M = 60ms, SE = 10ms, $p < 0.01$). Overall, these results support the assumption of stimulation effects only on day one.

Also, a Key main-effect indicated varying RT´s based on the executed key press, $F(1.48,41.56)=198.43$, $p < 0.01$, $\eta_p^2 = 0.88$. Pairwise comparisons showed significantly slower RT´s during the initiation of the sequence (R_1 -R_2: M = 433ms, SE = 29ms, $p < 0.01$). Also, the supposed phenomenon of the concatenation point was observed at R_4 (R_4-R_3: M = 48ms, SE = 13ms, p = 0.01; R_4 – R_5: M = 68ms, SE = 13ms, $p < 0.01$). In addition to the main effect of Key, the interaction of Sequence Structure and Key revealed slower performance at specific key presses, $F(2.99,83.68)=17.10$, $p < 0.01$, $\eta_p^2 = 0.38$. A pairwise comparison between keypresses showed increased RT´s at R_4–R_5, implying slowed performance at the proposed concatenation point. Whereas the R_4-R_5 performance did not differ for the 2x3 sequence (M = 46.59, SE = 15.55, p = 0.09), significantly increased RT´s were detected for the 1x6 sequence (M = 90ms, SE = 15ms, $p < 0.01$). This effect showed a slower reaction after the proposed concatenation point for the 1x6 sequence compared to the 2x3 sequence where this effect was not observable. This effect was observable for the RS and the SR group (RS: 1x6: M = 69ms, SE = 21ms, p = 0.04; 2x3: M = 53ms, SE = 22ms, p = 0.34; SR: 1x6: M = 111ms, SE = 21ms, $p < 0.01$; 2x3: M = 40ms, SE = 22ms, p = 1.00). Overall, the reported effects validate the assumption of different phases of motor sequence execution shown by the increased RT´s at R_1 (initiation) and R_4 (concatenation).

Accuracy:

A mixed ANOVA with Group (rTMS vs. cTBS) and Stimulation Order as between subject factors and the within subject factors Stimulation (2), Sequence Structure (2), Delay (4), and Key (6) was performed on the arcsine transformed error proportions, as an indicator of accuracy. The main effects of Group and Stimulation Order and the associated interaction revealed no significant effects, implying no differences of error rates between the experimental groups. A main effect for Key revealed a difference of error rates between the executed key presses, $F(3.02,84.41) = 8.71$, $p < 0.01$, $\eta_p^2 = 0.23$. A pairwise comparison of keypresses showed a significantly higher error rate after the proposed concatenation point at R_5 to R_6 (R_5: M = 0.59%, SE = 0.25%; R_6: M = 0.47%, SE = 0.08%, p = 0.03). Also, the

interaction of Stimulation and Key revealed an increased error rate at specific key presses due to the stimulation, $F(3.89,108.96) = 2.60$, $p = 0.03$, $\eta_p^2 = 0.09$. Pairwise comparisons of the interaction showed that the real stimulation increased the error rates for R_4 to R_5, ($M = 1.05\%$, $SE = 0.30\%$, $p = 0.03$), indicating an increased error rate at the concatenation point due to the stimulation. This effect was absent for the sham stimulation ($M = 0.17\%$, $SE = 0.26\%$, $p = 1.00$). Besides the mentioned effect, Stimulation had no effect on the error rates of the participants. Furthermore, the interaction of Sequence Structure and Key showed significant differences in error rates between the 1x6 and 2x3 sequence at specific key presses, $F(3.64,101.97) = 6.86$, $p < 0.01$, $\eta_p^2 = 0.20$. Pairwise comparisons of the keypresses revealed increased error rates during the execution of the 2x3 sequence for R_2 to R_3 (R_2: $M = 0.24\%$, $SE = 0.08\%$; R_3: $M = 0.99\%$, $SE = 0.21\%$, $p = 0.02$). In comparison higher error rates were observed for R_4 to R_5 during the execution of the 1x6 sequence, (R_4: $M = 0.37\%$, $SE = 0.19\%$; R_5: 2.37%, $SE = 0.42\%$, $p = 0.01$).

Awareness test

The results of the awareness test showed that 3 of the 16 participants in the rTMS group reproduced the 2 sequences without an error, in both the spatial and verbal tests. Also, 3 participants in the cTBS group reproduced the 2 sequences perfectly during the execution of all tasks. To investigate possible differences, a nonparametric mixed 2 (Group: cTBS vs. rTMS) x2 (Task) x2 (Sequence Structure) ANOVA with Group as between-subject variable on the numbers of correct responses per sequential position using the F1-LD-F2 design (of the nparLD package, Noguchi, Gel, Brunner, & Konietschke, 2012) was conducted in R Studio.

The results showed no difference at the Group level, $WTS(1) = 0.29$, $p = 0.59$, indicating no significant difference of the number of correct responses between the rTMS and cTBS group. However, a significant difference was revealed between the spatial and verbal task, $WTS(1) = 5.63$, $p = 0.02$. The obtained means showed more correct responses during the execution of the spatial task ($M = 5.36$ correct responses, with a maximum of 6 possible correct responses) compared to the verbal task ($M = 4.55$ correct responses, with a maximum of 6 possible correct responses).

Furthermore, significant differences were shown for the factor Sequence Structure, $WTS(1) = 4.46$, $p = 0.03$, indicating a significant difference of correct responses between the 1x6 and 2x3 sequence. The obtained means showed a higher rate of correctly performed responses for the 2x3 sequence ($M = 5.22$ correct responses, with a maximum of 6 possible

correct responses) compared to the 1x6 sequence (M =4.69 correct responses, with a maximum of 6 possible correct responses).

As the main results of this study showed a significant performance difference based on Stimulation Order (2: RS vs. SR), a further nonparametric mixed 2 (Stimulation Order) x 2 (Task) x 2 (Sequence Structure) ANOVA with Stimulation Order as a between-subjects variable on the number of correct responses per sequential position was conducted. The analysis showed no significant effect of Stimulation Order on the number of correctly performed responses, $WTS(1) = 0.09$, $p = 0.76$, implying no difference between the RS and SR group. As for the previously described nonparametric ANOVA, the results of the present ANOVA indicating a significant difference between the correctly performed sequences based on Task, $WTS(1) = 5.92$, $p = 0.02$ and Sequence Structure, $WTS(1) = 4.25$, $p = 0.04$. Thus, no overall differences between the SR and RS group exist.

Cognitive strategies during sequence reproduction

During the execution of the spatial task, 10 participants indicated to have remembered the order of the letters within the sequence, whereas 11 participants said they tapped the sequences in their mind. Six participants answered that they remembered the position of the squares on the screen and the associated position of the key. Two participants tapped the sequences on the table top and three participants just guessed. 13 of these participants were "very certain" about their correct responses, 9 stated they were "a little certain", seven "a little uncertain" and three subjects answered "very uncertain".

During the execution of the verbal task, 14 participants remembered the order of the letters within the sequence, whereas eight participants tapped the sequences in their mind. Five participants remembered the position of the squares on the screen and the associated position of the key. No participant tapped the sequences on the table top and five participants just guessed. Thirteen of these participants stated that they were "very certain" about their correct responses during the task, seven stated they were "a little certain", four "a little uncertain" and eight participants answered with "very uncertain".

Based on the reported results, the participants used different cognitive strategies to determine the successions of keypresses that they needed to press during the execution of the spatial and verbal task. At last, the participants were asked whether they had participated in a similar experiment (including key pressing sequences) during the last few months or years. Twenty-nine of the participants had not participated in a similar experiment before. However,

three participants answered that they had participated in an experiment including key pressing sequences, but with different sequences.

Discussion

The aim of the study was to investigate whether the 1 Hz rTMS and 50 Hz cTBS protocols would induce different inhibitory aftereffects implied by varying reaction times and error rates during DSP task execution when SMA is the target of stimulation. The stimulation parameters were chosen based on previous research that showed its inhibitory effects on cortical activity (e.g. Casula et al., 2014; Huang et al., 2005; Verwey et al., 2002) and therefore, the potential for functional investigation of brain structures (e.g., SMA). Also, taken into account the clinical relevance for treatment of neurological disorders (e.g., depression or stroke). However, whereas rather consistent evidence for the inhibitory effects of 1 Hz rTMS protocol is reported in the literature (e.g., Casula et al., 2014), inconclusive evidence regarding the same effects of the 50Hz cTBS protocol exists (e.g. Hamada et al., 2012; Huang et al., 2005). Thus, the present study examined the aftereffects of both stimulation protocols, in the context of motor sequence learning and performance to compare the effects of both protocols within the same experiment.

The results showed an inhibitory effect based on the day of stimulation application, indicating an inhibitory effect when applied on the first day compared to non-significant effects of the stimulation when applied on day two. Compared to the effects when stimulation was applied on the first day, no inhibitory effects of the stimulation were observed when the stimulation was applied on the second day.

Stimulation effects on day one

The results for the application of the 1 Hz rTMS protocol on day one showed an inhibiting effect on motor performance after the stimulation compared to the sham stimulation. This finding supports the assumption of inhibitory effects of 1 Hz rTMS reported in the literature (e.g., Casula et al., 2014). As the present study is a direct replication of the study by Verwey et al., (2002), a comparison of the study outcomes is made in the next paragraphs. Verwey et al., (2002) observed that the application of 1 Hz rTMS over SMAproper impeded the improvement of motor sequence performance. A similar effect of 1 Hz rTMS was found in the present study supporting the assumptions made by Verwey et al., (2002).

However, besides the similarity of impeded motor performance after active stimulation of SMAproper, a difference was observable between the effects of 1 Hz rTMS in the present study. More specifically, an increase of reaction times after the real stimulation was revealed in the present study, but was not shown in the study by Verwey et al., (2002). One possible explanation for the increased reaction time after the application of 1 Hz rTMS could be procedural differences between the two studies. The first procedural difference was the determination of the stimulation intensity. In the study by Verwey et al., (2002) the stimulation intensity was set to 90% of the motor threshold. The motor threshold was defined as the intensity at which a muscular reaction of the hand or thumb area was observable when single TMS pulses were applied to the hand area of M1. In the present study, the stimulation intensity was set to 90% of RMT defined by EMG measurements. During this procedure no visible muscular reaction was observable even at 100% RMT intensity. The observed muscular reaction observed in the study of Verwey et al. (2002) indicates that a stronger magnetic pulse was applied to the hand area of M1 compared to the applied pulses in the present study, where this visible muscle reaction was absent. This difference of the intensity could have led to the observed reaction time differences after stimulation.

A second procedural difference was the coil position for the stimulation of SMAproper. Whereas Verwey et al. (2002) chose a position of 10% the distance between inion and nasion i.e., 4cm anterior to Cz, the present study chose a coil position of 3cm anterior to Cz. As it is mentioned in the introduction, varying parametrization e.g., the position of the coil as well as the intensity of the applied pulses can lead to varying stimulation aftereffects (Lee et al.,2018; Klomjai et al., 2015).

In line with the results obtained for the application of the 1 Hz rTMS protocol on day one, the results shown for the 50 Hz cTBS seem to further support the assumption of motor sequence performance inhibition after the active stimulation. However, whereas 1 Hz rTMS produced an increase of reaction times directly after the stimulation, no reaction time increase was observable for the 50 Hz cTBS until 20 minutes after the intervention. However, no critical increase of performance could be observed for this group throughout the mentioned blocks compared to the sham stimulation on day two, where an increase of task performance was observable throughout the test blocks. This result reinforces the assumption of inhibited motor sequence performance during the conductance of the DSP task when SMA is the target of the stimulation utilizing 50 Hz cTBS. However, after forty minutes an increase of reaction times was observed, indicating a non-linear effect of time on aftereffects. This pattern of

aftereffects might be explained by the high inter-individual variability observed for the application of 50Hz cTBS (e.g., Hamada et al., 2012).

Hamada et al. (2012) tested two TBS protocols that were proposed by Huang et al. (2005), namely intermittent theta burst stimulation (iTBS) and cTBS. Compared to the proposed inhibitory effects of cTBS on cortical activity, iTBS is thought to induce faciliatory effects on cortical activity. Hamada et al., (2012) observed that only one quarter of the participants responded in the expected manner to both stimulation protocols. Only one quarter of the participants showed faciliatory effects after the application of iTBS and inhibitory effects after the application of cTBS. Furthermore, the results indicate that under a half of the participants responded to one of the protocols in the expected manner, but not to the other protocol. Thus, this varying effectiveness of the TBS protocol could have led to the aftereffects pattern observed in the present study. However, the underlying mechanisms of this pattern are yet to be investigated.

Stimulation effects on day two

Compared to the stimulation effects on day one, no inhibitory effects of the stimulation were observable on day two regardless of the applied stimulation protocol. A possible explanation for this non-significant effect of the stimulation on day two could be the consolidation of motor memories due to sleep. The consolidation of motor sequence memories after sleep was examined by Nettersheim et al., (2015). The authors investigated how sleep affects the performance of a new learned motor sequence task (finger sequence tapping task). They concluded, that sleep stabilized the new learned motor sequences, but without further improvements as it was often proposed in the literature (e.g., Walker et al., 2003). However, this stabilization of motor memories decreases the susceptibility for memory disruptions by procedures like rTMS (Krakauer, & Shadmehr 2006) and is thus a possible explanation for the absence of effects after the application of the stimulation on day two. A study by Kim et al., (2021) directly investigated the effects of non-invasive brain stimulation techniques (transcranial direct current stimulation) on motor consolidation. The results showed that faciliatory stimulation applied to M1 improved motor consolidation. Kim et al., (2021) conclude that increased neural activity caused by the faciliatory stimulation led to better motor consolidation. Thus, decreased neural activity of the SMA during task performance might be a reason for disrupted motor consolidation after task performance.

However, studies should further investigate the effects of non-invasive brain stimulation on motor consolidation as not a lot of research is available at this timepoint.

Limitations and directions of future research

One limitation of the present study is the inter-individual variability often observed between subjects (e.g., Hamada et al., 2012). Therefore, future studies should choose a full within-subject design to compare the effects of both stimulation protocols within the same sample. Additionally, future experiments should separate the practice phase from the testing phases to investigate the effects of consolidation on the effectiveness of non-invasive brain stimulation protocols. Furthermore, the experimental sessions should be separated by a time interval of at least one week in order to reduce carry-over effects of the stimulations.

At last, a more individualized study design would be appropriate, meaning the participants individual anatomical structure should be revealed by means of fMRI scans to localize SMA more efficiently.

Conclusion

The present study showed that both stimulation protocols produce inhibitory aftereffects on cortical excitability when the stimulation is applied on the first day, whereas these inhibitory aftereffects were not observable after the stimulation application at day two. These differences of inhibitory aftereffects based on the day of stimulation application might be explained by the stabilization of motor sequence memories due to sleep. Furthermore, it was shown that the pattern of aftereffects differed for the two stimulation protocols when applied on day one, namely for 1 Hz rTMS increased reaction times compared to baseline were observable directly after the stimulation and throughout all delays, whereas this effect for cTBS was only observable after 40 minutes when stimulation was applied on day one. Follow up studies should address this consolidation effects and the disruptive (or not disruptive) potential non-invasive brain stimulation techniques (e.g., TMS) within the motor sequence learning and performance domain. Additionally, the differences of aftereffects of 1 Hz rTMS and 50 Hz cTBS should be further investigated in this context.

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Appendix

Appendix 1: Medical examination pre-questionnaire

SCREENING FORMULAR NON-INVASIVE BRAIN STIMULATION

Studie:

Proband:

Dieses Formular muss vor Beginn des Experimentes komplett vom Versuchsteilnehmer ausgefüllt werden!

Bitte beantworten Sie die folgenden Fragen	**Ja**	**Nein**
1. Sind Sie unter 18 oder über 34 Jahre alt?		
2. Sind Sie schwanger oder besteht das Risiko, dass Sie schwanger sind?		
3. Hatten Sie jemals eine Gehirnverletzung, welche als Gehirnerschütterung diagnostiziert oder von einem Bewusstseinsverlust begleitet wurde?		
4. Hatten Sie jemals eine Gehirnoperation?		
5. Haben Sie irgendwelche Metallteile in/ an Ihrem Gehirn, Schädel oder sonst irgendwo an Ihrem Körper (z.B. Kleinteile, Clips etc.)? Wenn ja, geben Sie bitte den Ort und die Art des Metalls an:		
6. Haben Sie einen Schrittmacher oder intrakraniale Kabel?		
7. Haben Sie einen implantierten Neurostimulator (z.B. Tiefenhirnstimulator, epidural/ subdural, Vagus Nervenstimulation)?		
8. Haben Sie einen implantierten medizinischen IV?		

9. Leiden Sie unter Epilepsie oder hatten Sie je einen Anfall?		
10. Haben Sie unmittelbare Familienangehörige (Eltern, Geschwister, Kinder), die an Epilepsie leiden oder jemals einen Anfall hatten? Falls ja, geben Sie bitte weitere Informationen zu Verwandtschaft und Zeitpunkt an:		

11. Haben Sie eine Hautkrankheit oder Hautallergie? Falls ja, welche?		

Wenn Sie auf eine der oben genannten Fragen mit JA geantwortet haben, können Sie NICHT an diesem Experiment teilnehmen.

12. Haben Sie jemals TMS bekommen? Wenn ja, wann war das letzte Mal? Wie oft im letzten Monat? Gab es irgendwelche Probleme?		
13. Haben Sie jemals tDCS oder tACS bekommen? Wenn ja, wann war das letzte Mal? Wie oft im letzten Monat? Gab es irgendwelche Probleme?		

Auf Basis der oben gegebenen Antworten und der vorgegebenen Standards wird der Forschungsleiter bestimmen, ob Sie am Experiment teilnehmen dürfen und Ihnen diese Entscheidung mitteilen.

14. Haben Sie innerhalb der letzten zwei Wochen irgendwelche verschreibungspflichtigen Medikamente zur Behandlung oder zu Forschungszwecken eingenommen (Verhütungsmittel ausgenommen)? Falls ja, welche und wann?		
15. Haben/Hatten Sie jemals eine neurologische oder psychiatrische Erkrankung? Falls ja, welche und wann?		
16. Sind Sie jemals in Ohnmacht gefallen? Falls ja, geben Sie bitte Details zu den Umständen an:		
17. Leiden Sie unter Gehörproblemen oder hören Sie einen konstanten Piep Ton?		

18. Haben Sie ein Cochlea Implantat?		
19. Leiden Sie unter Migräne?		

Name:

Unterschrift:

Geburtsdatum:

Dieses Formular muss <u>vollständig</u> vom VERSUCHSLEITER ausgefüllt werden!

Projektnummer: Versuchspersonen Code:

Name: Datum:

Unterschrift:

Zu melden:

(Nebenwirkungen/ Zufallsbefunde) JA / NEIN

Falls ja, welche:

Appendix 2: medical tests

Code:

Gender:

Age:

Address:

Phone Number:

Neurological History:

Epilepsy (y/n):

Migraine (w/wo aura):

Heart complications. (y/n):

Others:

Medication:

HR: _____ BP (S/D): _____ /_____

(NE)

Mental status:

- Orientation

Cranial Nerve:

- Visual acuity
- Visual field
- Extraocular movements
- Facial nerve

Motor:

- Pronator drift
- Upper extremity
- Lower extremity

Sensory:

Reflexes:

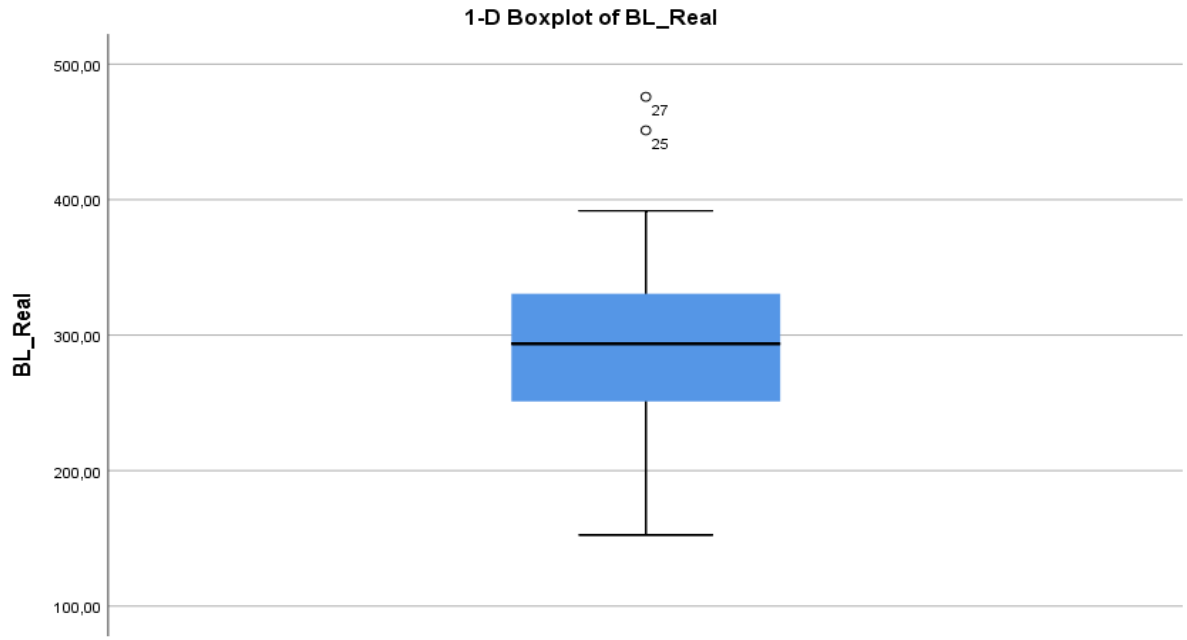
Coordination and Gait:

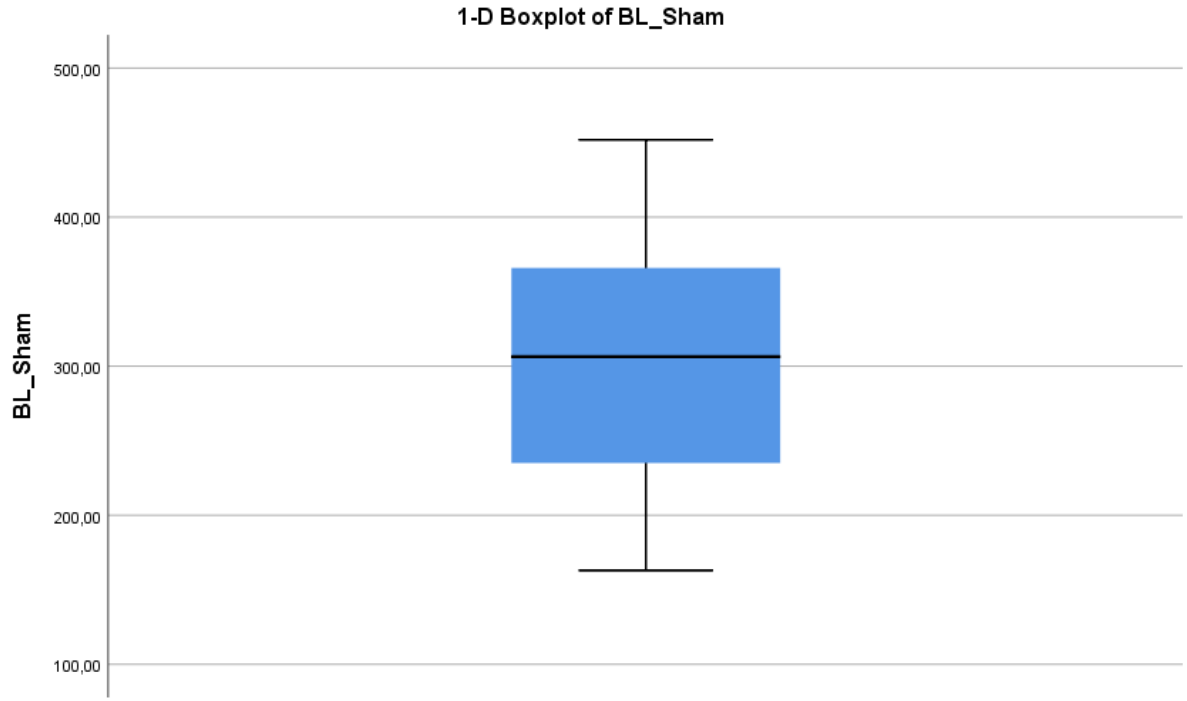
- Finger-to-nose
- Tandem walking

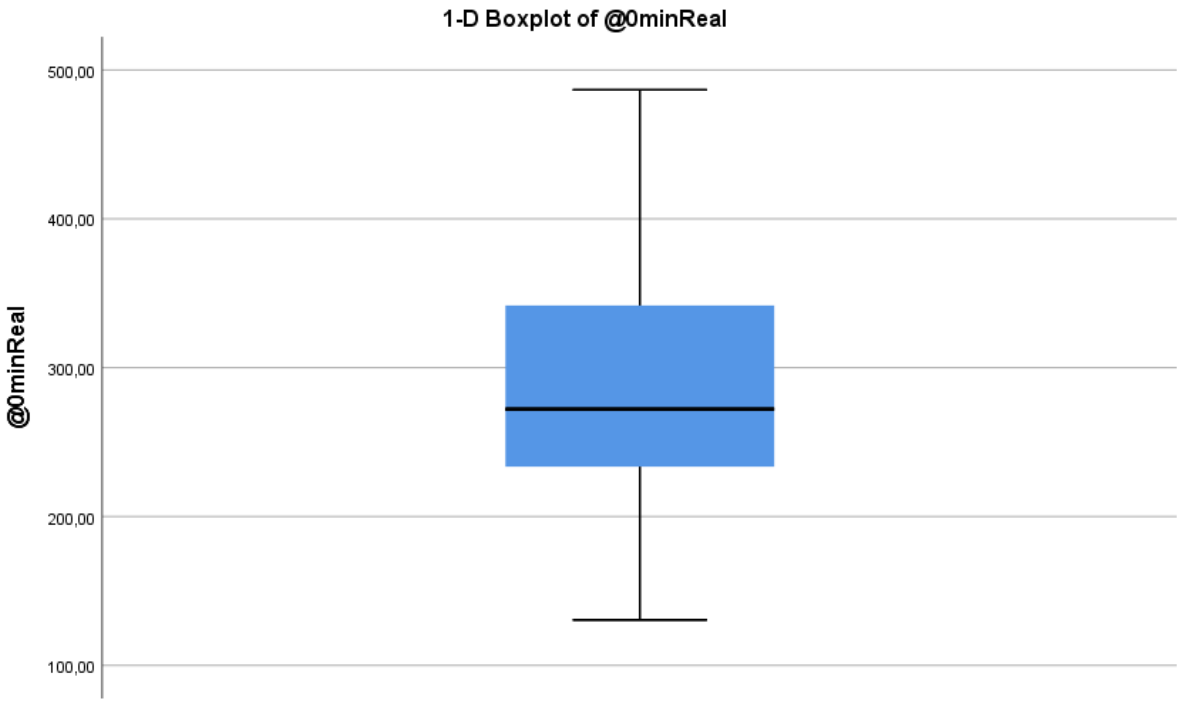
Date: _____ Signature: _____

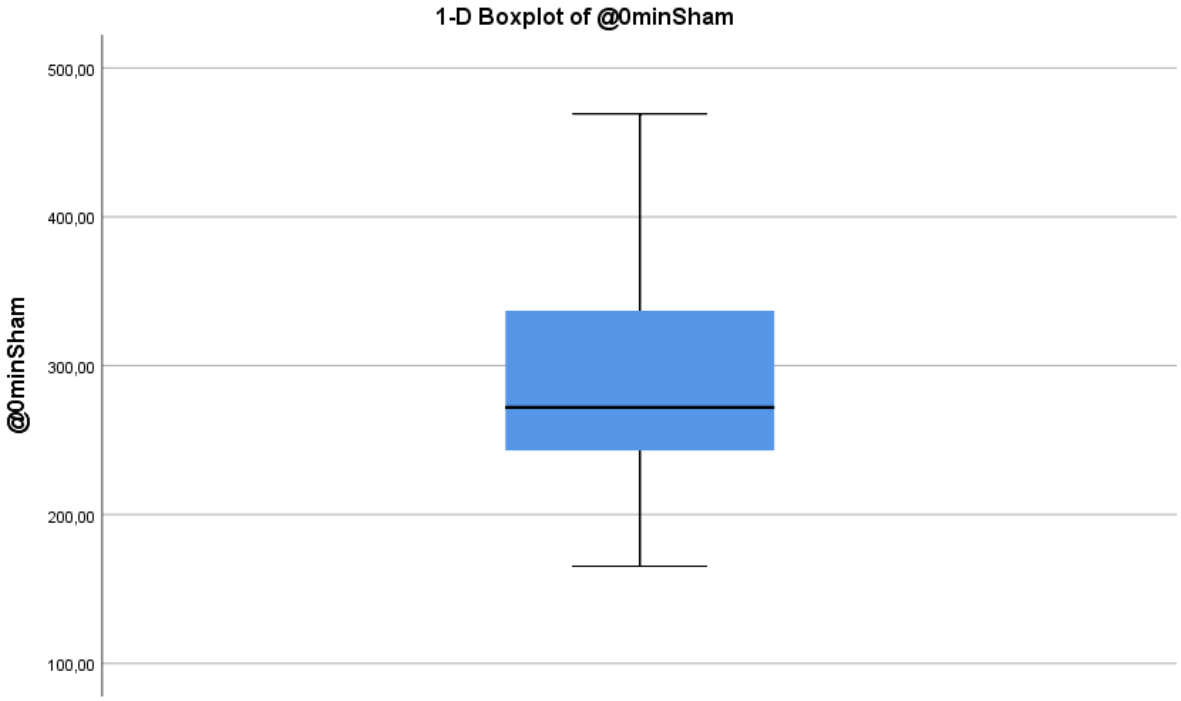
Appendix 3: Outlier detection Reaction Times Experimental and Practice

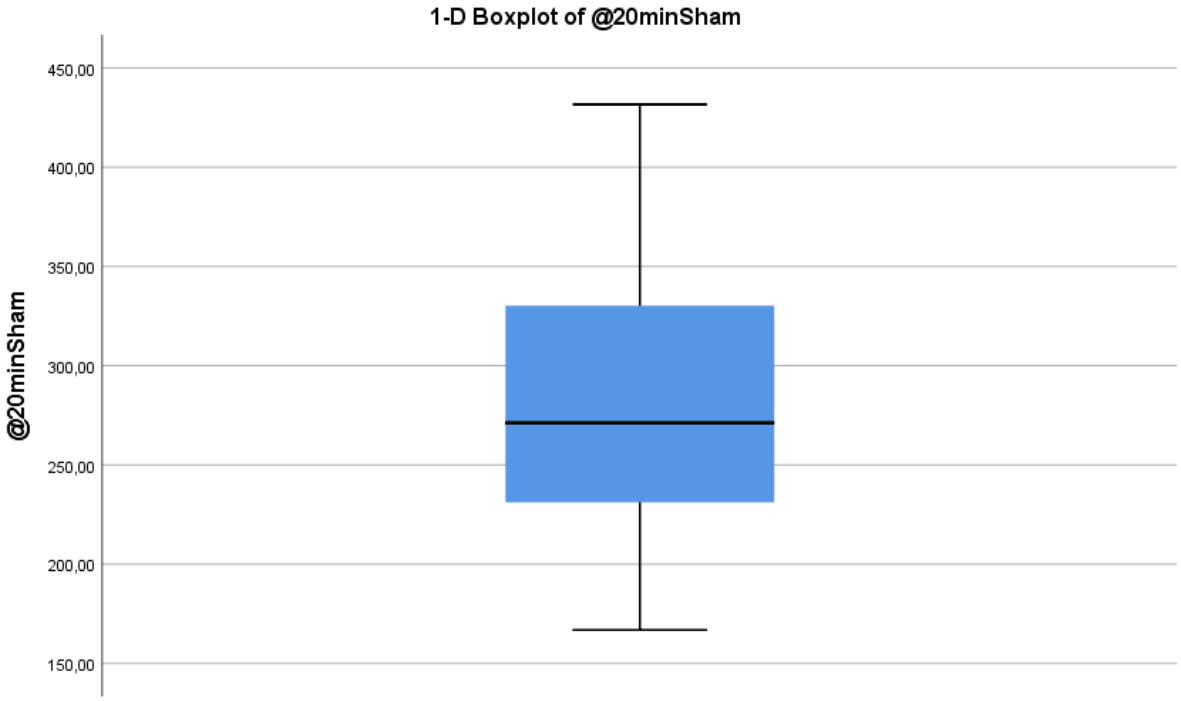
Experimental Reaction Times Outlier Detection

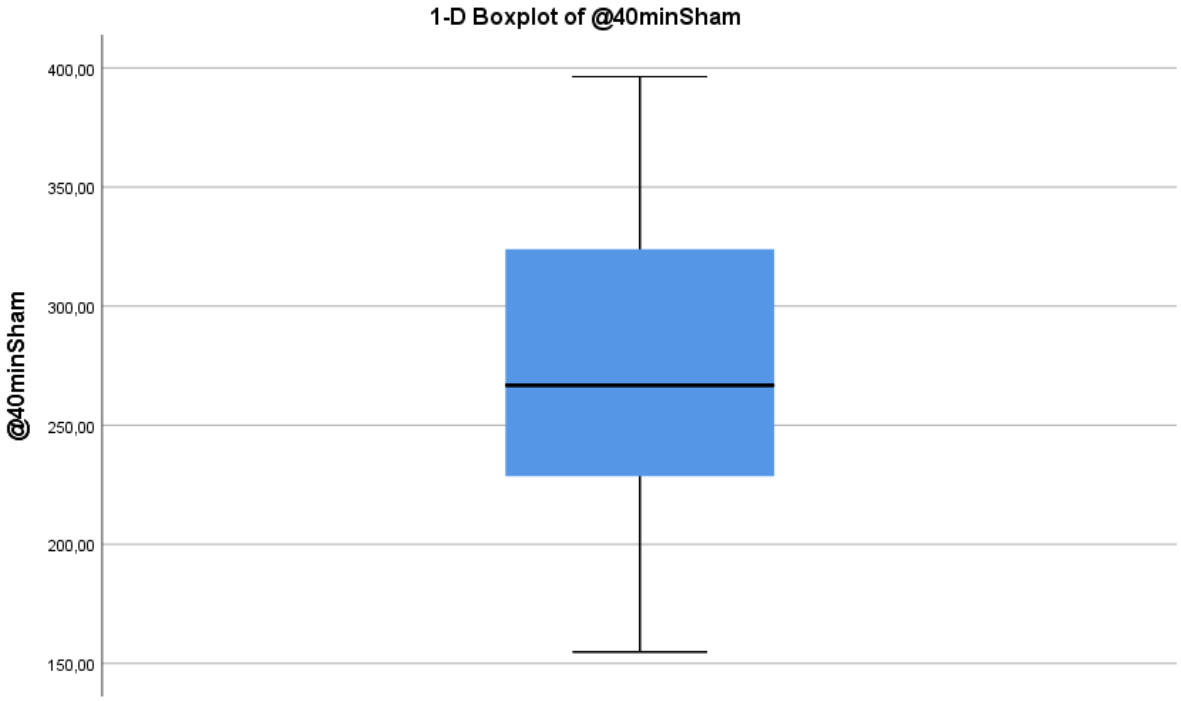




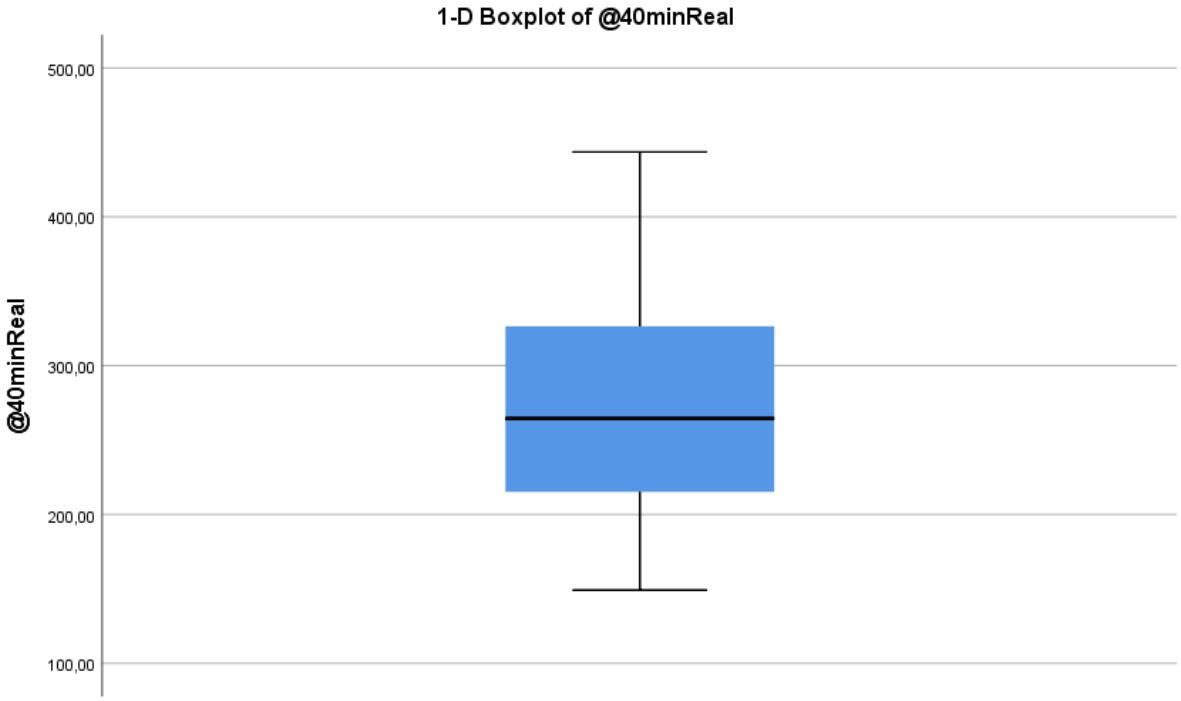

1-D Boxplot of @0minReal
@0minReal
500,00
400,00
300,00
200,00
100,00


1-D Boxplot of @0minSham
@0minSham
500,00
400,00
300,00
200,00
100,00

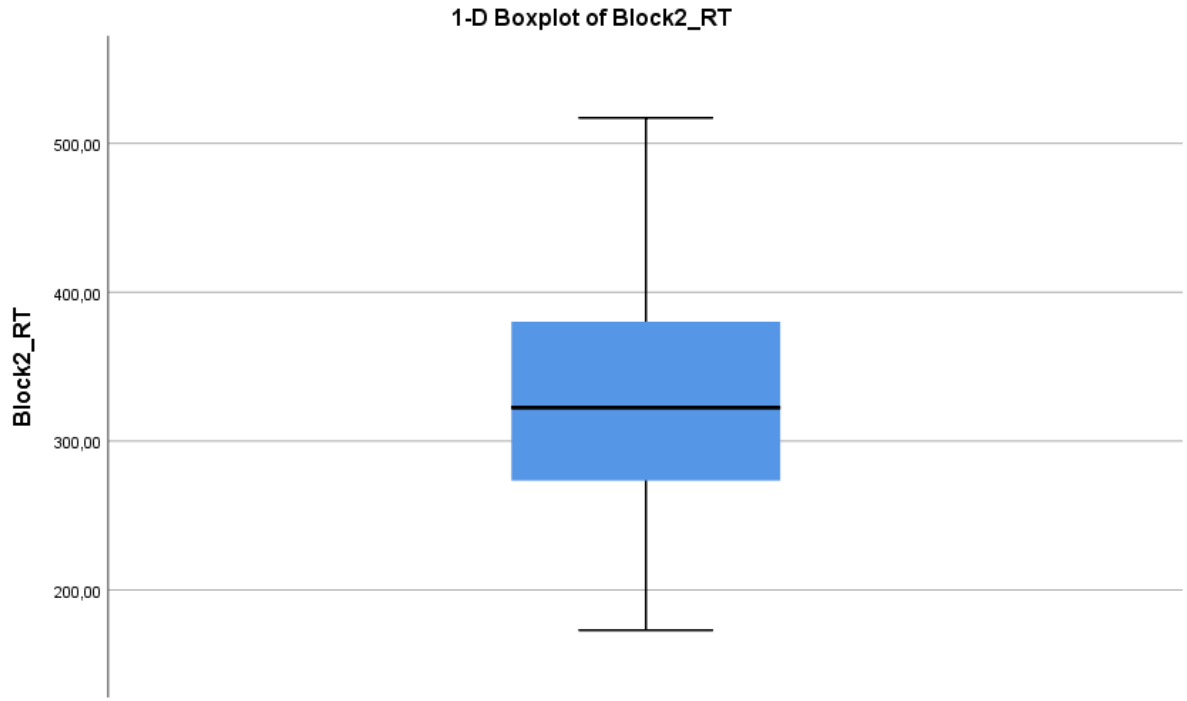

1-D Boxplot of @20minSham
@20minSham
450,00
400,00
350,00
300,00
250,00
200,00
150,00

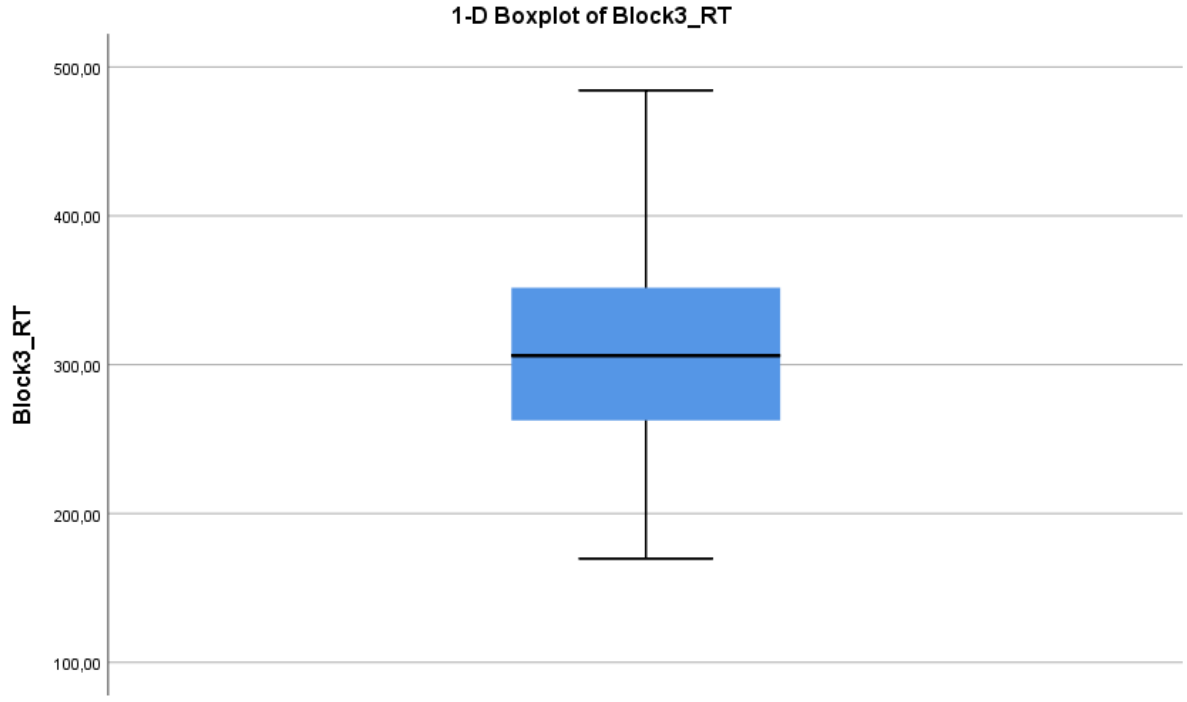

1-D Boxplot of @40minSham
@40minSham
400,00
350,00
300,00
250,00
200,00
150,00


1-D Boxplot of @20minReal
@20minReal
500,00
400,00
300,00
200,00
100,00


1-D Boxplot of @40minReal
@40minReal
500,00
400,00
300,00
200,00
100,00

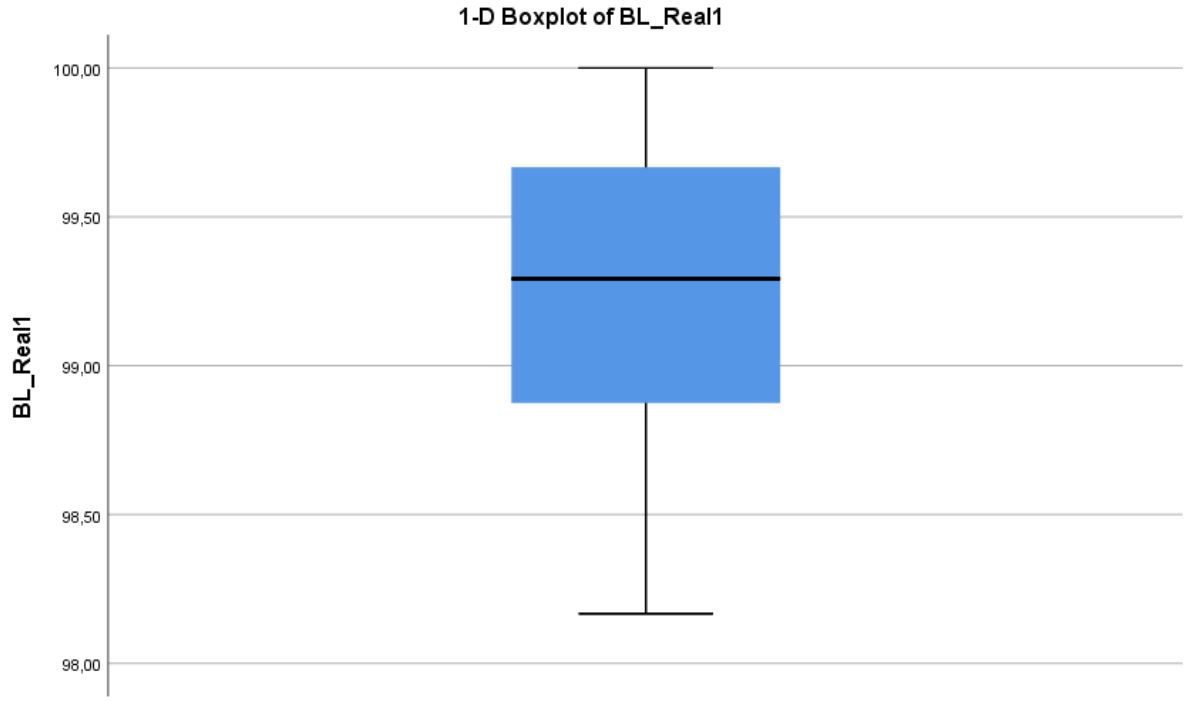
Practice Reaction Times Outlier Detection

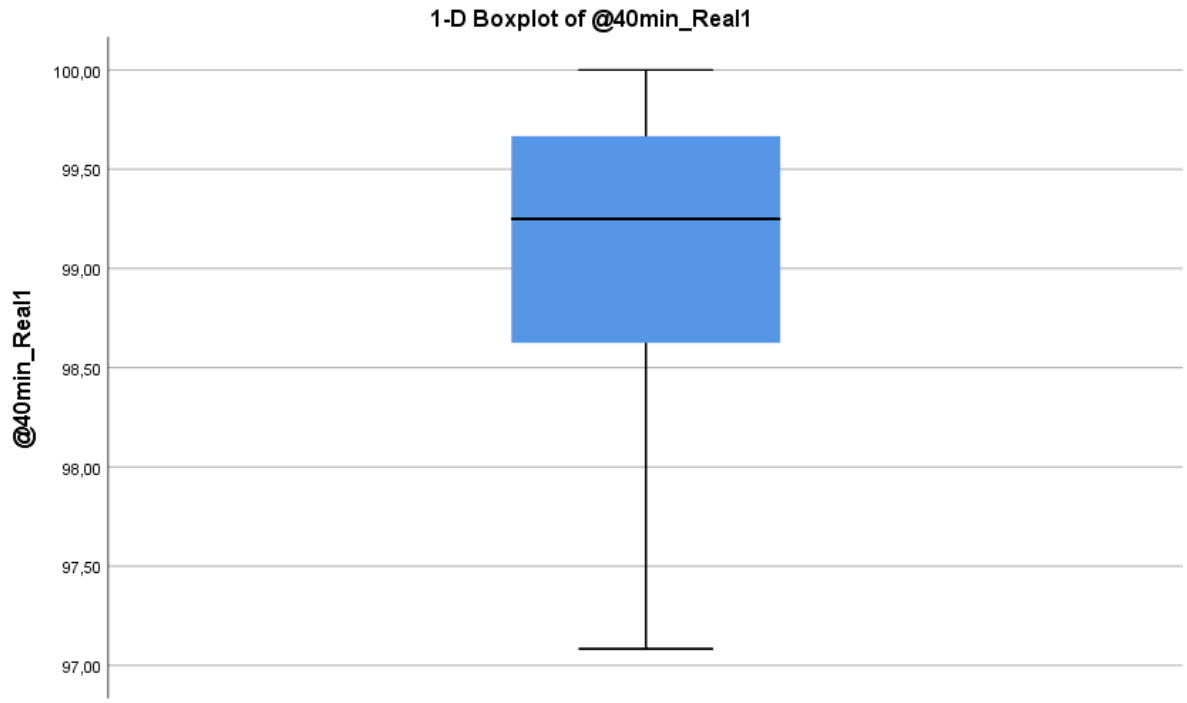


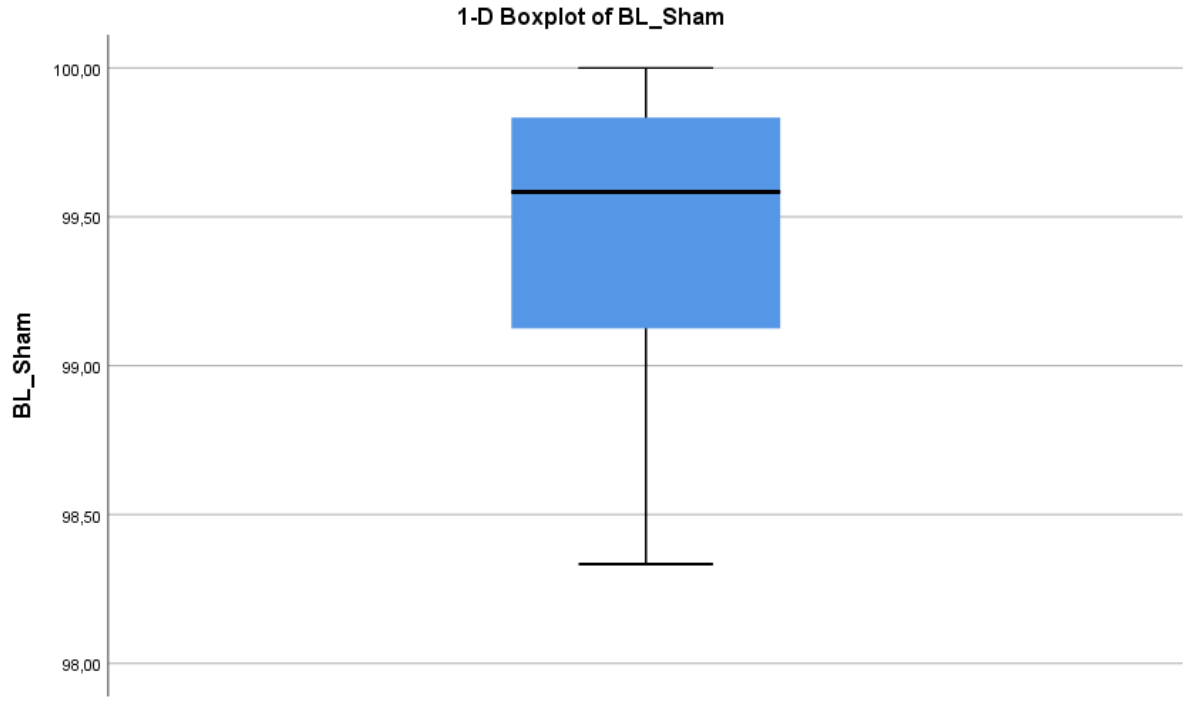


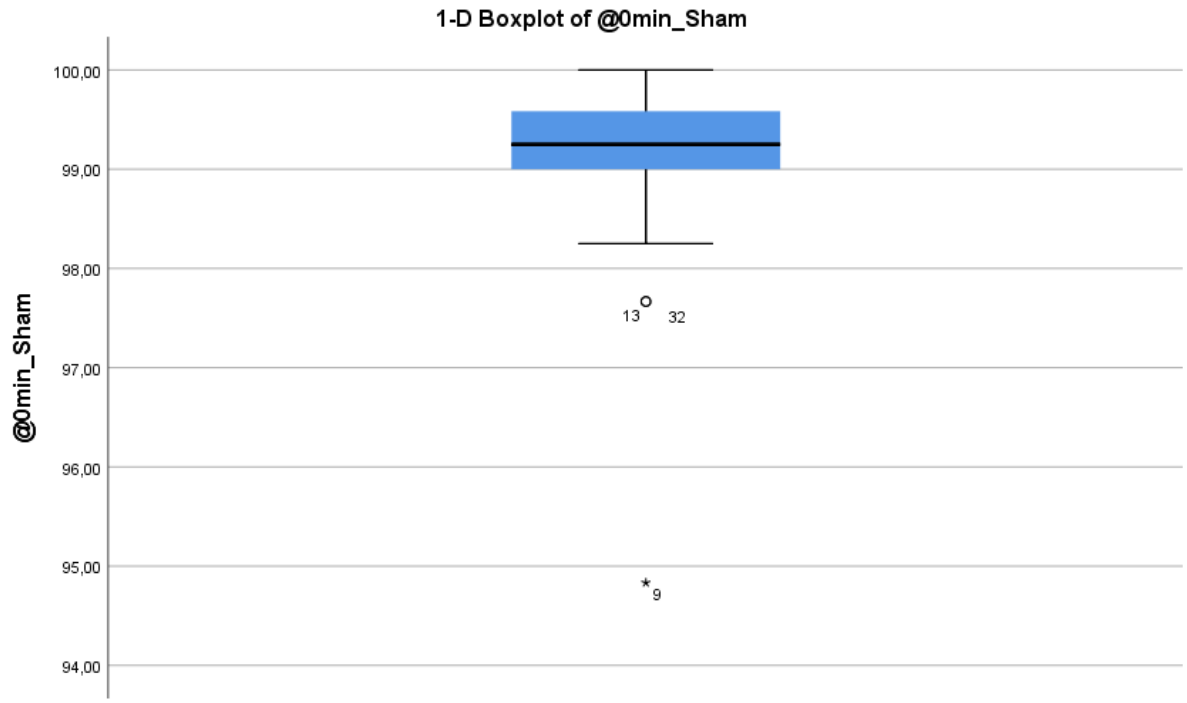
Appendix 4: Outliers Error Rates Experimental and Practice

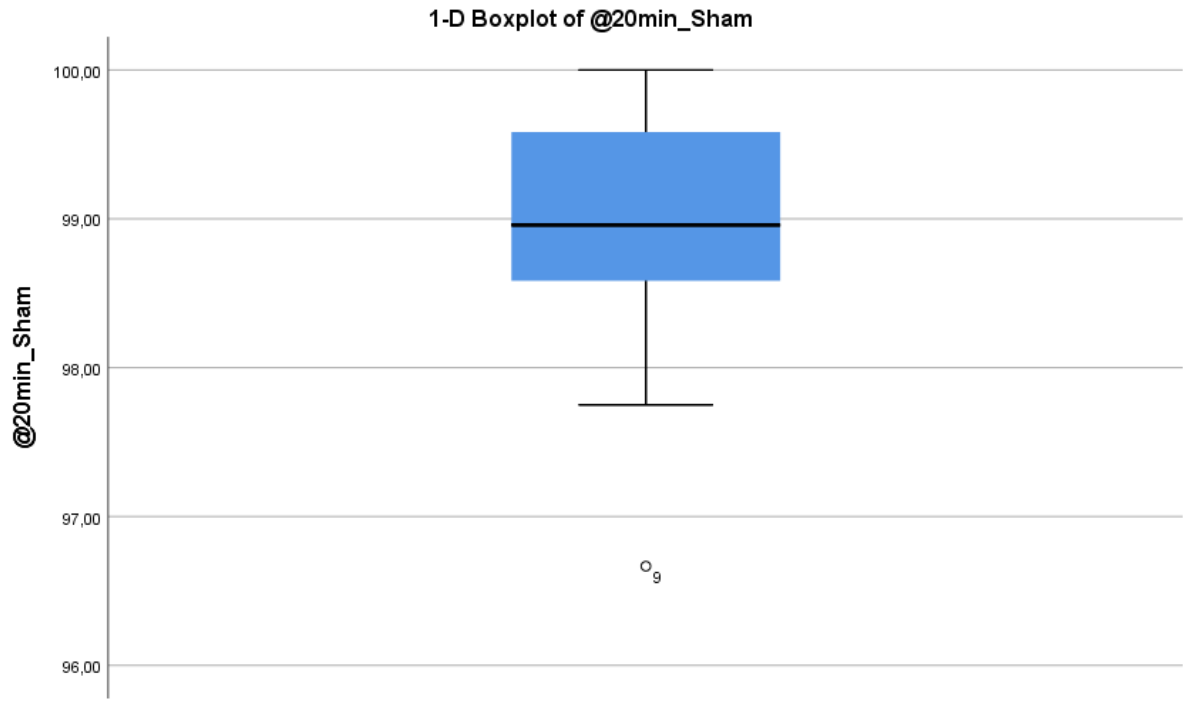
Outliers Experimental Errors

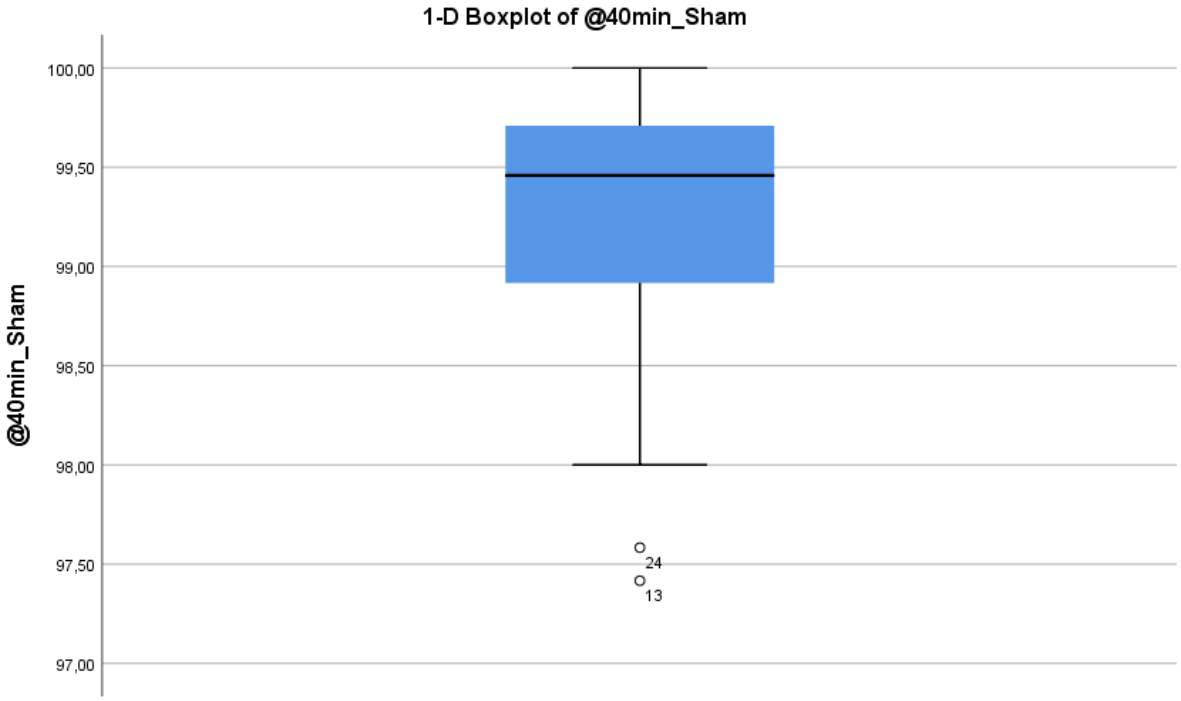



1-D Boxplot of @40min_Real1
@40min_Real1
100,00
99,50
99,00
98,50
98,00
97,50
97,00

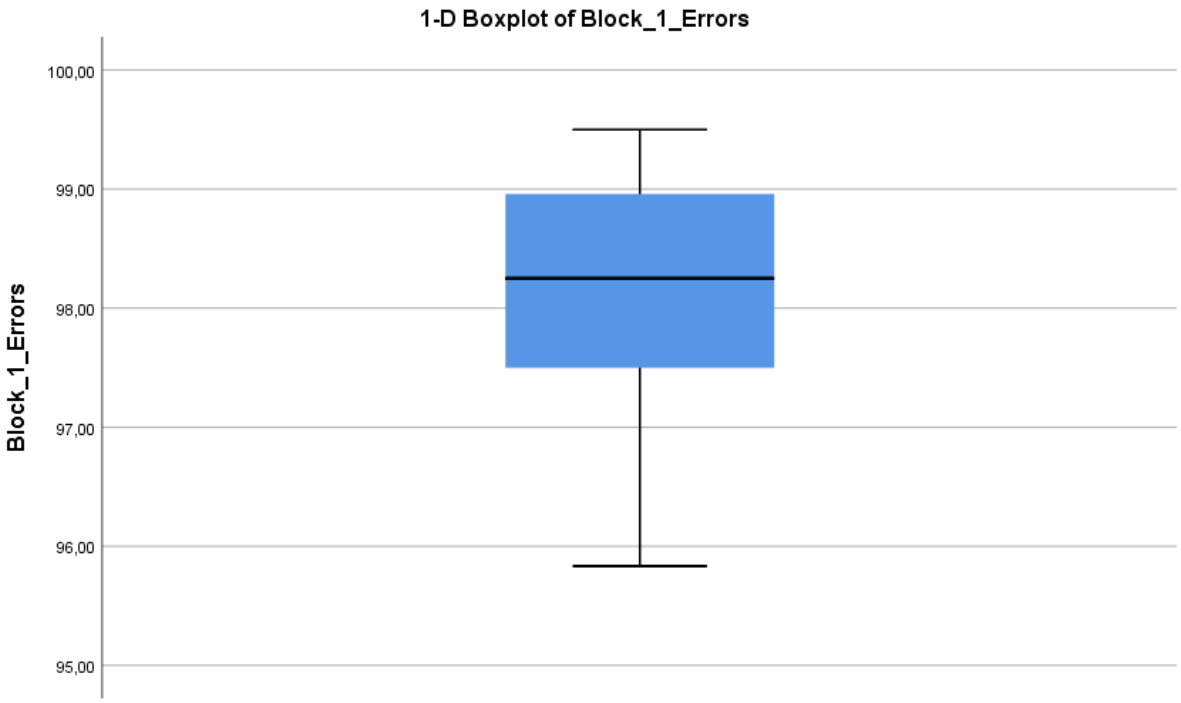

1-D Boxplot of BL_Sham
BL_Sham
100,00
99,50
99,00
98,50
98,00

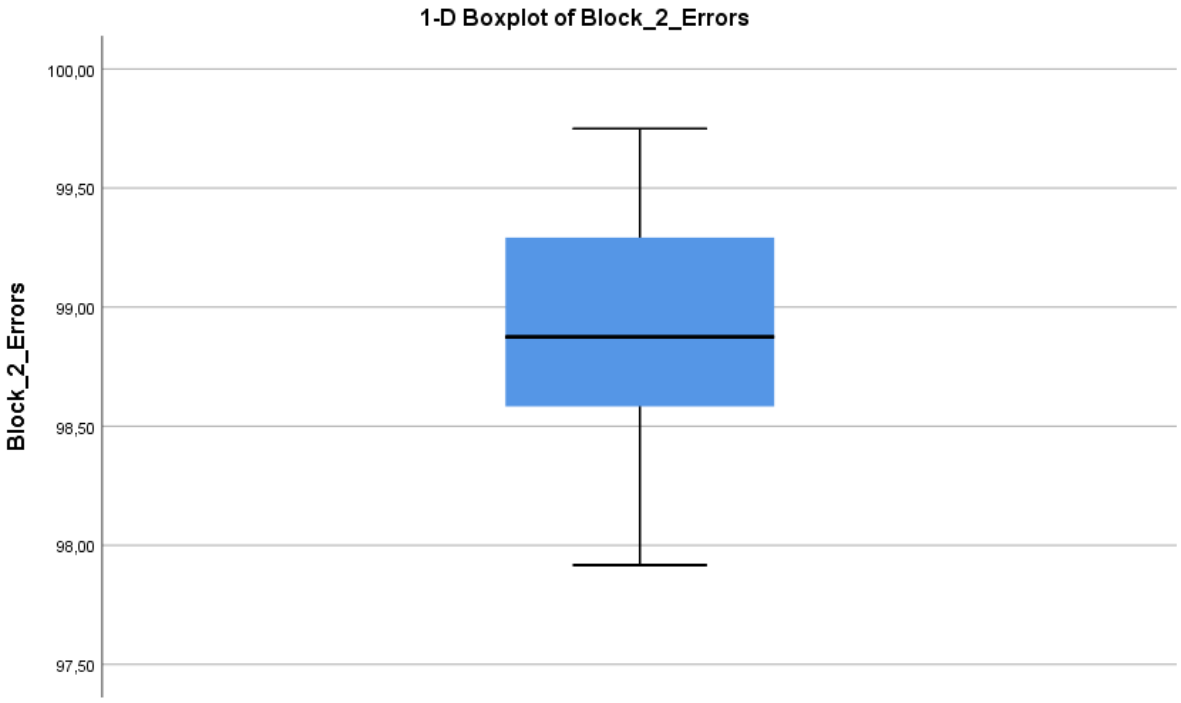

1-D Boxplot of @0min_Sham
@0min_Sham
100,00
99,00
98,00
97,00
96,00
95,00
94,00
13
32
9

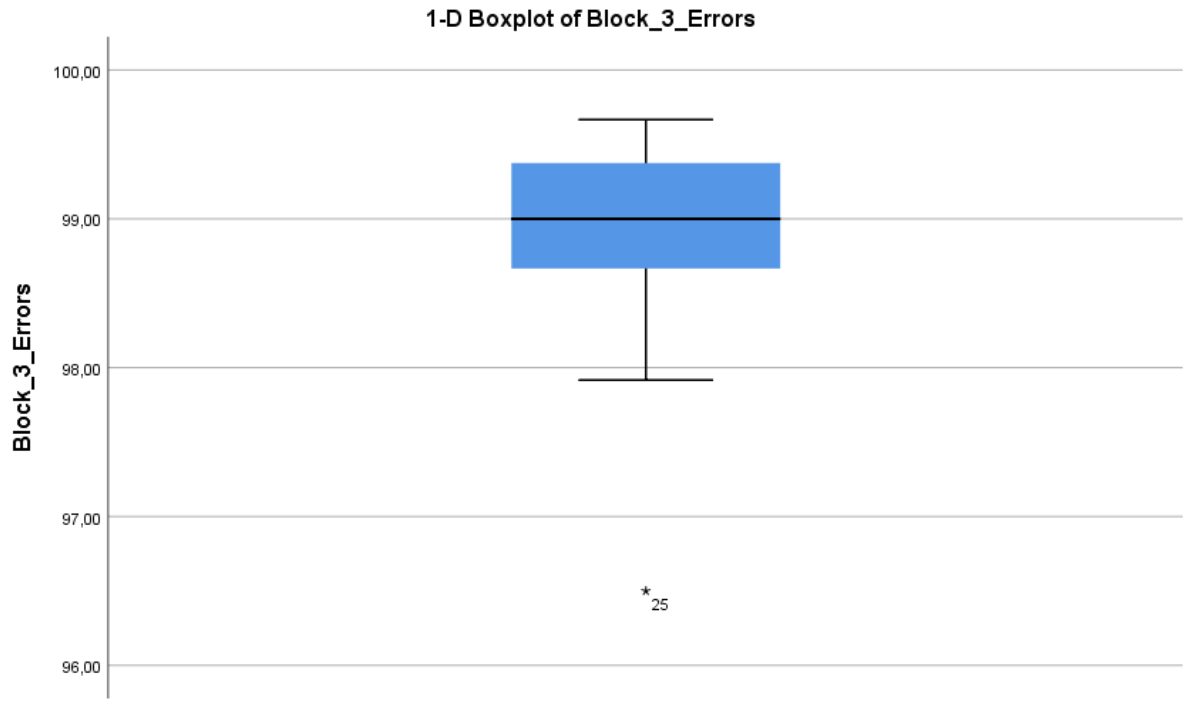

1-D Boxplot of @20min_Sham
@20min_Sham
100,00
99,00
98,00
97,00
96,00
9


1-D Boxplot of @40min_Sham
@40min_Sham
100,00
99,50
99,00
98,50
98,00
97,50
97,00
24
13

Outliers Practice Errors






1-D Boxplot of Block_3_Errors
Block_3_Errors
100,00
99,00
98,00
97,00
96,00
25

Appendix 5: Stimuli and sequences (these 7 element series were learned at home)

Participant	Pre-Stimulus	1x6 sequence	Pre-stimulus	2x3 sequence	Condition	Day 1	Day 2
1	O	bcvnvc	X	ncbncb	TMS	Real	Sham
2	O	bcvnvc	X	ncbncb	TBS	Real	Sham
3	E	nvbcbv	D	cvncvn	TMS	Sham	Real
4	E	nvbcbv	D	cvncvn	TBS	Sham	Real
5	G	cbnvnb	I	vbcvbc	TMS	Real	Sham
6	G	cbnvnb	I	vbcvbc	TBS	Real	Sham
7	L	vncbcn	M	bnvbnv	TMS	Sham	Real
8	L	vncbcn	M	bnvbnv	TBS	Sham	Real
9	X	bcvnvc	O	ncbncb	TMS	Real	Sham
10	X	bcvnvc	O	ncbncb	TBS	Real	Sham
11	D	nvbcbv	E	cvncvn	TMS	Sham	Real
12	D	nvbcbv	E	cvncvn	TBS	Sham	Real
13	I	cbnvnb	G	vbcvbc	TMS	Real	Sham
14	I	cbnvnb	G	vbcvbc	TBS	Real	Sham
15	M	vncbcn	L	bnvbnv	TMS	Sham	Real
16	M	vncbcn	L	bnvbnv	TBS	Sham	Real
17	M	bcvnvc	L	ncbncb	TMS	Real	Sham
18	M	bcvnvc	L	ncbncb	TBS	Real	Sham
19	I	nvbcbv	G	cvncvn	TMS	Sham	Real
20	I	nvbcbv	G	cvncvn	TBS	Sham	Real
21	D	cbnvnb	E	vbcvbc	TMS	Real	Sham
22	D	cbnvnb	E	vbcvbc	TBS	Real	Sham
23	X	vncbcn	O	bnvbnv	TMS	Sham	Real
24	X	vncbcn	O	bnvbnv	TBS	Sham	Real
25	L	bcvnvc	M	ncbncb	TMS	Real	Sham
26	L	bcvnvc	M	ncbncb	TBS	Real	Sham
27	G	nvbcbv	I	cvncvn	TMS	Sham	Real
28	G	nvbcbv	I	cvncvn	TBS	Sham	Real
29	E	cbnvnb	D	vbcvbc	TMS	Real	Sham
30	E	cbnvnb	D	vbcvbc	TBS	Real	Sham
31	O	vncbcn	X	bnvbnv	TMS	Sham	Real
32	O	vncbcn	X	bnvbnv	TBS	Sham	Real

Appendix 6: SPSS Syntax Reaction times experimental blocks

```
GLM BL_real_2x3_k1 BL_real_2x3_k2 BL_real_2x3_k3 BL_real_2x3_k4
BL_real_2x3_k5 BL_real_2x3_k6
    @0min_real_2x3_k1 @0min_real_2x3_k2 @0min_real_2x3_k3 @0min_real_2x3_k4
@0min_real_2x3_k5
    @0min_real_2x3_k6 @20min_real_2x3_k1 @20min_real_2x3_k2
@20min_real_2x3_k3 @20min_real_2x3_k4
    @20min_real_2x3_k5 @20min_real_2x3_k6 @40min_real_2x3_k1
@40min_real_2x3_k2 @40min_real_2x3_k3
    @40min_real_2x3_k4 @40min_real_2x3_k5 @40min_real_2x3_k6 BL_real_1x6_k1
BL_real_1x6_k2
    BL_real_1x6_k3 BL_real_1x6_k4 BL_real_1x6_k5 BL_real_1x6_k6
@0min_real_1x6_k1 @0min_real_1x6_k2
    @0min_real_1x6_k3 @0min_real_1x6_k4 @0min_real_1x6_k5 @0min_real_1x6_k6
@20min_real_1x6_k1
    @20min_real_1x6_k2 @20min_real_1x6_k3 @20min_real_1x6_k4
@20min_real_1x6_k5 @20min_real_1x6_k6
    @40min_real_1x6_k1 @40min_real_1x6_k2 @40min_real_1x6_k3
@40min_real_1x6_k4 @40min_real_1x6_k5
    @40min_real_1x6_k6 BL_sham_2x3_k1 BL_sham_2x3_k2 BL_sham_2x3_k3
BL_sham_2x3_k4 BL_sham_2x3_k5
    BL_sham_2x3_k6 @0min_sham_2x3_k1 @0min_sham_2x3_k2 @0min_sham_2x3_k3
@0min_sham_2x3_k4
    @0min_sham_2x3_k5 @0min_sham_2x3_k6 @20min_sham_2x3_k1
@20min_sham_2x3_k2 @20min_sham_2x3_k3
    @20min_sham_2x3_k4 @20min_sham_2x3_k5 @20min_sham_2x3_k6
@40min_sham_2x3_k1 @40min_sham_2x3_k2
    @40min_sham_2x3_k3 @40min_sham_2x3_k4 @40min_sham_2x3_k5
@40min_sham_2x3_k6 BL_sham_1x6_k1
    BL_sham_1x6_k2 BL_sham_1x6_k3 BL_sham_1x6_k4 BL_sham_1x6_k5
BL_sham_1x6_k6 @0min_sham_1x6_k1
    @0min_sham_1x6_k2 @0min_sham_1x6_k3 @0min_sham_1x6_k4 @0min_sham_1x6_k5
@0min_sham_1x6_k6
    @20min_sham_1x6_k1 @20min_sham_1x6_k2 @20min_sham_1x6_k3
@20min_sham_1x6_k4 @20min_sham_1x6_k5
    @20min_sham_1x6_k6 @40min_sham_1x6_k1 @40min_sham_1x6_k2
@40min_sham_1x6_k3 @40min_sham_1x6_k4
    @40min_sham_1x6_k5 @40min_sham_1x6_k6 BY Group RS_SR
  /WSFACTOR=state 2 Polynomial seq 2 Polynomial delay 4 Polynomial key 6
Polynomial
  /METHOD=SSTYPE(3)
  /EMMEANS=TABLES(Group*RS_SR*state*delay)
  /EMMEANS=TABLES(Group*state*delay)
  /EMMEANS=TABLES(RS_SR*delay)
  /EMMEANS=TABLES(RS_SR*seq*key)
  /EMMEANS=TABLES(Group)
  /EMMEANS=TABLES(RS_SR)
  /EMMEANS=TABLES(state)
  /EMMEANS=TABLES(seq)
  /EMMEANS=TABLES(delay)
  /EMMEANS=TABLES(key)
  /EMMEANS=TABLES(Group*RS_SR)
  /EMMEANS=TABLES(Group*state)
  /EMMEANS=TABLES(Group*seq)
  /EMMEANS=TABLES(Group*delay)
  /EMMEANS=TABLES(Group*key)
  /EMMEANS=TABLES(RS_SR*state)
  /EMMEANS=TABLES(RS_SR*seq)
```

```
  /EMMEANS=TABLES(RS_SR*key)
  /EMMEANS=TABLES(state*seq)
  /EMMEANS=TABLES(state*delay)
  /EMMEANS=TABLES(state*key)
  /EMMEANS=TABLES(seq*delay)
  /EMMEANS=TABLES(seq*key)
  /EMMEANS=TABLES(delay*key)
  /EMMEANS=TABLES(Group*RS_SR*state)
  /EMMEANS=TABLES(Group*RS_SR*seq)
  /EMMEANS=TABLES(Group*RS_SR*delay)
  /EMMEANS=TABLES(Group*RS_SR*key)
  /EMMEANS=TABLES(Group*state*seq)
  /EMMEANS=TABLES(Group*state*key)
  /EMMEANS=TABLES(Group*seq*delay)
  /EMMEANS=TABLES(Group*seq*key)
  /EMMEANS=TABLES(Group*delay*key)
  /EMMEANS=TABLES(RS_SR*state*seq)
  /EMMEANS=TABLES(RS_SR*state*delay)
  /EMMEANS=TABLES(RS_SR*state*key)
  /EMMEANS=TABLES(RS_SR*seq*delay)
  /EMMEANS=TABLES(RS_SR*delay*key)
  /EMMEANS=TABLES(state*seq*delay)
  /EMMEANS=TABLES(state*seq*key)
  /EMMEANS=TABLES(state*delay*key)
  /EMMEANS=TABLES(seq*delay*key)
  /EMMEANS=TABLES(Group*RS_SR*state*seq)
  /EMMEANS=TABLES(Group*RS_SR*state*key)
  /EMMEANS=TABLES(Group*RS_SR*seq*delay)
  /EMMEANS=TABLES(Group*RS_SR*seq*key)
  /EMMEANS=TABLES(Group*RS_SR*delay*key)
  /EMMEANS=TABLES(Group*state*seq*delay)
  /EMMEANS=TABLES(Group*state*seq*key)
  /EMMEANS=TABLES(Group*state*delay*key)
  /EMMEANS=TABLES(Group*seq*delay*key)
  /EMMEANS=TABLES(RS_SR*state*seq*delay)
  /EMMEANS=TABLES(RS_SR*state*seq*key)
  /EMMEANS=TABLES(RS_SR*state*delay*key)
  /EMMEANS=TABLES(RS_SR*seq*delay*key)
  /EMMEANS=TABLES(state*seq*delay*key)
  /EMMEANS=TABLES(Group*RS_SR*state*seq*delay)
  /EMMEANS=TABLES(Group*RS_SR*state*seq*key)
  /EMMEANS=TABLES(Group*RS_SR*state*delay*key)
  /EMMEANS=TABLES(Group*RS_SR*seq*delay*key)
  /EMMEANS=TABLES(Group*state*seq*delay*key)
  /EMMEANS=TABLES(RS_SR*state*seq*delay*key)
  /EMMEANS=TABLES(Group*RS_SR*state*seq*delay*key)
  /PRINT=DESCRIPTIVE ETASQ
  /CRITERIA=ALPHA(.05)
  /WSDESIGN=state seq delay key state*seq state*delay seq*delay
state*seq*delay state*key seq*key
    state*seq*key delay*key state*delay*key seq*delay*key
state*seq*delay*key
  /DESIGN=Group RS_SR Group*RS_SR.
```

Appendix 7: SPSS Syntax Reaction Times practice blocks

```
GLM Block1_1x6_K_1 Block1_1x6_K_2 Block1_1x6_K_3 Block1_1x6_K_4
Block1_1x6_K_5 Block1_1x6_K_6
    Block2_1x6_K_1 Block2_1x6_K_2 Block2_1x6_K_3 Block2_1x6_K_4
Block2_1x6_K_5 Block2_1x6_K_6
    Block3_1x6_K_1 Block3_1x6_K_2 Block3_1x6_K_3 Block3_1x6_K_4
Block3_1x6_K_5 Block3_1x6_K_6
    Block1_2x3_K_1 Block1_2x3_K_2 Block1_2x3_K_3 Block1_2x3_K_4
Block1_2x3_K_5 Block1_2x3_K_6
    Block2_2x3_K_1 Block2_2x3_K_2 Block2_2x3_K_3 Block2_2x3_K_4
Block2_2x3_K_5 Block2_2x3_K_6
    Block3_2x3_K_1 Block3_2x3_K_2 Block3_2x3_K_3 Block3_2x3_K_4
Block3_2x3_K_5 Block3_2x3_K_6 BY
    TxSCond D12RealShamShamReal
  /WSFACTOR=Block 3 Polynomial seq 2 Polynomial keys 6 Polynomial
  /METHOD=SSTYPE(3)
  /POSTHOC=TxSCond D12RealShamShamReal(TUKEY)
  /EMMEANS=TABLES(TxSCond) COMPARE ADJ(BONFERRONI)
  /EMMEANS=TABLES(D12RealShamShamReal) COMPARE ADJ(BONFERRONI)
  /EMMEANS=TABLES(Block) COMPARE ADJ(BONFERRONI)
  /EMMEANS=TABLES(seq) COMPARE ADJ(BONFERRONI)
  /EMMEANS=TABLES(keys) COMPARE ADJ(BONFERRONI)
  /PRINT=DESCRIPTIVE ETASQ
  /CRITERIA=ALPHA(.05)
  /WSDESIGN=Block seq keys Block*seq Block*keys seq*keys Block*seq*keys
  /DESIGN=TxSCond D12RealShamShamReal TxSCond*D12RealShamShamReal.
```

Appendix 8: SPSS Syntax Error Rates Experimental

```
GLM Arsin_BL_real_2x3_k1 Arsin_BL_real_2x3_k2 Arsin_BL_real_2x3_k3
Arsin_BL_real_2x3_k4
    Arsin_BL_real_2x3_k5 Arsin_BL_real_2x3_k6 Arsin_@0min_real_2x3_k1
Arsin_@0min_real_2x3_k2
    Arsin_@0min_real_2x3_k3 Arsin_@0min_real_2x3_k4 Arsin_@0min_real_2x3_k5
Arsin_@0min_real_2x3_k6
    Arsin_@20min_real_2x3_k1 Arsin_@20min_real_2x3_k2
Arsin_@20min_real_2x3_k3 Arsin_@20min_real_2x3_k4
    Arsin_@20min_real_2x3_k5 Arsin_@20min_real_2x3_k6
Arsin_@40min_real_2x3_k1 Arsin_@40min_real_2x3_k2
    Arsin_@40min_real_2x3_k3 Arsin_@40min_real_2x3_k4
Arsin_@40min_real_2x3_k5 Arsin_@40min_real_2x3_k6
    Arsin_BL_real_1x6_k1 Arsin_BL_real_1x6_k2 Arsin_BL_real_1x6_k3
Arsin_BL_real_1x6_k4
    Arsin_BL_real_1x6_k5 Arsin_BL_real_1x6_k6 Arsin_@0min_real_1x6_k1
Arsin_@0min_real_1x6_k2
    Arsin_@0min_real_1x6_k3 Arsin_@0min_real_1x6_k4 Arsin_@0min_real_1x6_k5
Arsin_@0min_real_1x6_k6
    Arsin_@20min_real_1x6_k1 Arsin_@20min_real_1x6_k2
Arsin_@20min_real_1x6_k3 Arsin_@20min_real_1x6_k4
    Arsin_@20min_real_1x6_k5 Arsin_@20min_real_1x6_k6
Arsin_@40min_real_1x6_k1 Arsin_@40min_real_1x6_k2
    Arsin_@40min_real_1x6_k3 Arsin_@40min_real_1x6_k4
Arsin_@40min_real_1x6_k5 Arsin_@40min_real_1x6_k6
    Arsin_BL_sham_2x3_k1 Arsin_BL_sham_2x3_k2 Arsin_BL_sham_2x3_k3
Arsin_BL_sham_2x3_k4
    Arsin_BL_sham_2x3_k5 Arsin_BL_sham_2x3_k6 Arsin_@0min_sham_2x3_k1
Arsin_@0min_sham_2x3_k2
    Arsin_@0min_sham_2x3_k3 Arsin_@0min_sham_2x3_k4 Arsin_@0min_sham_2x3_k5
Arsin_@0min_sham_2x3_k6
    Arsin_@20min_sham_2x3_k1 Arsin_@20min_sham_2x3_k2
Arsin_@20min_sham_2x3_k3 Arsin_@20min_sham_2x3_k4
    Arsin_@20min_sham_2x3_k5 Arsin_@20min_sham_2x3_k6
Arsin_@40min_sham_2x3_k1 Arsin_@40min_sham_2x3_k2
    Arsin_@40min_sham_2x3_k3 Arsin_@40min_sham_2x3_k4
Arsin_@40min_sham_2x3_k5 Arsin_@40min_sham_2x3_k6
    Arsin_BL_sham_1x6_k1 Arsin_BL_sham_1x6_k2 Arsin_BL_sham_1x6_k3
Arsin_BL_sham_1x6_k4
    Arsin_BL_sham_1x6_k5 Arsin_BL_sham_1x6_k6 Arsin_@0min_sham_1x6_k1
Arsin_@0min_sham_1x6_k2
    Arsin_@0min_sham_1x6_k3 Arsin_@0min_sham_1x6_k4 Arsin_@0min_sham_1x6_k5
Arsin_@0min_sham_1x6_k6
    Arsin_@20min_sham_1x6_k1 Arsin_@20min_sham_1x6_k2
Arsin_@20min_sham_1x6_k3 Arsin_@20min_sham_1x6_k4
    Arsin_@20min_sham_1x6_k5 Arsin_@20min_sham_1x6_k6
Arsin_@40min_sham_1x6_k1 Arsin_@40min_sham_1x6_k2
    Arsin_@40min_sham_1x6_k3 Arsin_@40min_sham_1x6_k4
Arsin_@40min_sham_1x6_k5 Arsin_@40min_sham_1x6_k6
    BY Group
  /WSFACTOR=Timepoint 4 Polynomial state 2 Polynomial SequenceStr 2
Polynomial Keys 6 Polynomial
  /METHOD=SSTYPE(3)
  /POSTHOC=Group(TUKEY)
  /PLOT=PROFILE(state*Timepoint*Group) TYPE=BAR ERRORBAR=NO
MEANREFERENCE=NO
  /PRINT=ETASQ
  /CRITERIA=ALPHA(.05)
  /WSDESIGN=Timepoint state SequenceStr Keys Timepoint*state
Timepoint*SequenceStr
```

```
    state*SequenceStr Timepoint*state*SequenceStr Timepoint*Keys state*Keys
Timepoint*state*Keys
    SequenceStr*Keys Timepoint*SequenceStr*Keys state*SequenceStr*Keys
Timepoint*state*SequenceStr*Keys
  /DESIGN=RSSR Group RSSR*Group.
```

Appendix 9: SPSS Syntax Error Rates Practice

```
GLM Arsin_Block1_2x3_k1 Arsin_Block1_2x3_k2 Arsin_Block1_2x3_k3
Arsin_Block1_2x3_k4
    Arsin_Block1_2x3_k5 Arsin_Block1_2x3_k6 Arsin_Block2_2x3_k1
Arsin_Block2_2x3_k2 Arsin_Block2_2x3_k3
    Arsin_Block2_2x3_k4 Arsin_Block2_2x3_k5 Arsin_Block2_2x3_k6
Arsin_Block3_2x3_k1 Arsin_Block3_2x3_k2
    Arsin_Block3_2x3_k3 Arsin_Block3_2x3_k4 Arsin_Block3_2x3_k5
Arsin_Block3_2x3_k6 Arsin_Block1_1x6_k1
    Arsin_Block1_1x6_k2 Arsin_Block1_1x6_k3 Arsin_Block1_1x6_k4
Arsin_Block1_1x6_k5 Arsin_Block1_1x6_k6
    Arsin_Block2_1x6_k1 Arsin_Block2_1x6_k2 Arsin_Block2_1x6_k3
Arsin_Block2_1x6_k4 Arsin_Block2_1x6_k5
    Arsin_Block2_1x6_k6 Arsin_Block3_1x6_k1 Arsin_Block3_1x6_k2
Arsin_Block3_1x6_k3 Arsin_Block3_1x6_k4
    Arsin_Block3_1x6_k5 Arsin_Block3_1x6_k6 BY RSSR Group
  /WSFACTOR=Block 3 Polynomial Seq 2 Polynomial Keys 6 Polynomial
  /METHOD=SSTYPE(3)
  /POSTHOC=RSSR Group(TUKEY)
  /PLOT=PROFILE(Keys*Seq) TYPE=LINE ERRORBAR=CI MEANREFERENCE=NO YAXIS=AUTO
  /PRINT=DESCRIPTIVE ETASQ
  /CRITERIA=ALPHA(.05)
  /WSDESIGN=Block Seq Keys Block*Seq Block*Keys Seq*Keys Block*Seq*Keys
  /DESIGN=RSSR Group RSSR*Group.
```

Appendix 10: R code for Figure 5

```
TMS_13 %>%
  group_by(Condition, Delay) %>%
  ggplot(aes(group = Condition,
             x = Delay,
             y = mean)) +
geom_line(aes(linetype=Condition))+
scale_color_manual(values = c("#330000","#330000","#330000","#330000"))+
labs(x = "Delay", y = "slowing effect of the stimulation (ms)")+
  theme_bw()+
  geom_errorbar(aes(ymin = mean - SE, ymax = mean + SE), width = 0.2)+
  scale_x_discrete(labels=c("1" = "BL", "2" = "D0","3" = "D20", "4" = "D40"))+
  theme(legend.text = element_text(size=10))+
  theme(legend.title=element_text(size=14))+
  guides(shape=guide_legend(override.aes = list(size = 5)))+
  guides(size = FALSE)
```

Appendix 11: R code for Figure 6

```
TMS_12 %>%
  group_by(RSSR, Day) %>%
  ggplot(aes(group = RSSR,
             x = Day,
             y = mean)) +
geom_point(aes(shape= RSSR, size = 0.1, colour = RSSR))+
  scale_shape_manual(values = c(15, 16, 17, 18))+
scale_color_manual(values = c("#330000","#330000","#330000","#330000"))+
  geom_line()+
labs(x = "Delay", y = "mean reaction time (ms)")+
  theme_bw()+
  geom_errorbar(aes(ymin = mean - SE, ymax = mean + SE), width = 0.2)+
  scale_x_discrete(labels=c("1" = "BD1", "2" = "0D1","3" = "20D1", "4" = "40D1", "5" = "BD2", "6" =
"0D2", "7" = "20D2", "8" = "40D2"))+
  geom_segment(aes(x = 1.5, y = 370, xend = 1.5, yend = 290), linetype = "dashed") +
  annotate("text", x= 2.25, y = 330, label= "real Stimulation", size = 3) +
  geom_segment(aes(x = 5.5, y = 275, xend = 5.5, yend = 240), linetype = "dashed") +
  annotate("text", x= 4.5, y = 240, label= "real Stimulation", size = 3) +
  theme(legend.text = element_text(size=10))+
  theme(legend.title=element_blank())+
  guides(shape=guide_legend(override.aes = list(size = 5)))+
  guides(size = FALSE)
```