The role of alpha oscillations in premotor-cerebellar connectivity in motor sequence learning: Insights from transcranial alternating current stimulation

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eingereicht von	Christine Viktoria Schubert geboren am 06.06.1994 in Hadamar
angefertigt an der	Medizinischen Fakultät der Universität Leipzig Universitätsklinikum Leipzig Klinik und Poliklinik für Neurologie
Betreuer	Prof. Dr. med. Joseph Claßen
Co-Betreuerin	Dr. rer. nat. Elinor Tzvi-Minker

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1 List of Abbreviations

BOLD	Blood oxygenation level dependent
CB	Cerebellum
dPMC	Dorsal premotor cortex
EEG	Electroencephalography
ERD	Event-related synchronization
ERS	Event-related desynchronization
fMRI	Functional magnetic resonance imaging
M1	Primary motor cortex
MSL	Motor sequence learning
PET	Positron emission tomography
PMC	Premotor cortex
RND	Random
S1	Primary somatosensory cortex
SEQ	Sequence
SM1	Sensorimotor cortex
SMA	Supplementary motor area
SMC	Supplementary motor cortex
SMP	Simple
SPL	Superior parietal lobule
SRTT	Serial reaction time task
tACS	Transcranial alternating current stimulation
- rCB	Right cerebellar tACS
- lM1	Left M1 tACS
tDCS	Transcranial direct current stimulation
TMS	Transcranial magnetic stimulation
TP	Time point

2 Introduction

The ability to acquire motor skills is fundamental throughout human lifetime. This includes children who are learning basic movements such as standing or walking but also adults who are continuously confronted to acquire new motor skills in divers fields of their daily life. Professional typing, playing the piano, tennis, or video games - as different as these movements appear, they are all based on similar strategies which enable the acquisition of a new motor skill. For patients with movement disorders such as Parkinson's disease or cerebellar ataxia as well as patients suffering from stroke it becomes specifically challenging to relearn motor skills as studies show that motor learning is impaired (Ustinova et al., 2000, Ding et al., 2002, Raghavan et al., 2007). Especially for those patients, rehabilitation programs are essential to regain motor functions. However, the mechanisms behind motor skill learning still need to be further investigated to develop both a profound understanding of the neurophysiological mechanisms of motor learning and subsequently therapeutic methods to facilitate rehabilitation processes.

Investigation of the neural underpinnings of motor learning has been a main interest in the neuroscience literature. A large number of studies focused on the detection of a motor network (Doyon et al., 2003, Doyon et al., 2009, Hardwick et al., 2013), its task-dependent activity patterns (Doyon et al., 2003, Penhune and Steele, 2012) and the structural-functional interplay of motor brain areas during learning (Tamas Kincses et al., 2008, Coynel et al., 2010). However, the mechanism of how communication within this motor network is implemented remains uncertain. Neuronal oscillations could serve as a possible mechanism through which interactions between brain regions take place.

In this dissertation, I aim to expand on the evidence linking neurophysiological parameters and motor learning, by investigating the role of alpha oscillations in a motor network underlying motor sequence learning (MSL). To this aim, I used a well-established paradigm, the serial reaction time task (SRTT; Nissen and Bullemer, 1987), to study motor sequence learning in young healthy subjects. Further, I combined transcranial alternating current stimulation (tACS), a non-invasive stimulation method that modulates ongoing brain rhythms (Herrmann et al., 2013), with electroencephalography (EEG) to analyse learning dependent changes on oscillations as well as connectivity within the motor network.

In the following, I will introduce the mechanisms underlying motor skill acquisition including the involved motor network and its functional connectivity patterns. I will then focus on the role of neuronal oscillations in network communication and in motor learning, and finally elaborate on modulation of brain activity by tACS.

2.1 Motor Learning Stages

Motor learning depends on the optimization of information processing through complex dynamic feedback systems. Learning proceeds in different successive stages. Early (within a single training session), late learning (multiple training sessions) and consolidation stages are followed by an automatization and retention stage (Doyon and Benali, 2005). In this process, learning is characterized by a gradually decreasing need for attention on task performance, decreasing susceptibility towards external interference or performance improvements without supplemental practice on the task (consolidation stage) and finally minimal cognitive efforts during task execution (automatization stage). As soon as a motor skill is sustainably internalized execution of the newly acquired motor skill is possible after long delays without further training (retention stage, Doyon and Benali, 2005). Behavioral studies focusing on motor learning showed that learning is accompanied by an increase in performance speed (Pascual-Leone et al., 1994), efficiency, as well as accuracy (Hikosaka et al., 1995, Penhune and Steele, 2012) and a change in the speed-accuracy tradeoff characteristic (Reis et al., 2008).

2.2 Motor Learning Tasks

The research field of motor learning is extremely diverse. To gain a deeper insight on different aspects of motor acquisition, various motor tasks have been developed. They serve to specifically investigate special types of motor learning, such as motor sequence learning and motor adaption. Motor sequence learning leads to the ability to perform several movements smoothly one after another (Doyon and Benali, 2005). One common paradigm for studying motor sequence learning is the serial reaction time task (SRTT, Nissen and Bullemer, 1987). Subjects are asked to react as quickly as possible to visually presented stimuli by pressing the corresponding buttons. MSL is then frequently defined as the shortening of reaction time and/or error reduction. Here, learning might take place on two different levels. On the one hand, associations between successive stimuli (perceptual learning), on the other hand associations between successive responses (motor domain) are possible (Robertson, 2007, Abrahamse et al., 2010). Moreover, visuomotor adaption tasks are sensorimotor tasks which are used to investigate motor adaptation capacities. These tasks require error-based learning of movement kinematics (e.g., movement speed and limb geometry) and the capacity to adapt to environmental perturbations (Doyon and Benali, 2005).

Size and activity of neuronal systems in motor learning highly depend on learning tasks and/ or stages. To meet all specific requirements for successful motor learning, not only one brain region but a communicating network is needed.

2.3 Motor Learning Network

Neuroimaging studies have shown that motor learning activates a network of cortical and sub-cortical structures (Doyon et al., 2003, Doyon et al., 2009, Hardwick et al., 2013). Using a meta-analysis of imaging studies, Hardwick and colleagues (2013) showed that a core motor network including dorsal premotor cortex (dPMC), supplementary motor cortex (SMC, comprising both the SMA-proper and pre-SMA), primary motor cortex (M1), primary somatosensory cortex (S1), superior parietal lobule (SPL), thalamus, putamen and the cerebellum (CB) is involved in motor learning. This core network showed constant activity during motor learning independent from the specific motor task. Basal ganglia and cerebellum seemed to be mainly active in sensorimotor adaptation tasks whereas cortical areas and thalamus seemed to have higher impact in MSL. Left dPMC showed pervasive activity in all motor paradigms (Hardwick et al., 2013).

Indeed, premotor cortex (PMC) seems to be a key structure in motor learning due to its function in motor planning and execution (Hoshi and Tanji, 2007) as well as visuomotor control (Hardwick et al., 2013), when motor responses are selected by adjusting them to sensory input like visual impulses (Grafton et al., 1998, Halsband and Lange, 2006, Hardwick et al., 2013). PMC is also said to be important for movement preparation and selection (Grafton et al., 1998) by organizing temporal information of sequential motor movements (Halsband et al., 1990, 1993, Hoshi and Tanji, 2007, Orban et al., 2010). Mice with lesioned PMC showed deficits in predicting action outcome revealing that PMC serves to process goal-directed movements (Gremel et al., 2013). Finally, Boyd and colleagues (2009) also found evidence that PMC plays an important role in motor consolidation (Boyd et al., 2009).

The motor cortex (M1) is predominantly responsible for movement execution (Penhune and Steele, 2012, Bhattacharjee, 2021), but also motor consolidation (Muellbacher, 2002, Robertson, 2005) and especially storage of new motor memories (Penhune and Steele, 2012), including learned sequential movements (Matsuzaka et al., 2007). Information output from M1 mainly controls contralateral limb movements. Thus, left-hand movements are associated with neural activity in the right motor cortex and right-hand movements with neural activity in the left motor cortex. Note that activity in ipsilateral M1 has also been reported, especially during complex, precise movements (Verstynen et al., 2005, Buetefisch et al., 2014). This effect is supposed to be mainly driven by interhemispheric influences between motor cortical areas (Allison et al., 2000, Kobayashi et al., 2003).

The basal ganglia comprise several nuclei: striatum (caudate nucleus and putamen), globus pallidus, substantia nigra and subthalamic nucleus. As these structures are highly connected to multiple motor network components, their role in motor learning is manifold. Striatum seems to be important for reward-based (Hikosaka et al., 2002) as well as associative learning (Penhune and Steele, 2012).

In motor learning, the cerebellum is important to enable learning and further optimize motor performance. Due to its multiple connections with subcortical and cortical areas, such as parietal, premotor, motor, and frontal cortex (Schmahmann et al., 1997, Middleton and Strick,

1998, Kelly and Strick, 2003), it guides learning through immediate feedback between actual input and output of motor and sensory information (Penhune and Steele, 2012). Therefore, internal models of movements are created in the way that a suitable movement and its sensory consequences are predicted, adjusted to the actual sensory input, and finally optimized and refined after an error assessment (Wolpert et al., 1998, Ohyama et al., 2003, Shadmehr and Krakauer, 2008, Penhune and Steele, 2012, Hardwick et al., 2013). Further evidence supporting the cerebellums' role in motor learning comes from studies with patients suffering from cerebellar damage. Although these patients reacted to a perturbation by adapting their movements, they were not able to sufficiently predict and learn movements (Bastian, 2006).

2.4 Theoretical Models of Motor Learning

Theoretical models of motor learning have been developed to integrate findings from imaging studies as well as behavioral studies to a broader concept of how motor learning takes place on a neurophysiological level. These learning models are usually based on constant feedback systems that are mediated or sustained by cortico-cerebellar and cortico-striatal loops (Hikosaka et al., 2002, Doyon et al., 2009, 2003, Penhune and Steele, 2012). They differ in that they focus either on learning components, learning stages or tasks.

Hikosaka and colleagues (2002) proposed a network model of parallel working, component-dependent loop circuits. In this model, motor learning is achieved through parallel learning of a spatial and a motor component. Acquisition of a spatial sequence is mediated by a loop between prefrontal cortex and associative areas of the basal ganglia and the CB, and is predominantly important during early learning. According to this model, once learning is established and motor execution becomes more important, information is mostly processed in a loop between M1 and motor regions of basal ganglia and CB (Hikosaka et al., 2002).

According to the learning-stage model by Doyon and colleagues (2009) both cortico-cerebellar and cortico-striatal loops are important in early learning stages. A network including striatum, parietal and motor cortices guides late learning, consolidation and retention (Doyon et al., 2009). This is in line with studies showing that cerebellar activity was predominantly found to decrease with learning (Doyon et al., 2002, Penhune and Doyon, 2005, Penhune and Doyon, 2010, Penhune and Steel, 2012). In a review of neuroimaging data from Lohse and colleagues (2014), a meta-analysis of 58 motor skill learning studies supported the role of a network including CB, prefrontal as well as premotor cortical regions in early learning, suitable with the need to form visual-spatial representations at this stage (Doyon et al., 2018). However, in M1 either increased activity (Karni et al., 1995, Penhune and Doyon, 2005, Floyer-Lea and Matthews, 2005, Lohse et al., 2014) or unvarying activity (Toni et al., 1998, Berlot et al., 2020) was found with practice, showing that present evidence from neuroimaging studies focusing on learning stage dependent motor network activity is still ambiguous.

According to the model by Doyon and colleagues (2003), a network of cortico-cerebellar structures primarily underlies learning in sensorimotor adaptation tasks while sequence learning tasks are processed through a cortico-striatal system (Doyon et al., 2003). This line

of evidence was expanded by Penhune and Steele in that they propose simultaneous activation of cortico-striatal and cortico-cerebellar loops that underlie motor learning. The degree of involvement of each loop is said to depend on both a particular learning phase and particular task demands (Penhune and Steele, 2012).

Theoretical models have been supported by not only analysis of spatial distribution of activity patterns in the brain, but also by the analysis of (functional) connectivity within the motor network.

2.5 Functional Connectivity of Motor Brain Regions

Non-human studies provided a first basis for the detection of anatomical projections in the motor network. Connections from the cerebellum via thalamus to cerebral cortex (including M1, premotor, prefrontal, posterior parietal areas and non-motor areas) have been detected as well as connections from cerebral cortex via pons to CB (Schmahmann and Pandya, 1997, Kelly and Strick, 2003, Bostan et al., 2013). For example, Strick and colleagues (2001, 2003) used genetically modulated viruses to reveal topographically distinct projections from prefrontal cortex (Middleton and Strick, 2001, Kelly and Strick, 2003) as well as from M1 (Kelly and Strick, 2003) to cerebellum, forming closed loops (Bostan et al., 2013).

Functional neuroimaging techniques were used to expand this line of evidence to humans. Functional connectivity is measured by spatio-temporally correlated activity of different brain regions (Ma et al., 2010). Allen and colleagues (2005) were able to demonstrate correlations of blood oxygenation level dependent signal (BOLD-signal, a measure of neuronal activity) between CB, parietal and prefrontal cortex, supporting the existence of cerebellar-parietal and cerebellar-prefrontal connections in humans at rest (Allen et al., 2005).
Moreover, studies showed that motor network expression and interaction dynamically change during motor learning (Sun et al., 2007, Tamas Kincses et al., 2008, Coynel et al., 2010).
Tamas Kincses and colleagues were able to demonstrate temporal changes in activity in a fronto-parieto-cerebellar network in the way that activity in a network decreased as MSL proceeded. Moreover, stronger network activation in parietal and premotor cortex was correlated with improved performance during sequence compared to random elements (Tamas Kincses et al., 2008). Early learning was also associated with stronger functional connectivity in the MSL network (Coynel et al., 2010). As soon as learning reached automatization stage, functional connectivity decreased between premotor or parietal cortex and connected subcortical areas, namely striatum and cerebellum (Coynel et al., 2010). These findings are consistent with results from bimanual motor learning studies demonstrating enhanced interregional coupling between SMA, sensorimotor and premotor cortex in early compared to late learning (Sun et al., 2007). Findings of a PET study revealed that activity in M1 correlated directly with activity in the CB during early learning, as indicated by an increase in cerebellar blood-flow associated with decreased blood flow in M1 (Penhune and Doyon, 2005).

Functional connectivity between M1 and CB was higher when comparing Day 5 to Day 1 during training of a temporal motor sequence task, which was interpreted to show that performance improvements modulate interactions between M1 and CB (Penhune et al., 2010). Consequently, motor learning seems to occur through dynamic changes in functional connectivity in a broad cortico-striato-cerebellar motor network.
However, activation patterns of brain regions measured by diverse neuroimaging techniques should be interpreted in a critical way to avoid inaccurate fallacies (Poldrack et al., 2006). A recent study by Berlot and colleagues reviewing fMRI signatures of MSL pointed out difficulties in interpreting activity increase or decrease as an absolute measure of function. Thus, the researchers advise that changes in activity should rather be interpreted as optimisation processes in terms of efficiency within the learning process than static parameters (Berlot et al., 2020).

2.6 Effective Connectivity of Motor Brain Regions

Measurement of effective connectivity expands on network analyses beyond correlated activity patterns (functional connectivity) by examining directed interactions between brain areas. Unilateral hand movements were shown to be accompanied by coupling of neuronal activity from contralateral premotor areas (SMA, PMC) and ipsilateral CB towards contralateral M1. Better motor performance was associated with stronger coupling (Pool et al., 2013). In subjects performing a modified version of a SRTT, connections from M1 to CB as well as from PMC to CB were negatively modulated by sequence learning blocks but not by random blocks (Tzvi et al., 2014). Moreover, consolidation of a motor sequence was associated with learning related negative modulation of connections from bilateral putamen to CB as well as less consistently from left M1 to CB. In addition, cerebellum showed positive forward connections to putamen and motor cortical areas during MSL (Tzvi et al., 2015). The direction of functional connections from CB to M1 gradually changed from positive to negative during training of a motor sequence for 4 weeks (Ma et al., 2010). These results support the cerebellums' role in error correction during early learning. In sum, it becomes clear that dynamic connections on a small as well as large scale within the motor network are essential for learning.
However, it remains an important object of research, how these connections are functionally mediated in the sense that an efficient way of communication is established. One possible neuronal phenomenon which serves to meet these demands are neuronal oscillations.

2.7 Oscillations in Neuronal Communication

Neuronal oscillations are rhythmical patterns of neuronal activity. They can be measured by electroencephalography (EEG) and are characterized by their phase, amplitude and frequency. In terms of frequency, they can be classified to the following frequency bands: delta (1–4 Hz), theta (4–8 Hz), alpha (8-13 Hz), beta (14–30 Hz), low gamma (30–70 Hz) and high gamma (70–150 Hz). Among these, oscillations in the alpha frequency range (8 - 13 Hz) are the

predominant oscillations of the primates' brain. The phase describes the actual deflection of an oscillation as follows $\varphi(t) = \omega t + \varphi_0$; ω *is pulsatance* $\left(\frac{rad}{s}\right)$. The power represents the amount of activity in a certain frequency band: $S_{XX}(\omega) := \lim_{T\to\infty} \frac{1}{2T} |F(f_T)(\omega)|^2$.

Information processing in the brain is organized by neurons oscillating synchronously in a time-, phase-, and frequency-specific way (Salinas and Sejnowski, 2001, Buzsáki and Draguhn, 2004, Fries, 2005, Hipp et al., 2011). One way of quantifying effective neuronal interaction between brain regions is through coherence (Fries, 2005). Communication through coherence is based on the theory that oscillatory activity is correlated when the respective phases or amplitudes show a constant relation (Fries, 2005, 2015, Hipp et al., 2011) or when the phase of a slower frequency is coupled to the amplitude of a higher frequency (Siegel et al., 2012). When groups of neurons oscillate coherently, they are communicating in the same communication channel and information exchange becomes highly efficient and flexible (Buzsáki and Draguhn, 2004, Fries, 2015). Thus, processing of information becomes less susceptible to internal or external distractions. In addition, network communication is not only provided by locally synchronized, but also long-range synchronized oscillatory activity (Hipp et al., 2011, Sadaghiani and Kleinschmidt, 2016). Slow oscillations, for example theta (Jensen et al., 2007, Klimesch et al., 2010) and alpha oscillations (Klimesch et al., 2010, Chapeton et al., 2019) are considered to be a marker of long-range neural communication. Patients suffering from Parkinson's disease show elevated oscillatory activity in the beta band especially in the basal ganglia (Little and Brown, 2014), as well as pathologically increased motor cortical beta/high-gamma phase-amplitude coupling (De Hemptinne et al., 2013, Gong et al., 2021). This increased coupling is said to underlie their motor control deficits (Voytek and Knight, 2015). Consequently, neural network communication seems to depend on a sophisticated interplay between local spiking activity and interregional coupling.

2.8 Alpha Oscillations

Alpha oscillations have been of broad interest in neuroscientific research. They have been found to be important in working memory (Klimesch et al., 1997, Klimesch et al., 2010, Bonnefond and Jensen, 2012, Sauseng et al., 2009), long term memory processes (Meeuwissen et al., 2011, Hanslmayr et al., 2012), attentional processes (Hanslmayr et al., 2011) and motor learning (Pollok et al., 2014, Tzvi et al., 2016, 2018). Further, alpha oscillations have been found to functionally guide information processing and communication (Başar et al., 2012, Klimesch et al., 2012, Jensen et al., 2014, Chapeton et al., 2019, Sadaghiani and Kleinschmidt, 2016). Alpha oscillations are thought to have an inhibitory effect (Klimesch et al., 2007, Mazaheri and Jensen, 2010). According to this theory, alpha oscillations serve to coordinate demand-related involvement of brain regions. Thus, alpha power increase is said to lead to inhibition of task-irrelevant regions and alpha power decrease supports involvement of relevant brain areas (Mazaheri and Jensen, 2010). A study by Sauseng and colleagues (2009) using a combined EEG-TMS approach showed that high alpha amplitudes were associated with low excitability of motor areas, corresponding to the targeted muscle (Sauseng et al.,

2009). Alpha power was also linked to suppression of distracting information in a memory task (Bonnefond and Jensen, 2012).
Further, alpha guided information processing was found to be highly dependent on the phase (Busch et al., 2009, Dugué et al., 2011, Mathewson et al., 2009, 2011, Bonnefond and Jensen, 2012). As the phase of an oscillation seems to be mainly responsible for timing of neuronal activity, inhibition and facilitation of information transfer were more effective depending on a particular phase of the alpha cycle (Dugué et al., 2011, Klimesch et al., 2010). For example, Mathewson and colleagues (2009) found in subjects performing a visual detection task that the probability of actively perceiving a stimulus was dependent on when the stimulus was presented relative to the alpha phase of oscillations recorded from the occipital cortex (Mathewson et al., 2009). Consequently, alpha oscillations seem to shape a functional framework for neuronal information processing by rhythmically synchronizing neuronal inhibition as well as excitation (Mathewson et al., 2011, Klimesch et al., 2010, Chapeton et al., 2019). In addition, coherent alpha oscillations were also found to mediate communication over long distances (Classen et al., 1998, Kujala et al., 2007, Klimesch et al., 2010, Palva and Palva, 2011, Chapeton et al., 2019). These alpha directed mechanisms seem to be crucial for optimal network communication by boosting efficiency and precision of information selection.
Alpha oscillations recorded over motor cortical regions are called mu (μ) oscillations. Mu oscillations have been found to be prominent during rest but suppressed as soon as a voluntary movement is imagined, planned, or performed (Arroyo et al., 1993), a phenomenon called event-related desynchronization (ERD, Pfurtscheller, 1992). ERD has been shown in task-relevant, activated brain regions and event-related synchronization (ERS) is said to be the neurophysiological correlate of decreased cortical excitability (Pfurtscheller, 1992).

2.8.1 Role of Alpha Oscillations in Motor Sequence Learning

Alpha oscillations also seem to play an important role in motor sequence learning. Alpha oscillations have been found to continually decrease over frontocentral areas with learning (Zhuang et al., 1997, Boenstrup et al., 2014). As soon as subjects gained explicit knowledge of a sequence, alpha power subsequently increased (Zhuang et al., 1997). Motor memory consolidation has been shown to be accompanied by an increase in alpha power measured by decreased alpha event-related desynchronization (Pollok et al., 2014). Moreover, mu oscillations were found to be associated with sequence complexity in a network between frontocentral areas and M1, such that unknown sequences resulted in greater alpha power decrease than pre-learned ones (Boenstrup et al., 2014). In perceptual learning, dynamic changes of alpha power over occipital-parietal areas seem to be of relevance. Right occipito-parietal alpha power was shown to decrease during visual sequence learning and increase at resting-state following learning (Moisello et al., 2013). Alpha power increased over right frontal areas shortly after stimulus presentation (Moisello et al., 2013) and over occipito-parietal areas shortly before response (Crivelli-Decker et al., 2018). Tzvi and colleagues

(2016, 2018) previously showed that during visuomotor sequence learning, a decrease in alpha power over occipito-parietal areas was associated with learning progress (Tzvi et al., 2016, 2018). Finally, Mehrkanoon and colleagues (2016) suggested a specific role for oscillations in alpha and beta frequency range in communication between motor cortex and CB in motor learning. Source-space analysis of EEG signals revealed that functional connectivity in alpha and beta band between CB und M1 was enhanced in post-training resting state (Mehrkanoon et al., 2016). In line with substantial evidence of the role of alpha oscillations in neuronal systems (see chapter 'Alpha Oscillations'), they also seem to guide functional recruitment of task-relevant and irrelevant brain regions in the motor network as well as in motor network communication over long distances. In visuomotor learning, alpha oscillations in occipito-parietal areas (e.g., location of visual cortex) might be of higher relevance due to task-dependent need of visuo-motor control (Tzvi et al., 2016, 2018). Moreover, decrease in alpha power in parietal areas was shown to be learning specific (Tzvi et al., 2016) and seems to play an important role in memory encoding (Moisello et al., 2013, Tzvi et al., 2018).
Further evidence supporting the role of alpha oscillations in motor learning comes from studies employing transcranial alternating current stimulation (tACS).

2.9 Transcranial Electric Stimulation

Transcranial electric stimulation is a non-invasive brain stimulation technique which is used to alter cortical excitability, cognition and behavior (Paulus et al., 2016). Transcranial direct current stimulation (tDCS) can be differentiated from transcranial alternating current stimulation (tACS) depending on the current waveform.
In motor sequence learning, tDCS applied over left M1 led to enhanced performance (Nitsche et al., 2003, Krause et al., 2016) as well as enhanced consolidation (Rumpf et al., 2017) in some studies, but had no effect in another study (Liebrand et al., 2020). Moreover, MSL was found to be improved during cerebellar tDCS in subjects performing a SRTT. This improvement was accompanied by an increase in learning-specific activity in right M1, left cerebellum lobule VI, left inferior frontal gyrus and right inferior parietal lobule compared to sham (Liebrand et al., 2020). Moreover, Liebrand and colleagues (2020) showed that cerebellar tDCS in this study elicited a network effect.
Whereas the effect of tDCS is based on the modulation of membrane polarization such as cathodal stimulation hyperpolarizes while anodal stimulation depolarizes the resting membrane potential, tACS is said to interfere with ongoing brain oscillations (Paulus, 2011).

2.9.1 Transcranial Alternating Current Stimulation (tACS)

By using a biphasic and sinusoidal current at a specific frequency, tACS offers the possibility to entrain endogenous brain oscillations in a frequency, amplitude, and phase specific way (Herrmann et al., 2013). A recent study in primates showed that tACS entrained frequency specific activity in single neurons over stimulated areas (Krause et al., 2019). To ensure successful entrainment, several stimulation criteria must be met (Thut et al., 2011).

For example, external modulation of a frequency depends on the internal presence of this frequency in the brain region and/or cognitive process (Thut et al., 2011). TACS at 10Hz is used to entrain ongoing alpha oscillations (8-13Hz). Supposedly, tACS does not only change activity in the directly stimulated area, but also influences network interactions between functionally connected regions (Herrmann et al., 2016, Cabral-Calderin et al., 2016). Thus, tACS is a useful method to reveal the role of specific oscillations in the targeted area and within a neuronal network. This could be achieved by analyzing the functional impact of externally driven changes on oscillatory activity. When combining tACS with EEG, behavioral influences can be correlated to stimulation induced oscillatory changes. Note, that combined EEG/tACS approaches are often limited by strong tACS-induced artifacts in EEG rendering online EEG-analyses ambitious (Herrmann et al., 2016).

Studies showed that 10Hz tACS leads to local entrainment of oscillatory activity over occipital areas (Zaehle et al., 2010, Helfrich et al., 2014) accompanied by phase-dependent modulation of cognitive functions (Helfrich et al., 2014). Motor sequence learning was found to be improved in healthy subjects receiving 10Hz tACS to left M1 compared to sham or other stimulation frequencies during learning (Antal et al., 2008, Pollok et al., 2015). Motor memory consolidation in healthy older adults was, however, disrupted when applying 10Hz tACS over M1 immediately after motor training compared to sham (Rumpf et al., 2019). Alpha-tACS applied over M1 during rest between motor training sessions revealed no changes in motor learning capacity (Krause et al., 2016, Sugata et al., 2018). Moreover, effects of alpha tACS seem to be age dependent (Fresnoza et al., 2020).

Despite its central role in motor learning and various evidence from cerebellar tDCS-studies, there are only few tACS-studies targeting the CB. So far, effects of cerebellar gamma-tACS are ambiguous, showing enhanced motor sequence learning during between-training stimulation (Naro et al., 2016) or no effects in acquisition or retention during online stimulation in subjects performing a sequential grip force task (Wessel et al., 2020). No effects on motor learning were found after cerebellar 10Hz tACS (Naro et al., 2016). Further, motor network communication between CB and M1 was influenced by simultaneous gamma-tACS over CB and M1 during training resulting in enhanced motor performance (Miyaguchi et al., 2018) and motor learning retention (Miyaguchi et al., 2020) in a visuomotor control task.

2.10 Summary of Study Rationale

Previous studies did not establish a causal link between motor learning and actual tACS-induced oscillatory entrainment in the alpha range. As alpha oscillations fulfill the theoretical requirements as mediators of network communication (Chapeton et al., 2019, also see chapter 'Alpha Oscillations') and have already been frequently shown to play an important role in higher cognitive processes (see chapter 'Alpha Oscillations' and 'Role of Alpha Oscillations in Motor Sequence Learning'), their role in MSL should be further investigated.

The goal of this dissertation was to investigate whether alpha oscillations mediate motor network communication and consequently modulate MSL. To this end, we used a combined

tACS-EEG approach to investigate behavioural as well as electrophysiological effects of alpha oscillations in a defined motor network during MSL. In our recent publication (Schubert et al., 2021), we showed that alpha spectral power over PMC and SM1 as well as alpha coherence between left PMC/SM1 and left cerebellar crus I gradually decreased during sequence learning in subjects performing a SRTT. Our aim was to expand this line of evidence by analysing how externally induced entrainment of alpha oscillations via 10Hz tACS would alter previously observed learning related changes in alpha power and alpha coherence in subjects performing a similar SRTT. The latter offers the possibility to draw more direct conclusions about the functional role of alpha oscillations in motor learning. Based on the importance of motor cortex and CB in MSL (Liebrand et al., 2020), we chose left M1 and right CB as stimulation locations in subjects performing a SRTT with their right hand. As online tACS leads to strong artifacts in EEG, our EEG analysis was focused on learning blocks immediately following stimulation.

Based on the evidence summarized above, we hypothesized that 10Hz tACS to either left M1 or right CB would lead to local entrainment of oscillatory alpha resulting in changes in learning-related alpha power and possibly affecting functional interactions in cortico-cerebellar loops, evident in experimental blocks following tACS. We expected that these oscillatory changes will subsequently affect learning performance during and following tACS.

3 Publication

Titel: Alpha oscillations modulate premotor-cerebellar connectivity in motor learning: Insights from transcranial alternating current stimulation

Authors: Christine Schubert[1], Alhuda Dabbagh[1], Joseph Classen[1], Ulrike M. Krämer[2,3,4], Elinor Tzvi[1]

[1] Department of Neurology, University of Leipzig, Liebigstraße 20, Leipzig 04103, Germany
[2] Department of Neurology, University of Lübeck, Ratzeburger Allee 160, Lübeck 23562, Germany
[3] Department of Psychology, University of Lübeck, Ratzeburger Allee 160, Lübeck 23562, Germany
[4] Center for Brain, Behavior and Metabolism, University of Lübeck, Ratzeburger Allee 160, Lübeck 23562, Germany

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Christine Schubert[a], Alhuda Dabbagh[a], Joseph Classen[a], Ulrike M. Krämer[b,c,d], Elinor Tzvi[a,*]

[a] Department of Neurology, University of Leipzig, Liebigstraße 20, Leipzig 04103, Germany
[b] Department of Neurology, University of Lübeck, Ratzeburger Allee 160, Lübeck 23562, Germany
[c] Department of Psychology, University of Lübeck, Ratzeburger Allee 160, Lübeck 23562, Germany
[d] Center for Brain, Behavior and Metabolism, University of Lübeck, Ratzeburger Allee 160, Lübeck 23562, Germany

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Alpha oscillations

ABSTRACT

Alpha oscillations (8-13 Hz) have been suggested to play an important role in dynamic neural processes underlying learning and memory. The goal of this study was to scrutinize the role of alpha oscillations in communication within a cortico-cerebellar network implicated in motor sequence learning. To this end, we conducted two EEG experiments using a serial reaction time task. In the first experiment, we explored changes in alpha power and cross-channel alpha coherence as subjects learned a motor sequence. We found a gradual decrease in spectral alpha power over left premotor cortex (PMC) and sensorimotor cortex (SM1) during learning blocks. In addition, alpha coherence between left PMC/SM1 and left cerebellar crus I was specifically decreased during sequence learning, possibly reflecting a functional decoupling in the broader motor learning network. In the second experiment in a different cohort, we applied 10Hz transcranial alternating current stimulation (tACS), a method shown to entrain local oscillatory activity, to left M1 (lM1) and right cerebellum (rCB) during sequence learning. We observed a tendency for diminished learning following rCB tACS compared to sham, but not following lM1 tACS. Learning-related alpha power following rCB tACS was increased in left PMC, possibly reflecting increase in local inhibitory neural activity. Importantly, learning-specific alpha coherence between left PMC and right cerebellar lobule VIIb was enhanced following rCB tACS. These findings provide strong evidence for a causal role of alpha oscillations in controlling information transfer in a premotor-cerebellar loop during motor sequence learning. Our findings are consistent with a model in which sequence learning may be impaired by enhancing premotor cortical alpha oscillation via external modulation of cerebellar oscillations.

Abbreviations

PMC	premotor cortex
SMA	supplementary motor area
SM1	sensorimotor cortex
tACS	transcranial alternating current stimulation
M1	primary motor cortex
CB	cerebellum
SRTT	serial reaction time task
EEG	electroencephalography
SMP	simple
RND	random
SEQ	sequence
rmANOVA	repeated-measures analysis of variance
DICS	dynamic imaging of coherent sources
TP	time point
TPJ	temporo-parietal junction
fMRI	functional magnetic resonance imaging

1. Introduction

Neural oscillations have gained increasing interest as a potential key mechanism underlying cognitive functions. Specifically, alpha oscillations (α, 8-13Hz) have been linked to working memory (Bonnefond and Jensen, 2012; Jensen, 2002; Sauseng et al., 2010) as well as long-term memory processes (Meeuwissen et al., 2011). Oscillations in the α frequency range over motor areas, also known as μ oscillations, were shown to decrease with motor sequence learning (Boenstrup et al., 2014) as well as with motor memory consolidation (Pollok et al., 2014). In previous work, we investigated oscillatory activity underlying visuomotor sequence learning (Tzvi et al., 2018, 2016). We found a relative increase in α power during the early learning phase over occipito-parietal areas, as well as α power decrease as learning progressed. This evidence suggested that dynamic changes in α power at functionally relevant sites may play a critical role in motor sequence learning.

While these findings provided merely correlative evidence, conclusions about a causal role of oscillations can be sought by employing

* Corresponding author.
E-mail address: elinor.tzvi-minker@medizin.uni-leipzig.de (E. Tzvi).

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transcranial alternating current stimulation (tACS), which leads to entrainment of local oscillatory activity to an externally applied frequency (Zaehle et al., 2010) and modulates behavior (Helfrich et al., 2014). A comprehensive study in primates showed that while tACS did not affect firing rates of individual cells or cell populations, it did entrain activity in single neurons, specific to the targeted frequency and spatial location (Krause et al., 2019). Moreover, a recent study in anesthetized rats showed that Purkinje cells recorded extracellularly from cerebellar vermis lobule 7, were strongly entrained by AC stimulation in a large range of frequencies (Asan et al., 2020). Thus, it appears that not only cortical but also cerebellar neuronal networks can be entrained by external AC stimulation. In motor sequence learning, 10Hz tACS (i.e. in the mid frequency range of α) to left primary motor cortex (M1) led to enhanced performance in some studies (Antal et al., 2008; Pollok et al., 2015) but disrupted consolidation in another study (Rumpf et al., 2019). While these studies suggest a link between learning and α oscillations, the exact role that α oscillations play in motor learning is not yet clear.

Potentially, α oscillations could be important for large-scale communication between brain regions (Chapeton et al., 2019). For motor sequence learning, interactions in a cortico-cerebellar loop appear to be critical as shown by functional magnetic resonance imaging (fMRI) studies (Bonzano et al., 2015; Penhune and Doyon, 2005; Steele and Penhune, 2010; Tzvi et al., 2015, 2014). Using source-space analysis of EEG signals, Mehrkanoon and colleagues (2016) suggested a specific role for oscillations in α and the β frequency range in communication between motor cortex and cerebellum underlying motor learning.

Against this background, we here investigated whether α oscillations mediate cortico-cerebellar interactions and thereby enable motor learning. To this end, we performed two EEG experiments in independent cohorts, using a modified version of the serial reaction time task (SRTT, Nissen and Bullemer, 1987). In the first experiment, we investigated α power changes during motor sequence learning, as well as α-dependent connectivity as indexed by coherence of oscillatory signals. Based on evidence above, we expected to find learning-related changes to α power in motor cortical areas as well as the cerebellum. In the second experiment, we tested whether externally applied α oscillation influences learning through local α entrainment in M1 and cerebellum, and connectivity within the cortico-cerebellar network. To this end, we applied 10Hz tACS to left M1 and right cerebellum while subjects performed the SRTT. Based on previous evidence stressing the contribution of both M1 and cerebellum to motor sequence learning, we hypothesized that tACS at both locations would lead to changes in learning-related α power, measured following tACS.

2. Materials and methods

2.1. Experiment 1

2.1.1. Participants

Twenty-six healthy participants (mean age: 23 years, range 18-38; 9 males) took part in the first experiment after giving informed consent. Participants were financially compensated or received course credit. All participants were right-handed, had normal or corrected to normal vision with no color deficiency. We excluded participants who regularly played a musical instrument or computer games. The study was approved by the Ethics Committee of the University of Lübeck and was performed in accordance with the Declaration of Helsinki. One participant was excluded due to an error in data acquisition. An additional participant was excluded due to high error-rates (see behavioral analysis). For the EEG data analysis, two participants were excluded due to artifacts in the recorded EEG signal, resulting in a sample of 22 participants for the EEG analyses of Experiment 1.

2.1.2. Experimental paradigm and task design

During the EEG recordings and task performance, participants were seated comfortably in front of a 17" screen, about 1 m away, on which visual stimuli were presented. Before and after the experimental blocks, we recorded resting-state sessions (200 s each), which were not further analyzed for the present work. Participants performed a modified version of the serial reaction time task (SRTT). They were explicitly informed about the presence of a sequence within the task. In each trial, four squares were presented on a grey background in a horizontal array, with each square (from left to right) associated with one of four fingers of the right hand (Fig. 1A). At stimulus onset, one of the squares turned blue and the rest remained black. Participants were instructed to respond to this blue colored square with the corresponding button, as precisely and quickly as possible. The stimulus remained on the screen until a button press was registered. In case of a wrong button press, the blue colored square turned to red to mark the error. The response-stimulus interval was 500ms (Fig. 1B). Trials were counted as correct when the appropriate key was pressed within 1000ms after stimulus onset. In case no button was pressed within this time frame, a text appeared on the screen requesting the participants to be faster ("Schneller!").

The task consisted of three different conditions: simple (SMP), random (RND), and sequence (SEQ). In SMP, stimuli were presented in a simple order of button presses 4-3-2-1-4-3-2-1. In RND, stimuli were presented in a pseudorandom order, generated using Matlab (The Mathworks®, Natick, MA), such that items appeared exactly twice, were not repeated and pairs of consecutive stimuli were followed by some other stimuli, thereby preventing learning by pairwise associations (Curran, 1997). In SEQ, stimuli were organized in an 8-items-sequence (4-1-4-2-3-1-3-2) also preventing pairwise associations. The task contained a total number of 11 blocks, separated by 20 s breaks. There was one SMP block at the beginning or the end of the task (counter-balanced between subjects), and five RND blocks and five SEQ blocks in an alternating order (see Fig. 1D for an exemplary order). Each of the SEQ blocks contained 15 repetitions of the 8-element sequence summing to a total of 120 trials per block. The SMP block contained 10 repetitions of the simple sequence summing to a total of 80 trials. Each of the RND blocks contained 80 trials. Note that RND blocks were constructed to be shorter than SEQ blocks such that learning is not disrupted for longer than necessary. The total duration of the task was ~25 min, depending on the individual performance.

Next and after a short (~5 min) break, participants performed a completion task to assess explicit knowledge of the sequence. This task was identical to the SRTT, only that here the 8-element sequence was repeated 32 times (summing to a total of 256 trials) in one short block and the other conditions were omitted. In each repetition, one regular trial was substituted by a completion trial. In a completion trial, the target square was replaced by a question mark and subjects had to press a button corresponding to one of the 4 squares which they believed should be the next target square. Each position in the sequence was therefore tested four times producing 32 completion trials. After guessing, subjects were asked whether they were sure of their choice and gave a YES/NO answer. This procedure enabled us to differentiate between a correct response and a correct assured response.

2.1.3. Behavioral analysis

We computed reaction times (RT) and error-rates (ER) for each of the experimental conditions (SEQ, RND and SMP). Both wrong button-presses and missing responses were regarded as errors. RT were averaged across mini-blocks of 40 trials, corresponding to five repetitions of the 8-element sequence in SMP and SEQ. This sub-division resulted in two mini-blocks for each RND and SMP block and three mini-blocks for each SEQ block. We excluded trials in which the participants made an error as well as trials in which RT deviated by more than 2.7 standard deviations (SD) from the mini-block's averaged RT (corresponding to 1% of the normal distribution tail). This resulted in an averaged exclusion of 1.4±1.5% of the trials in each mini-block. We defined five time points (TP) in which sequence learning was assessed at the transition from RND to SEQ (see Fig. 1C). RTs from mini-blocks at this transition were subjected to a 2 × 5 repeated measures ANOVA (rmANOVA) with

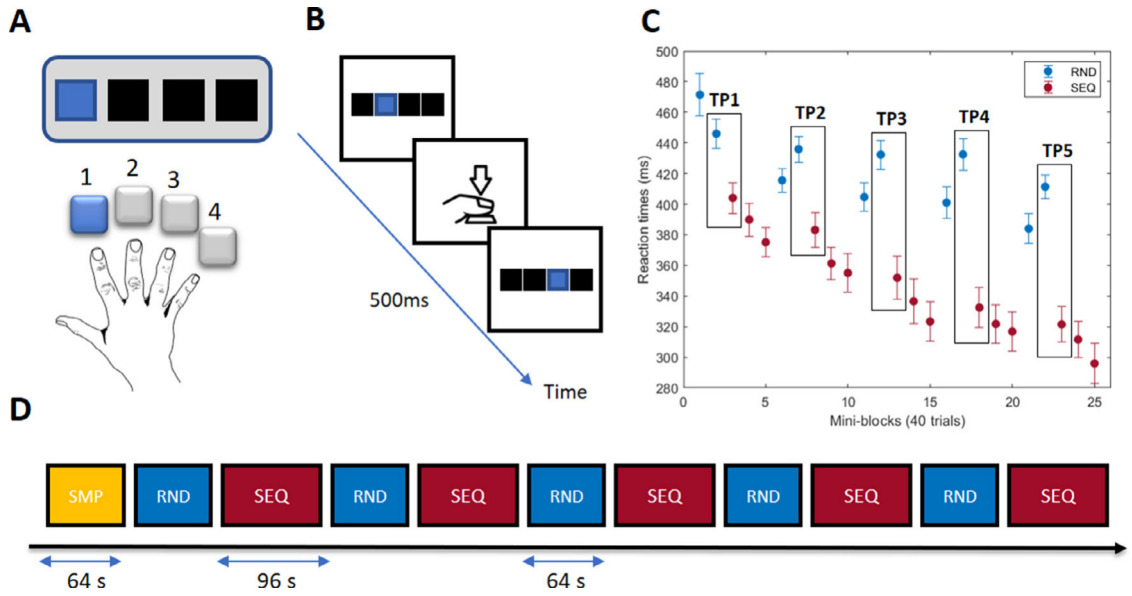

Fig. 1. Experiment 1: experimental design and behavioural results. (A) Serial reaction time task. In each trial, 4 black squares were presented. At stimulus onset, one of the squares turned blue and subjects were instructed to press the button corresponding to the blue square with the respective finger. (B) Task timeline. (C). Reaction times averaged across subjects in each mini-block with 40 trials, and each condition. Error bars are standard errors of the mean. RND – random trials. SEQ – sequence trials. TP – time points represent the mini-blocks used for analysing learning effects. D Subjects performed five RND blocks of 80 trials and five SEQ blocks of 120 trials in an alternating order. A simple block (SMP – yellow) with 80 trials appeared before or after the main task.

factors COND (SEQ, RND) and TP (TP1-TP5). Note that the SMP block was not included in this analysis.

ER were computed by dividing the number of errors in each mini-block by 40. As ER were not distributed normally, we were not able to apply rmANOVA as with the RTs. We, therefore, assessed condition differences at each transition point (TP). These differences were normally distributed which allowed us to evaluate whether ER difference between conditions changed over time, using a 1-way rmANOVA with factor TP (TP1-TP5).

2.1.4. EEG recordings

EEG was recorded with Ag/AgCl electrodes and two 32-channel BrainAmp MR plus amplifiers (Brain Products GmbH) with a sampling rate of 250Hz, band-pass filter of 0.1-1000Hz, and amplitude resolution of 0.1μV. Electrodes were placed according to an extension of the international 10–20 system. Vertical and horizontal eye movements were recorded, using electrodes placed below the right eye and a frontopolar electrode (vertical), as well as electrodes located on the outer canthus of each eye (horizontal). The EEG was recorded against a reference electrode placed on the right earlobe and a ground electrode at FPz location. All electrode impedances were kept below 5kΩ.

2.1.5. EEG pre-processing

Pre-processing and all subsequent analyses were performed using in-house Matlab scripts and the EEGLAB toolbox. EEG signals were re-referenced offline to the averaged signal of two earlobe electrodes. A high-order band-pass filter (F_{cutoff} = 1 – 49 Hz) was applied to the signals to remove slow drifts and power line noise. Then, signals were segmented into 3s epochs (-1s to 2s around stimulus onset). An independent component analysis (ICA) procedure was then applied to the signals to identify components related to eye blinks and horizontal eye movements based on their topography and signal shape. In most subjects we removed 2-3 components. In two subjects, noisy electrodes at locations Fp1, Fp2, T7, T8 were identified and interpolated prior to ICA. In those subjects we accounted for the rank-deficiency problem, due to interpolation of 4 channels, by producing 57 ICs (instead of 61 ICs). In these two subjects up to 6 components were rejected. Additional artifacts were removed in the entire cohort using a simple threshold (-80μV, +80μV) on the filtered data.

2.1.6. Spectral power and source analysis

Next, we computed the power spectrum of the EEG signals using the Morlet wavelet as implemented in the Fieldtrip toolbox (http://www.fieldtriptoolbox.org). Signals were filtered to obtain oscillatory power at 1 - 49Hz using wavelets of 7 cycle length. Frequency resolution was set to 2Hz and time resolution to 50ms. Similar to previous work (Tzvi et al., 2018, 2016), the effect of learning on EEG power was computed using power differences between SEQ and RND trials in each consecutive block (time point TP1-TP5). Based on evidence from a study using a similar SRTT showing that sequence knowledge is reflected by increased fronto-central negativity at ~300ms (Verleger et al., 2015), we expected to find the strongest effect around ~300ms and therefore focused our analysis on a 0 - 300 ms time window following stimulus onset. Note that these time windows were also selected based on averaged RT at the end of the task to minimize possible overlap with button presses and thus motor activity. Signals were averaged separately across a pre-stimulus period in a -200 - 0 ms time window (0ms being stimulus onset), and across a post-stimulus period in a time window of 0 - 300 ms. No baseline correction was applied. There was no specific hypothesis regarding the pre-stimulus period. Statistical tests were performed on power differences (SEQ-RND) in TP1-TP5 to a 1-way rmANOVA, as implemented in the Fieldtrip function 'ft_statfun_desamplesFunivariate.m', and cluster-based Monte Carlo permutation testing with 1000 randomizations (described by Maris and Oostenveld, 2007). Clusters were specified using the Fieldtrip function 'ft_prepare_neighbours' as channel neighbors with a distance smaller than 40mm. On average, for each channel, 4.1 neighboring channels were specified. Cluster statistics were calculated for the α (8-13 Hz) frequency band and in accordance with our hypothesis (see above). We report the maximal t-value (peak voxel) for each cluster identified in this analysis and the p-value at the peak voxel. Note that the purpose of the source analysis is to allocate in source space the significant clusters from the electrode-space analysis. Therefore, correction for multiple comparisons is not necessary. Control analyses were performed in other

frequency bands (θ: 4 - 8 Hz; β: 13 - 30 Hz; γ: 30 - 49 Hz). Significant clusters were defined based on a p-level of 0.05 (one-tail test).

For source reconstruction, we used a beamformer technique as implemented in the Fieldtrip toolbox (Oostenveld et al., 2011). As a first step we created a head model which was used to estimate the electric field measured by the EEG electrodes. Since individual MRI scans were not available, a standard MRI template was used to construct the boundary element model. Note that using a template limits the accuracy of the head model. To this end, we segmented a template provided by the Fieldtrip toolbox into three tissue types: brain, skull and scalp. Next, we estimated for each tissue type a boundary triangle mesh (brain: 3000 points, skull: 2000 points and scalp: 1000 points). Based on this geometry, a volume conduction model was specified (using standard tissue conduction values) using a Boundary Element Method. For each grid point (3780 grid points in total), we calculated a lead field matrix which was then used to calculate the inverse spatial filter.

Following the pre-processing steps described above, the signals were re-referenced to a common-average reference and the spectrum was calculated separately for pre-stimulus period (-200 - 0 ms) and post-stimulus period (0 - 300 ms), with a center frequency of 10Hz, spectral smoothing factor of 3Hz and a Hanning taper. Then, we computed an inverse filter across all conditions and blocks and applied this filter, during source reconstruction with dynamic imaging of coherent sources (DICS) algorithm, for each condition and block separately. We specified power differences similarly to the electrode-space power analysis described above and performed identical statistical tests (1-way rmANOVA for TP1-TP5), only that clusters here represent grid points. Note that since these analyses were performed twice, on the electrode space and on the source space, some inconsistencies between clusters showing the strongest statistical differences and electrode clusters can be expected, especially when multiple sources are involved. To plot the reconstructed sources, we interpolated the t-values to a template structural MRI. The anatomical labels of the sources were determined using an MNI-based AAL atlas (Tzourio-Mazoyer et al., 2002).

To study synchrony between sources, we computed inter-regional spectral coherence as follows:

$$coh_{xy}(f) = \frac{\left|S_{xy}(f)\right|}{\sqrt{S_{xx}(f)S_{yy}(f)}}$$

where x and y stand for the two signals at frequency f. Note that this type of coherence measure does not account for possible spurious coherence between two channels, that may arise from the same source due to volume conduction (Bastos and Schoffelen, 2016). The algorithm used for source reconstruction (DICS) was specifically formulated to localize sources coherent with a specific signal and this can be achieved by estimating the cross-spectral density between all EEG channels. Therefore, to compute the coherence with a specific source, we require to specify a dipole as reference. The dipole was thus selected to be the peak voxel in the clusters showing power differences across TP1-TP5 in the analysis above. Here as well, cross-spectral density matrices were calculated for a pre-stimulus period (-200 - 0 ms) and a post-stimulus period (0 - 300 ms) with the same specifications as above. Next, the coherence differences (SEQ-RND) at each time point (TP1-TP5) were submitted to the same 1-way rmANOVA, to evaluate learning-related changes to α coherence.

2.2. Experiment 2

2.2.1. Participants

Twenty-five healthy participants (mean age: 24.8 years, range 20-31; 11 males) took part in the second experiment after giving informed consent. None of the participants had been included in Experiment 1. Participation was financially compensated. All participants were classified as right-handed by means of the Edinburgh Handedness Inventory (Oldfield, 1971) and had normal or corrected to normal vision with no color deficiency. Participants were non-smokers and did not suffer from any mental or neurologic disorder (by self-report). We excluded participants who regularly played an instrument or computer games as well as professional type writers. For the EEG data analysis, one participant was excluded due to a technical error in the data acquisition resulting in a total sample of 24 participants. The participants were blinded to the conditions of the task (as presented below). Experiment 2 was approved by the Ethics Committee of University of Leipzig and was performed in accordance with the Declaration of Helsinki.

2.2.2. Experimental design

Each participant completed three sessions at intervals of at least one week between sessions (Fig. 2A). In each session, participants received 10 Hz tACS to either left M1 (lM1), right cerebellum (rCB), or sham, with the stimulation location counterbalanced between participants. In each session, participants performed a modified version of the SRTT. A different 8-element sequence was practiced in each session to prevent cross-over effects. Participants were explicitly informed about the presence of a sequence in the task and were explicitly instructed not to actively search for the sequence. Before and after the experimental blocks, we recorded resting-state sessions (200s each), which were not further analyzed for the present work. EEG was collected throughout the experiment in each session. During EEG recordings and task performance, participants were seated comfortably in front of a 17" screen, about 74 cm away, on which visual stimuli were presented. The design of the SRTT in Experiment 2, described in Fig. 2C, was similar to Experiment 1. These are the two differences between both experiments: (1) the response-stimulus interval was shorter (300ms) (2) the number of SEQ and RND blocks was different (see Fig. 2C). Similar to experiment 1, RND blocks entailed stimuli in a pseudorandom order such that items appeared exactly twice, were not repeated and pairwise associations were prevented. In SEQ (Sequence 1: 4-1-4-2-3-1-3-2, Sequence 2: 3-2-4-2-1-4-3-1, Sequence 3: 1-3-4-3-1-2-4-2), stimuli were organized in an 8-items-sequence preventing as well pairwise associations.

Prior to stimulation, two RND blocks (80 trials each) were practiced. During tACS, we introduced three smaller RND blocks with 40 trials each, used as a behavioral marker for learning during tACS. At stimulation onset, a SMP block (40 trials) was performed, followed by the first smaller RND block. Then four blocks of SEQ with 120 trials each (15 × 8-element sequence) were performed. Here the second RND block was introduced, followed by three blocks of SEQ, the third RND block and a last block of SEQ. Once the stimulation finished, participants performed two large RND blocks (80 trials each) and two SEQ blocks alternately (see Fig. 2C). The total task duration was ~35 min, depending on the individual performance. Next and after a short (~8 min) break, participants performed the completion task which was identical to the completion task in Experiment 1.

2.2.3. Behavioral analysis

We computed reaction times (RT) and error-rates (ER) for each of the experimental conditions. Both wrong button-presses and missing responses were regarded as Errors. RT were averaged across mini-blocks of 40 trials, corresponding to five repetitions of the 8-element sequence. We excluded trials in which the subject made an error as well as trials in which RTs were bigger and/or smaller than 2.7 SD (corresponding to ~1% of the normal distribution tail) of the mini-block's average RT. This resulted in an averaged exclusion of 1.9 ± 1.6 % for rCB tACS, 1.9 ± 1.6 % for lM1 tACS and 2.0 ± 1.6 % for sham of the trials in each mini-block. We defined four time points (TP) in which sequence learning was assessed by an interruption of a RND block. A Learning Index was calculated for each TP as follows: the average RT of one or two RND mini-blocks subtracted from the average RT of two surrounding (preceding and following) SEQ mini-blocks (see Fig. 2D). This ensures that the Learning Index is not influenced by fatigue and practice effects. In addition, we also calculate a baseline index by subtracting the last RND mini-block prior to stimulation from the first SEQ mini-block at

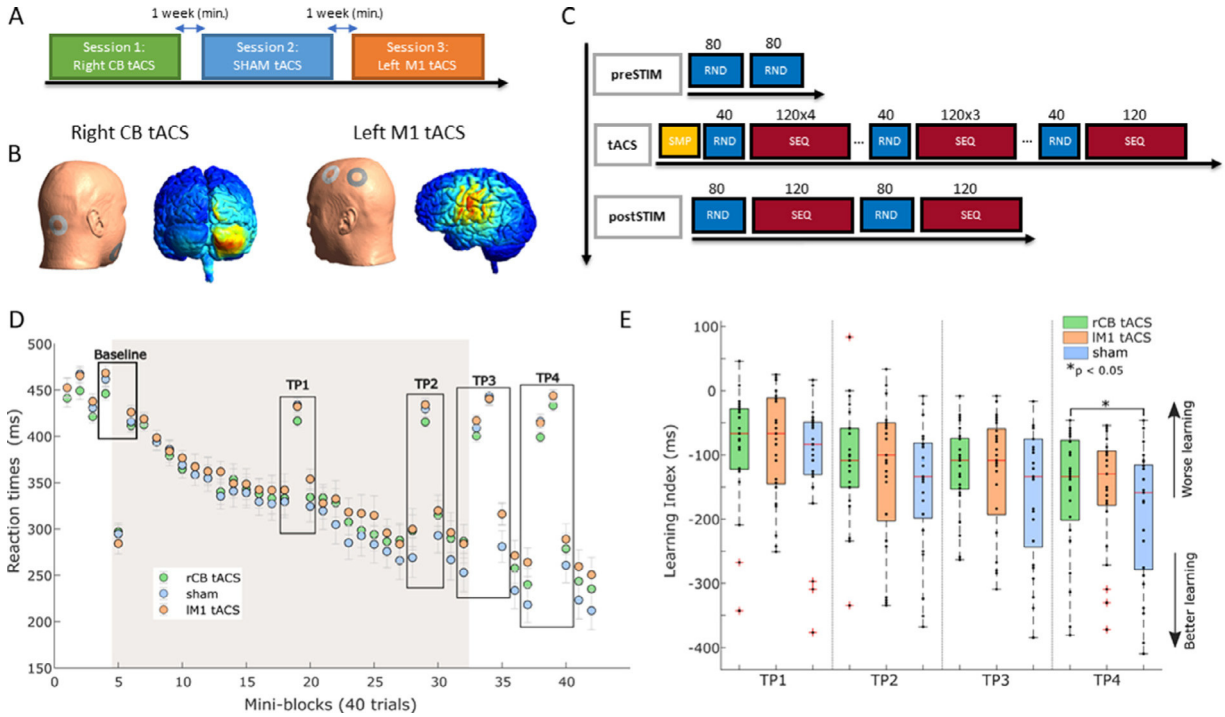

Fig. 2. Experiment 2: experimental design and behavioural results. (A) Experimental sessions were kept at least a week apart and were counter-balanced between subjects. (B) computational modelling of electric field distribution in left M1 and right cerebellum during tACS. (C) Design of serial reaction time task in each session. EEG was recorded throughout the experiment. (D) Reaction times (RT) in mini-blocks averaged across 40 trials each, for each stimulation protocol. The duration of tACS is marked in beige. Time points (TP) for RT analysis are marked with a black frame. Error bars are standard error of the mean (SEM) across subjects. E Boxplots for the learning index, evaluated as the difference between SEQ and RND as shown in D, at each TP. Significant differences are marked with a star ($p < 0.05$). Black points are individual data points from each subject.

the beginning of the stimulation (Fig. 2D). We then assessed the effect of tACS on motor sequence learning by comparing the baseline index and the Learning Index at end of the task (TP4) across stimulation protocols using a 2-way rmANOVA. We then explored post-hoc the effect of tACS on the Learning Index at TP4. As the Learning Index was not normally distributed (see Fig. 2D), we submitted the values to the non-parametric Friedman's test which is used as rmANOVA by ranks. The Wilcoxon signed rank test was used for post-hoc testing of significant effects. The error-rate (ER) of each task block (SEQ and RND) was computed as the number of errors divided by the number of trials in each block. Here as well, we specified a Learning Index for each time point, exactly as for the RT (see above). As this Learning Index, too, was not normally distributed we used the Friedman's test to assess the effect of tACS on the ER-based Learning Index.

2.2.4. Computational modelling of M1 and cerebellar-stimulation locations

We used the SimNIBS software package (http://simnibs.org/), version 2.1 (Saturnino et al., 2019), to simulate the optimal electrode montage for focal left M1 and right cerebellum electric stimulation. The head model, provided by the software package, was created using finite element modeling on T1- and T2-weighted MRI images of an exemplary subject, resulting in a high-resolution tetrahedral head mesh model containing 6 tissue types (grey matter (GM), white matter (WM), cerebrospinal fluid (CSF), skull, skin and eye balls). We set the following standard conductivity values for the 6 tissue types: WM: 0.126 S/m, GM: 0.275 S/m, CSF: 1.654 S/m, skull: 0.01 S/m, eye balls: 0.500 S/m as well as the following conductivity values for the electrode rubber = 29.4 S/m and the electrode gel = 1.0 S/m. All tissues were treated as isotropic. The electrical field $\vec{E}$ was determined by taking the numerical gradient of the electric potential. For both montages, we used ring-shaped electrodes with 48mm outer diameter, 24mm inner diameter and 3mm thickness. The size and geometry of the electrodes were incorporated into the forward model. The total current injected was 1mA. For lM1 tACS montage, electrodes were placed at EEG locations FC3 and CP3 (Fig. 2B, right montage). For rCB tACS montage, one electrode was placed 1cm below and 3cm right to the inion and the other over right mandibula (Fig. 2B, left montage).

2.2.5. Transcranial alternating current stimulation (tACS) protocol

Transcranial alternating current stimulation (tACS) was applied via two ring-shaped conductive rubber electrodes (outer diameter 48mm, inner diameter 24mm, area: 15cm^2, DC- Stimulator PLUS, NeuroConn, Ilmenau, Germany) with an intensity of 1mA at 10Hz (peak-to-peak-amplitude; sinusoidal waveform; 0.07 mA/cm^2 current density) for a total duration of 20 min. Ring-shaped stimulation electrodes were used to allow placement around EEG recording electrodes (see below). Prior to electrode placement, the skin surface was treated with high-chloride abrasive electrolyte gel for lowering skin impedance. Impedances were kept below 5kΩ. Stimulation electrodes were placed either to stimulate rCB or lM1. For rCB tACS, one electrode was placed on the right mandibula and the other 1cm below and 3cm right to the inion. For lM1 tACS, one ring-shaped electrode was placed around electrode FC3 and one around CP3 rendering the current flow as precisely as possible to C3. For sham, the current was ramped up for 30s, then stayed at 1mA for 10s and ramped down for another 30s, in order to effectively blind the participants to the experiment protocol. For sham, stimulation electrodes were either placed over M1 or cerebellum in a pseudo-randomized order across subjects. We used a questionnaire to estimate subjects' personal assessment of whether they received real stimulation or sham.

2.2.6. EEG recordings and pre-processing

EEG was recorded using Ag/AgCl electrodes embedded in a 64-channel cap and connected to an eego™ amplifier (ANT Neuro b.v., Hengelo, the Netherlands) with a sampling rate of 512Hz and 24bit resolution. A low-pass filter was applied at 0.26*sampling rate ($f_c \approx 133\ Hz$). Eye movements were recorded with an electrooculogram below the left eye. Electrodes were placed according to an extension of the international 10–20 system. The EEG was recorded against an online reference electrode in location CPz and ground in location AFz. All electrode impedances were kept below 5kΩ.

Pre-processing and all subsequent analyses were performed using in-house Matlab scripts and the EEGLAB toolbox. EEG signals were analyzed before and after tACS, but not during tACS due to non-linear artifacts in the EEG signals (Noury et al., 2016). We first applied a high-order band-pass filter ($F_{cutoff} = 1$ - 49Hz) to remove slow drifts and power line noise. Signals were then re-referenced offline to the average of the signal from left and right mastoids and the signal from electrode CPz was re-calculated. Next the signals were segmented into 3s epochs (-1s to 2s around stimulus onset). Based on ICA, we visually identified 3-4 components related to eye blink and horizontal eye movement artifacts and removed them. Additional artifacts were removed using a simple threshold (-70μV, +70μV) on the filtered data.

2.2.7. EEG spectral power and source analysis

We computed the power spectrum of the EEG signals using the Morlet wavelet as implemented in Fieldtrip, in the exact same procedure as in Experiment 1. To assess the effect of tACS on oscillatory power during motor sequence learning, we computed power differences between SEQ and RND trials at TP4, when behavioral differences between stimulation protocols were observed (see results, Section 2.2.1). Here as well signals were averaged across a pre-stimulus period in a -200 - 0 ms time window (0ms being stimulus onset) and a post-stimulus period in a time window of 0 - 200 ms. Statistical analyses were performed using non-parametric cluster-based Monte Carlo permutation testing with 1000 randomizations, comparing power differences between rCB tACS and sham, in accordance with the behavioral results (see results, Section 2.2.1). Sources responsible for producing oscillatory activity and inter-regional spectral coherence were identified exactly as in Experiment 1 (see Section 2.1.6 above for further details). The reference dipole for the coherence analysis was selected to be the peak voxel in the cluster showing power differences between rCB tACS and sham.

3. Results

3.1. Experiment 1

3.1.1. Behavioral results

One subject was excluded from further analyses due to large error-rates, more than 2.7 SD from the group mean, resulting in a sample of 24 subjects. To assess motor sequence learning, we subjected the reaction times (RTs) at each time-point (TP) to a repeated measures ANOVA (rmANOVA) with factors COND (SEQ, RND) and TP (TP1-TP5). We found a main effect of COND ($F_{1,23} = 156.5$, $p < 0.001$) and main effect of TP ($F_{4,92} = 30.6$, $p < 0.001$) as well as a COND x TP interaction ($F_{4,92} = 10.2$, $p < 0.001$), suggesting that subjects improved their performance in sequence blocks compared to the random blocks with time (Fig. 1C).

To assess learning effects in error rates (ER), we evaluated changes in ER differences with time, i.e., across TP. No main effect was evident ($p > 0.8$). ER rates were however significantly larger in RND (mean ± standard deviation: 0.042 ± 0.037) compared to SEQ (0.024 ± 0.035) at TP4 ($t_{23} = 2.8$, $p = 0.01$, corrected for multiple comparisons across TP1-TP5), possibly because subjects subjects were biased toward performing the sequence during RND blocks (see similar effects in Liebrand et al., 2020; Tzvi et al., 2016, 2015).

Following the SRTT, we examined subjects' knowledge of the sequence using a completion task. Subjects were tested on 32 trials and gave correct and correct assured (a correct response followed by a "yes" answer to the question: "are you sure?") responses. The correct response rate was 67.2% ± 15.9% (chance level is 33%) and correct assured responses was 40.3% ± 26.9%, suggesting that most subjects could successfully reproduce at least some of the sequence, in accordance with the RT results above.

3.1.2. Learning-related effects on post-stimulus power

We investigated learning related changes in oscillatory power using a time-frequency analysis. Based on our hypotheses outlined above, we examined differences in learning-related power across time points (TP1-TP5), focusing on α (8-13 Hz) frequency band. To this end, power differences (SEQ-RND) at TP1-TP5 were subjected to a 1-way rmANOVA and cluster-based permutations. No clusters were evident when signals were averaged across the entire 0 – 300 ms time window, however, when averaging across a 200 – 300 ms time window (i.e., shortly before the fronto-central effect reported by Verleger and colleagues, 2015), we found a similar central cluster (Fig. 3D) showing a significant effect (cluster level $p = 0.04$) reflecting learning-related (SEQ-RND) α power changes across TP1-TP5 (Fig. 3A). No clusters were observed in periods 0 - 100 ms and 100 - 200 ms post-stimulus. No correction was performed across time windows. Note that we plot the percentage power difference at each time point compared to a pre-stimulus baseline (-200 - 0 ms). In a post-hoc Wilcoxon signed rank test, corrected for multiple comparisons using the false-discovery rate (FDR), we compared α power in this cluster between task baseline at TP1 and TP2-TP5. We found a significant α power decrease in TP2-TP5 compared to TP1 (all $Z > 2.1$, $p < 0.04$, Fig. 3A). These differences compared to TP1 resulted from a specific α power increase in RND (TP2, TP3, and TP5: $Z > 2.0$, $p < 0.04$, Fig. 3C), as well as a specific α power decrease in SEQ (TP5: $Z = 3.2$, $p = 0.001$, Fig. 3B).

Importantly, we tested whether learning-related power changes across TP1-TP5 were specific to the α frequency band by subjecting power differences (SEQ-RND) in other frequency bands (θ: 4 - 8 Hz, β: 13 - 30 Hz, γ: 30 - 49 Hz) to a 1-way rmANOVA and cluster-based permutations, similar to the analysis above. No significant clusters were found (all $p > 0.1$) suggesting that these effects were specific to α. In Fig. 3E we plot the power differences across the spectrum (5 - 49 Hz) for the cluster shown in Fig. 3D.

Together, these results suggest that motor memory encoding is characterized by an early α power increase as well as a late α power decrease. There were no significant correlations between learning-related α power changes and RT differences between conditions (all $p > 0.09$).

Using the beamformer technique, we reconstructed possible sources of learning-related α power across time (TP1-TP5) using the same rmANOVA at the electrode-level described above. Activity was evident in left PMC, left sensorimotor cortex (SM1), and left temporo-parietal junction (TPJ, not shown, all peak voxels at $F_{4,21} = 3.6$, see Fig. 3F). Note that the left PMC cluster was closest to the interhemispheric fissure, suggesting that PMC best represents the central cluster result in the electrode-space analysis (Fig. 3D).

We also tested whether learning-related oscillatory α power changes were evident after the motor response and before the next stimulus appears. Here, we had a constant 500 ms response-stimulus interval (see methods above). Using the same statistical analysis as with the post-stimulus α power above, we found no significant clusters showing learning-related α power across time (TP1-TP5).

3.1.3. Connectivity between neural sources

Next, we computed the coherence between neural sources by specifying peak voxels in left PMC and left SM1 as sources. We then asked

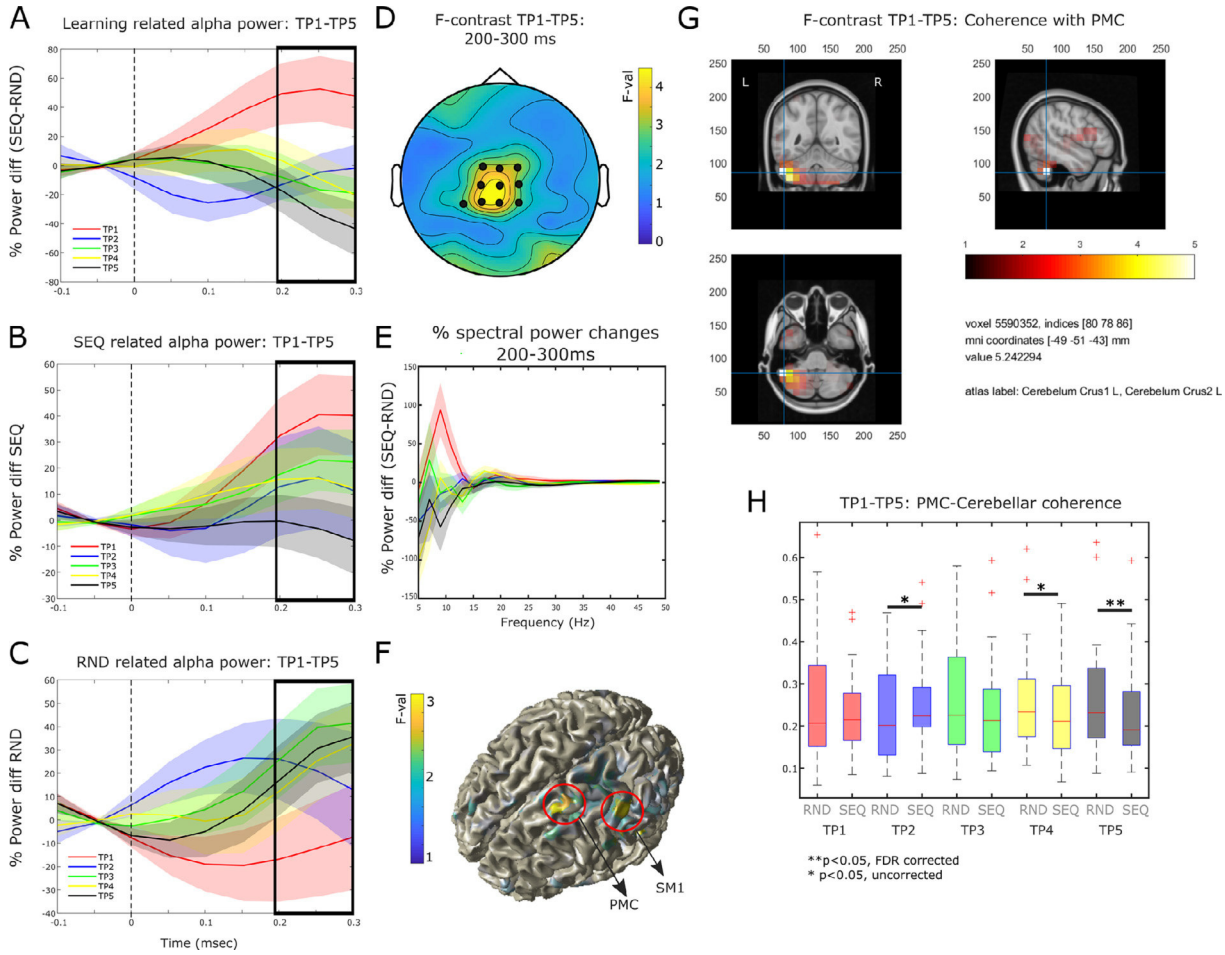

Fig. 3. Experiment 1: learning related alpha power modulation and coherence. (A-C) Time resolved percentage alpha (8-13Hz) power compared to a pre-stimulus baseline for each time point (TP1- TP5). (A) for the difference between SEQ and RND blocks (B) for SEQ only (C) for RND only. Lines are the mean across subjects and the shade is standard error of the mean (SEM). Significant effects shown in D were found 200-300 ms (black frame) after stimulus onset (dashed line). (D) Topographic map of rmANOVA analysis across TP1-TP5. Electrodes marked with black circle show significant changes with learning. (E) Percentage power differences between SEQ and RND blocks, across different frequencies, for each time point (TP1- TP5, same colour code as in A) in the cluster shown (D). Values were averaged across 200-300 ms. (F) Source reconstructed activity corresponding to the effects shown in (D). (G) Coherence changes across TP1-TP5 with the left PMC cluster shown in (F), evident in a left cerebellar crus I cluster. (G) Coherence effects between left PMC and left cerebellar crus I cluster across TP1-TP5 (same colour code as in A).

whether learning-related (SEQ-RND) α coherence changed as learning progressed, similar to learning-related α power. To this end, we submitted the coherence differences (SEQ-RND) at across TP1-TP5 to the same rmANOVA analysis above. Coherence differences across TP1-TP5 with the source in left PMC were evident in a left cerebellar crus I cluster (peak voxel at $F_{4,21} = 5.2$, $p < 0.001$, Fig. 3G). Coherence differences across TP1-TP5 with the source in left SM1 were evident in a single voxel in right temporal pole ($F_{4,21} = 3.8$, $p = 0.005$, data not shown), and in left cerebellum crus II (peak voxel at $F_{4,21} = 3.2$, $p = 0.01$, data not shown). We compared coherence in SEQ to RND using post-hoc Wilcoxon signed-rank tests in each TP (as data were not normally distributed) and corrected for multiple comparisons across TP1-TP5 using FDR. The results showed that coherence between left PMC and left cerebellum was stronger in RND compared to SEQ at TP5 ($Z = 2.45$, $p = 0.01$, Fig. 3H). On trend level ($p < 0.05$, uncorrected), this effect was larger in RND compared to SEQ at TP4 ($Z = 2.42$, $p = 0.016$, Fig. 3H), and larger in SEQ compared to RND at TP2 ($Z = 2.0$, $p = 0.046$, Fig. 3H). Coherence between left SM1 and left cerebellum was stronger in RND compared to SEQ at TP5 ($Z = 3.04$, $p = 0.002$, data not shown), but not at TP1-TP4 ($p > 0.2$). These results suggest that connectivity in a network comprising motor cortical regions and cerebellum was reduced during late learning.

3.2. Experiment 2

3.2.1. General tACS effects

None of the subjects reported any adverse effects during or after stimulation. We asked subjects whether they experienced phosphenes, which could be a sign of visual cortex stimulation (Kar and Krekelberg, 2012). Seven out of 25 subjects reported phosphenes during either lM1 tACS (N = 2) or rCB tACS (N = 5). Three of the seven claimed to see phosphenes during sham. There were no reports on pain or dizziness due to stimulation. During real tACS when asked whether the session was sham, subjects correctly answered "no" in 48% of all real tACS sessions. During sham, 19 out of 25 subjects answered correctly that the session was sham, which might indicate that they were aware of the intervention. However, it seems that the formulation of the question ("was this session sham?") has led to this large number of subjects correctly identifying the sham session. For example, five out of 25 subjects answered "yes" in all sessions, and 13 out of 25 subjects answered "yes" in two out of three sessions.

3.2.2. Right cerebellar tACS leads to a trend for diminished learning

To assess the effect of rCB and lM1 tACS on performance in the motor sequence learning task, we subjected the baseline index and the Learn-

ing Index at the end of the task (TP4, see Fig. 2D) in each stimulation protocol (rCB tACS, lM1 tACS and sham), to a 2-way rmANOVA. We found a trend for a main effect of the stimulation protocol ($F_{1,24} = 2.9$, $p = 0.06$), but no interaction ($p = 0.54$). These results suggest that learning of the sequence tended to differ between the stimulation protocols but there was no effect of tACS on learning improvement from BL to TP4. To resolve these complex effects without making any firm conclusions, we explored these two time points separately using a 1-way Friedman's test. At TP4, a main effect ($\chi^2 = 6.1$, $p = 0.048$) suggested that sequence learning significantly differed between stimulation protocols, whereas no such effects were evident at baseline ($p > 0.1$). We then explored post-hoc using the Wilcoxon signed rank test, whether rCB tACS or lM1 tACS differed from sham at TP4. We found a tendency (significance level: $p = 0.025$) for reduced learning following rCB tACS compared to sham ($Z = 2.1$, $p = 0.035$, uncorrected, Fig. 2D) but not following lM1 tACS compared to sham ($Z = 1.5$, $p = 0.143$).

Next, we analyzed the effect of rCB and lM1 tACS on error-rate (ER) differences between conditions. Here as well, we evaluated a Learning Index for ER exactly as for the RT and tested the differences between the stimulation protocols using a Friedman's test. There were no differences in error-rates between stimulation protocols ($\chi^2 = 0.8$, $p = 0.66$).

Performance in the completion task was high across stimulation protocols with median percentage correct responses for rCB tACS: 83.3%, lM1 tACS: 87.5% and sham: 87.5%. No differences in completion task performance between the stimulation protocols were evident (Friedman's test: $\chi^2 = 2.1$, $p = 0.35$). Note that performance in the completion task here was enhanced (across all stimulation protocols) compared to Experiment 1, probably due to differences in the experimental design.

As subjects performed the task a total of three times, we also examined whether repeated task sessions, which could affect the extent of explicit knowledge of the sequence, affected sequence learning by subjecting the Learning Index at TP4 in each session to a Friedman's test. No main effect was evident ($\chi^2 = 2.5$, $p = 0.29$), suggesting no effect of session order on motor sequence learning in this experiment. This also means that different levels of explicit knowledge of the task across sessions has not affected sequence learning per se.

In addition, we examined whether performance of the three different sequences was comparable by subjecting the Learning Index at TP4 for each sequence (Sequence 1, Sequence 2, and Sequence 3) to a Friedman's test. There was no main effect of sequence ($\chi^2 = 2.9$, $p = 0.24$) suggesting the three different sequences were comparable in their complexity.

3.2.3. Modulation of alpha power following 10Hz tACS

We excluded one subject from all EEG analyses described below due to extreme outliers in α power across conditions (more than 2.7 SD than the group's median), resulting in a total of 24 subjects. In accordance with previous reports (see above), we expected that 10Hz tACS would modulate local oscillatory activity, reflected by increased α power in experimental blocks directly following the stimulation. To this end, we first computed α power differences between a RND block performed pre-tACS and a RND block performed post-tACS. We then compared these differences between stimulation protocols. No significant clusters were found. As these changes might be affected by both tACS and learning, we directly compared α power differences between stimulation protocols (rCB tACS vs. sham, lM1 tACS vs. sham), during the first post-stimulation RND block. A significant α power increase in electrodes O2 and Oz ($p = 0.006$, Fig. 4A and B) was evident following rCB tACS compared to sham. No differences were found following lM1 tACS compared to sham. Further, we tested whether this effect was specific to α, by analyzing power differences in θ (4 - 8 Hz), β (13 - 30 Hz) and γ (30 - 49 Hz) frequency bands using the same approach above. We found significant power differences also in the θ frequency band (4 - 8 Hz) in these electrodes, expanding to electrodes O1 and O3 as well as to a left centro-parietal cluster (see supp. Fig. 1). No effects were found in β (13 - 30 Hz) or γ (30 - 49 Hz) frequency bands (all $p > 0.1$).

We then used beamforming to locate α power changes between rCB tACS and sham. We found a significant cluster in left inferior parietal cortex (Fig. 4C, peak voxel at $t_{23} = 5.2$, cluster level $p = 0.02$, corrected for multiple comparisons). Sources over right cerebellar crus I (peak voxel at $t_{23} = 3.7$) as well as middle occipital gyrus (peak voxel at $t_{23} = 3.6$) were also observed (as well as others, see Fig. 4D), but on an uncorrected p-level threshold of 0.001.

3.2.4. Effects of rCB tACS on learning-related α power

Next, we asked whether rCB tACS affected learning-related (SEQ-RND) α power in a 0 - 200 ms time window following stimulus onset. Note that here we chose a narrower time window compared to experiment 1, since averaged RT at the end of the task was around 200 ms for sham (Fig. 2D). To this end, we compared α power following rCB tACS to α power following sham at TP4. We found a significant increase in learning-related α power in a left fronto-central cluster (cluster level $p = 0.04$, Fig. 5A), when comparing rCB tACS to sham (Fig. 5B). Source reconstruction (in a 0 – 200 ms time window) revealed that this effect originated from a cluster in left PMC (peak voxel $t = 3.1$, Fig. 5D). A similar analysis as with α power was performed in θ, β and γ frequency bands but no effects were found (all $p > 0.1$, see also supp. Fig. 2 for learning-related power in this cluster across different frequencies). No clusters were found comparing lM1 tACS to sham.

As learning effects may be evident even prior to stimulus presentation, we also tested whether tACS affected learning-related α power in a pre-stimulus period (-200 – 0 ms). We found that α power in electrodes F5 and FC5 was stronger ($p = 0.02$ and $p = 0.03$ resp., data not shown) following rCB tACS compared to sham, suggesting that rCB tACS effect on learning-related α power is not specific to the period prior to the motor response, but is probably a general effect.

To investigate how changes in α power may relate to individual performance, we correlated for each stimulation protocol, the RT-based Learning Index at TP4 with learning-related α power post-stimulus (0 – 200 ms, no baseline correction was applied) in the fronto-central cluster (Fig. 5A). We found that learning-related α power significantly correlated with the Learning Index following sham ($r = 0.6$, $p = 0.003$, Fig. 5C) but not following rCB tACS ($p > 0.1$). This means that stronger learning-related α power decrease in sham was associated with better learning (larger Learning Index) across the subjects.

3.2.5. Effects of rCB tACS on premotor-cerebellar connectivity

Next, we computed α coherence between neural sources by specifying the peak voxel in left PMC as source. We then compared the differences in coherence between rCB tACS and sham at TP4 (both SEQ-RND). Larger coherence differences with the left PMC source were found in a right cerebellar lobule VIIb cluster when comparing rCB tACS to sham (peak voxel at $t_{23} = 2.65$, $p = 0.007$, uncorrected, Fig. 5E). Post-hoc t-tests showed that coherence following rCB tACS was stronger in SEQ when compared to RND ($t_{23} = 2.1$, $p < 0.05$, uncorrected, Fig. 5F), with no such differences following sham ($p > 0.2$). These results suggest that rCB tACS led to increased connectivity between left PMC and right cerebellar lobule VIIb during SEQ blocks.

4. Discussion

In this study we demonstrated a strong link between alpha oscillations (α, 8-13 Hz) in the premotor-cerebellar loop and motor sequence learning. In the first experiment, we showed that α power over left premotor cortex (PMC) and sensorimotor cortex (SM1) increased early-on and decreased as learning progressed. At the same time, α coherence between a left PMC/SM1 seed and left cerebellar crus I, was weaker in sequence (SEQ) compared to random (RND) blocks in late learning blocks. This could indicate a functional decoupling within a cortico-cerebellar loop guided by α oscillations and underlying motor learning. In a second experiment, we showed that 10Hz transcranial alternating current stimulation (tACS) to right cerebellum (rCB) during sequence

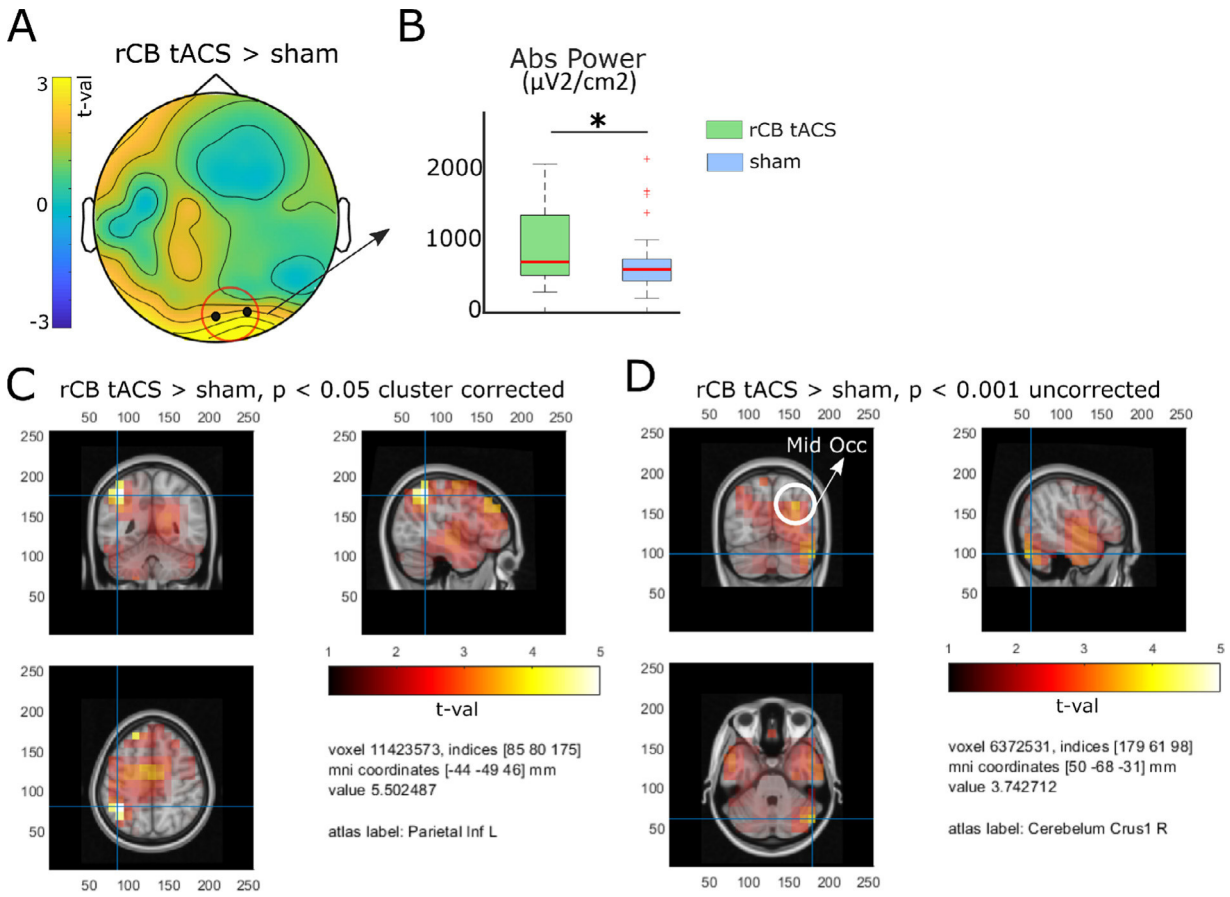

Fig. 4. Experiment 2: Alpha power modulation following 10 Hz tACS. (A) Topographic map for alpha (8-13Hz) power differences between rCB tACS and sham in the first RND block following stimulation. Significant electrodes are shown in black and marked with a red circle. (B) Boxplots for the absolute power ($\mu V^2/cm^2$) averaged across electrodes O2 and Oz (shown in A). The star marks a significant difference ($p = 0.006$). (C-D) Source reconstruction of power differences between rCB tACS and sham, corresponding to the map in A showing activity in (C) left inferior parietal cortex on a $p < 0.05$, cluster level corrected and in (D) right cerebellar curs I and middle occipital gyrus on a $p < 0.001$ uncorrected level.

learning tended to interfere with learning and led to a specific learning-related α power increase over left PMC. Importantly, condition differences in α coherence between left PMC and right cerebellar lobule VIIb were evident following rCB tACS but not following sham stimulation, through a specific increase during SEQ blocks. Together these results suggest that: (1) α oscillations in the cerebellar-motor loop are modulated during motor sequence learning (2) application of 10Hz tACS over cerebellum interferes with motor learning, through increased α coherence in premotor-cerebellar connections and increased α power in left PMC.

4.1. Learning modulates α power in premotor- and sensorimotor cortex

In Experiment 1, we found that learning-related α power in left PMC and SM1 increased early-on and decreased as learning progressed. Importantly, this effect was found prior to the response with no apparent changes to α power in a similar period pre-stimulus. This corresponds to previous findings showing that the more a movement is performed automatically, the less α power desynchronizes over motor areas (Boenstrup et al., 2014). Rueda-Delgado et al. (2019) have recently shown that following bimanual coordination task, α power in resting-state EEG was also decreased in sensorimotor and premotor areas. Based on the functional inhibition theory by Jensen and Mazaheri (2010), postulating that decrease in α oscillations reflects release of inhibition of task-relevant areas, it is plausible that α power decrease represents activation, by disinhibition, of PMC during encoding of the motor sequence. Indeed, previous fMRI studies of motor sequence learning consistently show increased activity in PMC as learning progresses (Bapi et al., 2006; Grafton et al., 2002; Orban et al., 2010).

We further found that α coherence between left PMC/SM1 and left cerebellar crus I was significantly weaker during SEQ compared to RND during late learning. Previously, we showed that reduced α phase coupling in SEQ compared to RND, interpreted to reflect global functional decoupling, is important for encoding the sequence into memory (Tzvi et al., 2018). Evidence from our previous fMRI studies of motor sequence learning suggests that learning of a motor sequence induces connectivity changes between motor and premotor areas and bilateral cerebellum (Tzvi et al., 2015, 2014) as part of the cortico-striato-cerebellar network. Functional connectivity as measured using coherence, does not directly implicate anatomical connections but rather a "net" effect between two regions. Therefore, coherence between left PMC and left cerebellum found here, could be indicative of the distributed motor learning network. We, therefore, suggest that α decoupling between left PMC/SM1 and left cerebellar crus I serves to integrate motor representations in motor cortical areas, and internal model representations in the cerebellum (Ito, 2006), during motor learning.

4.2. Modulation of posterior α oscillations following 10Hz tACS to right cerebellum

We found increased α power in occipital electrodes, in RND blocks directly following rCB tACS compared to sham. The source of this effect

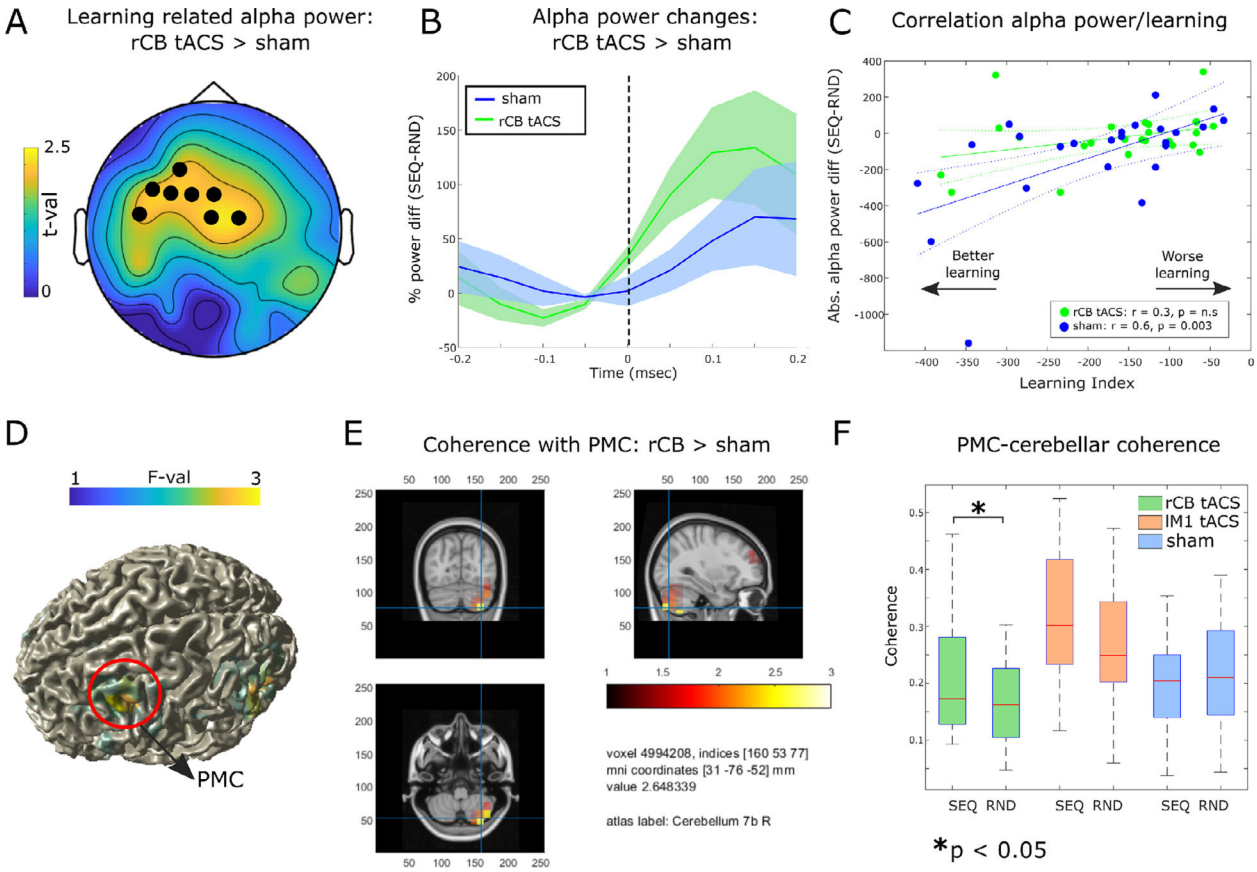

Fig. 5. Experiment 2: the effect of rCB tACS on learning-related alpha power. (A) Topographic map for learning-related (SEQ-RND) alpha power effects (expressed in t-values) between rCB tACS and sham, averaged across a 200 ms time frame following stimulus onset (t = 0 ms). Significant electrodes are marked in black. (B) Time resolved % power differences between SEQ and RND blocks in rCB tACS and sham averaged across the cluster shown in (A). Lines are the mean across subjects and the shade is the standard error of the mean. Stimulus onset is marked with a dashed line. (C) Correlation between the Learning Index (described in 1.2.3) and power differences (SEQ-RND) averaged across 0-200 ms following rCB tACS (green) and sham (blue), in TP4. Note that each data point represents one session per subject. (D) Source reconstruction corresponding to the maps in (A). (E) Cerebellar cluster showing coherence effects with the seed in left PMC, shown in (D). (F) Boxplots for coherence effects between left PMC and right cerebellum. Significant differences ($p < 0.002$) are marked with a star.

was probably widespread with a maximal effect in left inferior parietal lobe (IPL), suggesting a network effect, elicited by modulation of cerebellar oscillatory activity following rCB tACS. The IPL is important for sensory processing and sensorimotor integration and is directly connected to the cerebellum (Clower et al., 2001). Thus, in accordance with the behavioral results showing a tendency for improved general task performance following rCB tACS, it is plausible that modulation of cerebellar α oscillations resulted in changes to IPL-cerebellar loop underlying visuomotor processes. This speculation should be further investigated in future studies focusing on modulation of stimulus-response processes. Interestingly, in a group of Parkinson's disease and essential tremor patients, alpha tACS to the cerebellum led to a significant entrainment of tremor frequency (Brittain et al., 2015). Given that rCB tACS was applied superficially, it is likely that the cerebellar cortex is the most affected by the stimulation, although it remains a possibility that entrainment, if it occurred, was due to oscillatory peripheral stimulation. Purkinje cells, as the sole output of the cortex, were strongly entrained by AC stimulation in a large range of frequencies, shown by extracellular recordings from vermis lobule 7 in anesthetized rats (Asan et al., 2020). Of note, 10Hz rCB tACS has modulated θ power in occipital and centro-parietal electrodes, in addition to the effect in the α band reported above. This finding indicates that non-learning "offline" effects by tACS, may potentially involve different frequency bands. Future studies could explore the direct mechanism in which 10Hz tACS to cerebellum may influence cortical and cerebellar oscillations in other frequency bands.

4.3. *The effect of 10Hz tACS on motor learning performance*

The results of Experiment 1 led us to hypothesize that modulation of α power in a (bilateral) network consisting of PMC/SM1 and cerebellar crus I, through external stimulation, would disrupt learning. Indeed, in Experiment 2, we found a tendency for declined learning performance following 10Hz rCB tACS, but not lM1 tACS, when compared to sham. Only few studies investigated the effect of cerebellar tACS on motor performance (Miyaguchi et al., 2018; Naro et al., 2016). These studies show that higher frequency tACS to the cerebellum led to improved motor performance, but contrary to our results, found no effect at 10Hz. Notably, 10Hz CB tACS was applied at rest, whereas we stimulated during motor learning which is likely to critically impact the results (Ruhnau et al., 2016).

4.4. *The effect of 10Hz tACS on learning-related α oscillations*

Comparing between rCB tACS to sham, we found a relative increased learning-related (SEQ-RND) α power over fronto-central electrodes following rCB tACS. Correlation between this effect and behavioural measures of learning revealed that better learners had a stronger α decrease, following sham but not rCB tACS. This result further corroborates the findings of Experiment 1, linking α power decrease with encoding of the motor sequence and suggests that rCB tACS might interfere with this effect. Source reconstruction of the effect over fronto-central electrodes revealed a left PMC cluster, similar to the results of Experiment 1. Thus,

despite the differences between the experiments in terms of design, changes in left PMC were found in both experiments on a whole-brain level, stressing the robustness of these results. The relative learning-related power increase following rCB tACS could indicate reduced activity in PMC resulting in impaired learning.

Importantly, we found that coherence between left PMC and right cerebellum was stronger in SEQ compared to RND following rCB tACS but not following sham stimulation. We propose that modulation of cerebellar oscillations by tACS led to increased coherence in the premotor-cerebellar loop during learning, and ultimately lead to increased α power at PMC. Projections from cerebellum to premotor cortex, shown in macaques using retrograde trans-synaptic transport of a neurotropic virus (Hashimoto et al., 2010) suggest that cerebellum strongly impacts higher cognitive functions represented in PMC. A study in mice showed that disruption of cerebellar output by optogenetic stimulation of Purkinje cells, could disinhibit sensory LFP responses in S1 and M1 as well as S1-M1 coherence in theta (4 - 8 Hz) and low gamma (30 – 49 Hz) bands. In addition, we previously found using fMRI, negative modulation of interactions from motor and premotor cortex to cerebellum by motor sequence learning (Tzvi et al., 2015, 2014), as well as specific interaction from right cerebellum to left PMC, leading to increased left PMC activity, associated with learning in a visuomotor adaptation task (Tzvi et al., 2020). We thus speculate that α mediated communication in the PMC-cerebellar loop serves to integrate perceptual and motor components for motor learning. Taking the results of both experiments together, we propose the following scenario: modulation of cerebellar α oscillations by 10Hz cerebellar tACS leads to increased cerebellar output activity, which results in enhanced α coherence with PMC. The resulting α power increase in PMC, supposedly reflecting PMC inhibition is linked to the observed diminished sequence learning.

4.5. Limitations

The within-subject design of experiment 2 was constructed in order to circumvent strong inter-subject variability in the effect of tACS. Note however that in motor sequence learning tasks, such a design could result in different levels of explicit knowledge which may affect learning. Thus, future experiments should consider accounting for inter-subject variability in the effect of tACS by implementing individualized “dose” control (Evans et al., 2020) which can effectively reduce the variability in electric field intensities at the target stimulation site.

The computational model suggested that the montage we used for cerebellar tACS elicits a response mostly in the cerebellum. Possibly, such a montage may also elicit a response in the near-by visual cortex. However, we believe that this not the case in Experiment 2 since phosphenes, a perception of continuously flickering light, previously shown to be reliably elicited by tACS over the occipital cortex (Kanai et al., 2008), was experienced by very few subjects only under cerebellar tACS.

For technical reasons, it is impossible to know if local cerebellar oscillations were entrained by rCB tACS. We showed, however, significant modulation of α oscillations at occipital electrodes, also predicted by the computational model (Fig. 3B), which were closest to the stimulation electrode targeting the cerebellum. This suggests that electrodes placed inferior to occipital electrodes might be able to detect cerebellar oscillations (Andersen et al., 2020; Todd et al., 2018). In addition, we demonstrated modulation of α oscillations following rCB tACS at left IPL, which is tightly connected to right cerebellum (Bostan et al., 2013), as well as coherence between PMC and cerebellum, suggesting that tACS targeting the cerebellum could entrain local oscillatory activity.

Surprisingly, 10Hz tACS to left M1 did not significantly affect learning compared to sham as we had expected from previous work (Fresnoza et al., 2020; Pollok et al., 2015). In previous studies, stimulation electrodes were placed over left M1 (at C3 or lM1 measured by TMS) and right orbita. This may have led to a distributed effect over not just M1 but PMC and large portions of prefrontal areas (e.g. Opitz et al., 2015), which might have led to the positive learning effects. However, as we found no evidence for local modulation of μ oscillations following lM1 tACS, we suspect that placement of stimulation electrodes over FC3 and CP3 might have led the current to be shunted across the scalp, due to the relative proximity of the stimulation electrodes. This may have resulted in a weaker electric field strength at M1.

Is should also be noted here that there is a current debate whether tACS effects could be mediated by transcutaneous and not by transcranial stimulation (Asamoah et al., 2019). A very recent work using single-cell recordings in alert monkeys showed that tACS entrains local neural activity also when somatosensory input is blocked with topical anesthetic, suggesting that peripheral stimulation is not required for tACS to entrain neuronal activity (Vieira et al., 2020). In this work, we did not apply topical anesthetics prior to tACS, which means we cannot rule out that the effects observed here are mediated by transcutaneous stimulation. However, we find it unlikely that the effects observed here are mediated only through peripheral stimulation due to the specific spatial pattern of α (and θ) power increase following rCB tACS, which does not follow the expected anatomical representation of somatosensory stimulation.

The results of experiment 1 in which effects were specific to α frequency band have motivated the selection of the stimulation frequency (i.e., 10Hz) in experiment 2. Since a within-subject design with a learning component prevents the use of multiple sessions, we decided to not include a stimulation session in a control frequency (e.g. 20Hz), limiting the interpretation of stimulation effects on motor sequence learning specifically to α. Notably, we found no evidence that 10Hz tACS affects learning-related power in other frequency bands. Future studies focusing on cerebellar stimulation could test for the specificity of 10Hz tACS by including control stimulation in other frequency bands.

5. Conclusions

The results of this study suggest that α oscillations mediate interactions in the premotor-cerebellar loop and thus modulate motor sequence learning. Specifically, a gradual decrease of learning-related α power in left premotor cortex, and weaker learning-specific coherence with right cerebellum suggests a functional decoupling within the premotor-cerebellar loop underlying motor learning. When 10Hz tACS was applied to right cerebellum, learning-related α power increased in left premotor cortex and was more coherent with right cerebellum compared to sham. The latter provides evidence for a unique causal link between α oscillations in a premotor-cerebellar loop and motor sequence learning. These results suggest that interactions within a premotor-cerebellar loop, underlying motor sequence learning, can be modulated through external modulation of cerebellar oscillations, and this may lead to both behavioral and physiological changes.

Data and code availability statement

The data and the code used in this study would be made available upon request.

Credit authorship contribution statement

Christine Schubert: Data curation, Investigation, Formal analysis, Writing – original draft. **Alhuda Dabbagh:** Investigation, Software. **Joseph Classen:** Writing – review & editing, Supervision. **Ulrike M. Krämer:** Conceptualization, Writing – review & editing. **Elinor Tzvi:** Conceptualization, Methodology, Software, Investigation, Writing – original draft, Funding acquisition.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.neuroimage.2021.118410.

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4 Summary

Dissertation
in partial fulfillment of the requirements for the degree of
Dr. med.

Alpha oscillations modulate premotor-cerebellar connectivity in motor learning: insights from transcranial alternating current stimulation

Submitted by	Christine Viktoria Schubert
Written at	University of Leipzig, Faculty of Medicine University Hospital Leipzig Department of Neurology
Supervised by	Prof. Dr. med. Joseph Claßen Dr. rer. nat. Elinor Tzvi-Minker

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The ability to acquire motor skills is essential in our daily life. Neuroimaging studies have shown that motor learning activates a network of cortical and sub-cortical structures (Doyon et al., 2003, Doyon et al., 2009, Hardwick et al., 2013) including inter alia premotor cortex, primary motor cortex and cerebellum (Hardwick et al., 2013). According to several theoretical models of motor learning, learning is based on constant feedback systems mediated by cortico-cerebellar and cortico-striatal loops (Hikosaka et al., 2002, Doyon et al., 2009, 2003, Penhune and Steele, 2012) serving to provide efficient network communication. Neuronal oscillations have been considered to mediate network communication (Salinas and Sejnowski, 2001, Buzsáki and Draguhn, 2004, Fries, 2005, Hipp et al., 2011). The predominant oscillations in the human brain are alpha oscillations (8-13 Hz). Alpha oscillations have been found to be important in higher cognitive processes like working memory (Klimesch et al., 1997, Klimesch et al., 2010, Bonnefond and Jensen, 2012, Sauseng et al., 2009) and motor learning (Pollok et al., 2014, Mehrkanoon et al., 2016, Tzvi et al., 2016, 2018). One way of quantifying effective neuronal interaction between brain regions is through coherence which arises when oscillations show a constant relation of their phases or amplitudes (Fries, 2005, 2015, Hipp et

al., 2011). During motor learning, alpha oscillations were found to dynamically change over functional cortical areas (Boenstrup et al., 2014, Pollok et al., 2014, Tzvi et al., 2016, 2018) and were shown to be important for communication in the cerebellar-motor loop (Mehrkanoon et al., 2016, Schubert et al., 2021). However, the causal role of alpha oscillations in motor learning remains elusive. Transcranial alternating current stimulation (tACS) is a non-invasive brain stimulation technique that offers the possibility to locally entrain endogenous oscillatory activity (Herrmann et al., 2013, Krause et al., 2019, Zaehle et al., 2010) and influence large-scale interactions between functionally connected brain regions (Herrmann et al., 2016, Cabral-Calderin et al., 2016, Chapeton et al., 2019).

The goal of this dissertation was to investigate whether alpha oscillations mediate motor network communication and consequently modulate motor sequence learning.

To this end, we used a combined tACS-EEG approach to investigate behavioral as well as electrophysiological effects of externally modulated alpha oscillations in the motor network during motor learning. Since motor cortex and cerebellum are important in motor sequence learning (Hardwick et al., 2013), we chose left M1 and right cerebellum as stimulation locations in 25 healthy, right-handed subjects performing a serial reaction time task (SRTT; Nissen and Bullemer, 1987) with their right hand. TACS was applied with an intensity of 1 mA at 10Hz (corresponding to mean range of alpha oscillations). A 64-channel EEG was recorded throughout the experiment. The SRTT included an underlying 8-element sequence (SEQ) as well as blocks of random patterns (RND). Each subject took part in three experimental sessions including two stimulation sessions and one sham stimulation in a counterbalanced, single-blinded order. For each session, a different underlying sequence was used.

Based on the evidence summarized above, we hypothesized that 10Hz tACS to either left M1 or right cerebellum would lead to local entrainment of oscillatory alpha, resulting in changes in learning-related alpha power and interactions in the cortico-cerebellar loop. We expected that these oscillatory changes would subsequently affect learning performance during and following tACS.

On a behavioral level, learning performance differed between stimulation protocols at a defined time point such that learning showed a tendency to be reduced following right cerebellar tACS (rCB tACS) compared to sham. No such effect was found following tACS over left M1 (lM1 tACS) compared to sham. On an electrophysiological level, 10Hz cerebellar tACS effected alpha power and premotor-cerebellar connectivity. Learning-related alpha power was found to be increased in left premotor cortex (PMC) after rCB tACS. Moreover, learning-related alpha coherence between left PMC and right cerebellum was stronger following rCB tACS but not following sham. No such effects were found comparing lM1 tACS to sham.

We propose that cerebellar oscillations were modulated by rCB tACS which consequently led to increased coherence in the premotor-cerebellar loop during learning and ultimately increased alpha power in left PMC compared to sham. As alpha oscillations have been shown to functionally guide involvement and disinvolvement of task-relevant regions, assumingly

due to their inhibitory effect (Jensen and Mazaheri, 2010), increased alpha power in left PMC after rCB tACS might have led to a functional inhibition of this area. Since PMC plays an important role in motor learning (Grafton et al., 2002, Bapi et al., 2006, Hoshi and Tanji, 2007, Orban et al., 2010), especially in visuo-motor control (Hardwick et al., 2013) and temporal organization of sequential movements (Halsband et al., 1990, 1993), impaired learning after rCB tACS may result from reduced activity in PMC.

In sum, this study suggests a strong link between alpha oscillations (8-13 Hz) in the premotor-cerebellar loop and motor sequence learning. Moreover, our results show that interactions within a premotor-cerebellar loop during motor sequence learning can be modulated through external modulation of cerebellar alpha oscillations leading to behavioral as well as electrophysiological changes during motor learning. Our work contributes to the development of a deeper understanding of alpha oscillations in motor networks and consequently provides a basis for further research in the field of non-invasive brain stimulation and motor network analysis.

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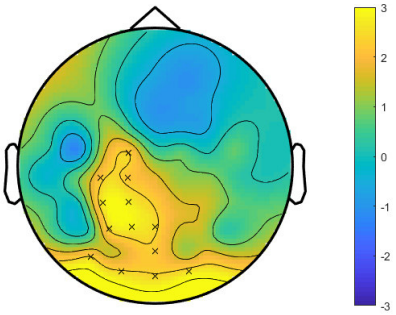
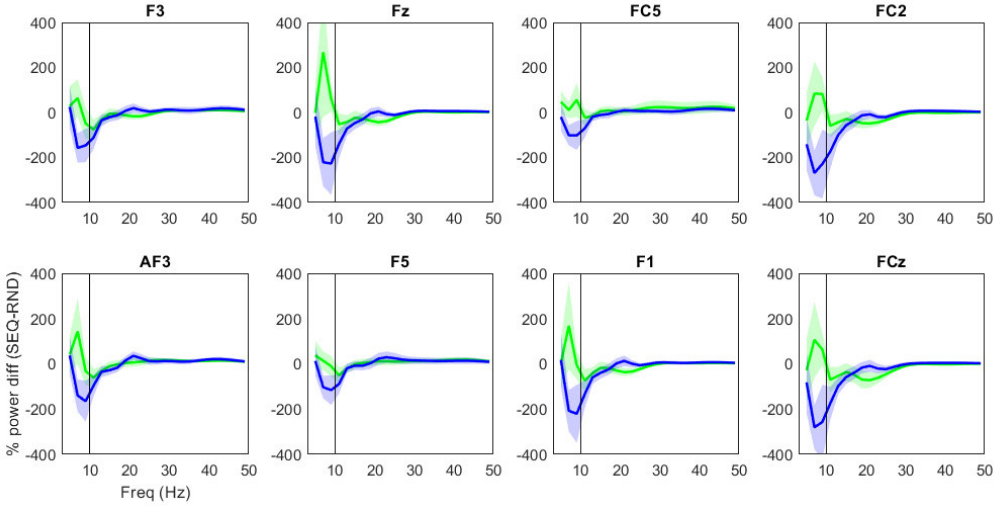
6 Supplementary Materials



Supp. Fig 1. Topographic map for theta power differences between rCB tACS and sham in the first RND block following stimulation. Significant electrodes are marked with an 'x'.



Supp. Fig 2. Percentage power differences at TP4, across the spectrum, following rCB tACS (green) or sham (blue). Electrodes correspond to the cluster shown in Fig. 5A (publication). Signals were averaged across 0-200 ms time-window around stimulus onset. 10Hz is marked with a black line.

7 Contribution of Authors / Darstellung des eigenen Beitrags

Christine Schubert	Data collection and management, investigation, formal analysis, writing of original draft and dissertation manuscript
Alhuda Dabbagh:	Investigation, software
Joseph Claßen:	Review and editing, supervision
Ulrike M. Krämer:	Conceptualization, review and editing
Elinor Tzvi:	Conceptualization, methodology, software, investigation, writing of original draft, funding acquisition

Christine Schubert .....
signature

Alhuda Dabbagh .....
signature

Prof. Dr. med. Joseph Claßen .....
signature

Prof. Dr. rer. nat. Ulrike M. Krämer .....
signature

Dr. rer. nat. Elinor Tzvi-Minker .....
signature

8 Declaration of Authorship / Erklärung über die eigenständige Abfassung der Arbeit

Hiermit erkläre ich, dass ich die vorliegende Arbeit selbstständig und ohne unzulässige Hilfe oder Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe. Ich versichere, dass Dritte von mir weder unmittelbar noch mittelbar eine Vergütung oder geldwerte Leistungen für Arbeiten erhalten haben, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen, und dass die vorgelegte Arbeit weder im Inland noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde zum Zweck einer Promotion oder eines anderen Prüfungsverfahrens vorgelegt wurde. Alles aus anderen Quellen und von anderen Personen übernommene Material, das in der Arbeit verwendet wurde oder auf das direkt Bezug genommen wird, wurde als solches kenntlich gemacht. Insbesondere wurden alle Personen genannt, die direkt an der Entstehung der vorliegenden Arbeit beteiligt waren. Die aktuellen gesetzlichen Vorgaben in Bezug auf die Zulassung der klinischen Studien, die Bestimmungen des Tierschutzgesetzes, die Bestimmungen des Gentechnikgesetzes und die allgemeinen Datenschutzbestimmungen wurden eingehalten. Ich versichere, dass ich die Regelungen der Satzung der Universität Leipzig zur Sicherung guter wissenschaftlicher Praxis kenne und eingehalten habe.

22.11.22
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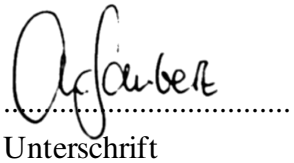
Datum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Unterschrift

9 Curriculum Vitae

Christine Viktoria Schubert

Contact christine.schubert94@googlemail.com

Education

University of Leipzig | since 08/2018
Doctorate, Clinic and Policlinic of Neurology, working group for motor control and neuronal plasticity
Title: „Alpha oscillations modulate premotor-cerebellar connectivity in motor learning: insights from transcranial alternating current stimulation"
Paper published in NeuroImage (2021)

University of Leipzig | 10/2013 – 06/2021
Studies in Medicine

Berlin, November **2022**

10 Publication and Presentation

Publication:
Schubert, Christine; Dabbagh, Alhuda; Classen, Joseph; Krämer, Ulrike M.; Tzvi, Elinor (2021): Alpha oscillations modulate premotor-cerebellar connectivity in motor learning: Insights from transcranial alternating current stimulation. In: *NeuroImage* 241, S. 118410. DOI: 10.1016/j.neuroimage.2021.118410.

Poster presentation:
"The role of alpha oscillations in a premotor-cerebellar loop in motor learning", Christine Schubert, 64. Jahrestagung der DGKN, 7th International Conference on Non-Invasive Brain Stimulation and 4th European Conference of Brain Stimulation in Psychiatry, 10.-14. November 2020

11 Acknowledgement / Danksagung

Bereits in den ersten Semestern meines Medizinstudiums war es vor allem die Neuroanatomie, die mein authentisches Interesse weckte. Seitdem fortbestehend entwickelte sich der grundlegende Wunsch, das menschliche Gehirn und seine Funktion ergründen und verstehen zu wollen. Mit dieser Arbeit bin ich diesem Wunsch ein Stück näher gekommen.
An dieser Stelle möchte ich all denjenigen danken, die mich auf diesem Weg begleitet und unterstützt haben.