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DISSERTATION

Schwellenmodulation als Mechanismus transkranieller elektrischer Hirnstimulation

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Inhalt

1. Zusammenfassung (Deutsch)

Hintergrund: Während Techniken nicht-invasiver Hirnstimulation (*Non-invasive Brain Stimulation*, NiBS), einschließlich transkranieller elektrischer Stimulation (*transcranial Electrical Stimulation*, tES), inzwischen zum festen Repertoire von Neurowissenschaftlern und Klinikern gehören, verbleiben die Wirkungsmechanismen nach wie vor unklar. Für die oszillatorische transkranielle Wechselstromstimulation (*transcranial Alternating Current Stimulation*, tACS) werden als Mechanismen sowohl *Entrainment*- als auch *Rebound*-Effekte vorgeschlagen, während für die neuartige und vielversprechende transkranielle Rauschstromstimulation (*transcranial Random Noise Stimulation,* tRNS) der Mechanismus der stochastischen Resonanz vermutet wird. Eine Gemeinsamkeit verschiedener Hypothesen zu den Wirkmechanismen ist eine mögliche Verstärkung intrinsischer Oszillationen über Schwellenmodulation.

Wir prüften zunächst die Sicherheit der retinofugalen Wechselstromstimulation (*retinofugal Alternating Current Stimulation*, rACS) (Studie 1), bevor wir mit dieser Methode die Wirkmechanismen der ACS auf intrinsische Oszillationen im umschriebenen visuellen System untersuchten (Studie 2). Um letztlich die Effekte unterschiedlicher Aktivität kortikaler Netzwerke auf die Auswirkungen der Stimulation zu analysieren, stimulierten wir den motorischen Kortex mit tRNS während der Ausführung inhibitorischer und exzitatorischer motorischer Aufgaben (Studie 3).

Methoden: In Studie 1 sammelten wir Stimulationsparameter und subjektive Berichte über unerwünschte Ereignisse von 20 Probanden, die mit 10 Hz rACS stimuliert wurden, und analysierten die Sicherheit zusätzlich mittels Finite-Elemente-Modellierung.

In Studie 2 stimulierten wir 30 Probanden in einem doppelblinden, randomisiert-kontrollierten Paradigma entweder mit 10 Hz rACS oder mit Sham-Stimulation, wobei wir die okzipitale α-Power und die individuelle α-Spitzenfrequenz im EEG untersuchten.

In Studie 3 stimulierten wir den dominanten motorischen Kortex entweder mit tRNS oder Sham-Stimulation (doppelblind, randomisiert-kontrolliert) während der Ausführung von zwei motorischen

Aufgaben (Fingertapping und Go/No-Go-Aufgabe) und untersuchten dabei die i) kortikospinale Erregbarkeit (über motorisch evozierte Potentiale) sowie die ii) Aufgabenperformance.

Ergebnisse: Studie 1 lieferte Belege für die gute Sicherheit und Verträglichkeit von rACS. Studie 2 zeigte einen Anstieg der α-Power nach rACS im Vergleich zu Sham, aber bemerkenswerterweise keine Verschiebung des individuellen α-Peaks in Richtung der Stimulationsfrequenz. In Studie 3 fanden wir aufgabenspezifische Effekte auf die Aufgabenleistung und CSE für tRNS im Vergleich zu Sham.

Schlussfolgerung: Zusammengenommen liefern diese Studien Belege für eine Verstärkung der zugrundeliegenden Oszillationen (speziell α-Oszillationen im visuellen System), höchstwahrscheinlich über einen schwellenmodulierenden Effekt, als eine der Ursachen für NiBS-Effekte auf neuronale Oszillationen. Dieser Effekt könnte durch Rebound- oder Burst-Firing-Mechanismen für ACS bzw. einen stochastischen Resonanzeffekt für RNS bedingt sein.

2. Abstract (Englisch)

Background: While Non-invasive Brain Stimulation (NiBS) techniques, including transcranial electric stimulation (tES), have become part of the repertoire of neuroscientists and clinicians alike, the mechanisms of action remain unclear. For the oscillatory transcranial alternating current stimulation (tACS), proposed mechanisms include entrainment as well as rebound effects, whereas the novel and promising transcranial random noise stimulation (tRNS) is suspected to employ the mechanism of stochastic resonance. A common feature of several hypotheses on the mechanisms of action is a possible amplification of intrinsic oscillations via threshold modulation.

We first tested the safety of retinofugal alternating current stimulation (rACS) (Study 1) before employing this method to investigate the mechanisms of action of ACS on intrinsic oscillations in the circumscribed visual system (Study 2). Finally, to investigate the effects of different cortical

network activity on the effects of stimulation, we stimulated the motor cortex with tRNS during the performance of inhibitory and excitatory motor tasks (Study 3).

Methods: In study 1, we gathered stimulation parameters and subjective report of adverse events from 20 subjects stimulated with 10 Hz rACS and further analyzed the safety via finite element modeling.

For study 2, we stimulated 30 subjects with either 10 Hz rACS or Sham-stimulation in a double-blind randomized-controlled paradigm while assessing occipital α power and individual α peak frequency in the EEG.

For study 3, we stimulated the dominant motor cortex with either tRNS or sham stimulation (double-blind, randomized-controlled) during the execution of two motor tasks (fingertapping and go/no-go task) while assessing i) corticospinal excitability (via motor evoked potentials) as well as ii) task performance.

Results: Study 1 presented evidence for the good safety and tolerability of rACS. Study 2 showed an increase in α power after rACS compared to Sham, but a notable absence of a shift of the individual α peak towards stimulation frequency. In study 3, we found task specific effects on task performance and CSE for tRNS compared to Sham.

Conclusion: Taken together, these studies provide evidence for an enhancement of underlying oscillations (especially α oscillations in the visual system) as one of the causes behind NiBS effects on neural oscillations, most likely via a threshold-modulating effect. This effect could include rebound- or burst firing mechanisms for ACS and a stochastic resonance effect for RNS, respectively.

3. Einführung

3.1 Nicht-invasive Hirnstimulation – ein Überblick

Techniken der nicht-invasiven Hirnstimulation (Non-invasive Brain Stimulation, NiBS), einschließlich transkranieller Elektrostimulation (tES) zählen inzwischen zu etablierten Instrumenten im Repertoire klinischer Neurologen und Neurowissenschaftler.

Speziell seit der Jahrtausendwende wächst das Interesse an verschiedenen Techniken der tES infolge der Beschreibung dauerhafter und polaritätsspezifischer Änderungen der kortikalen Erregbarkeit nach transkranieller Gleichstromstimulation (transcranial Direct Current Stimulation, tDCS)(1). Diesen vielversprechenden Ergebnissen folgten klinische Studien für ein breites Spektrum an Indikationen (2).

Zuletzt kam es jedoch verstärkt zu Kritik an der großen Effektvariabilität und geringen Reproduzierbarkeit der Ergebnisse einiger tDCS-Studien (3). Um dieser Herausforderung zu begegnen, wurden neue Techniken der transkraniellen elektrischen Stimulation eingeführt (4) und optimiert (5). Eine dieser Varianten ist die transkranielle Wechselstromstimulation (transcranial Alternating Current Stimulation, tACS). Die beschriebenen Effekte dieser oszillatorischen Stimulationstechnik reichen von psychophysischen Veränderungen (6-8), Verbesserung des Arbeitsgedächtnisses, Lernen und langfristiger Gedächtnisbildung (9-11) bis hin zu klinischen Effekten, einschließlich einer Verlangsamung von Tumorwachstum (12) sowie Tremorsuppression bei Patienten mit Morbus Parkinson (13).

3.2 Transkranielle elektrische Stimulation - Chancen und Herausforderungen

Trotz dieser vielversprechenden Ergebnisse sind tACS-Anwender ebenso mit den Herausforderungen der Variabilität und geringen Reproduzierbarkeit konfrontiert (8, 14, 15). Das unvollständige Wissen um die Wirkmechanismen von tACS (14) erschwert zudem eine genaue Eingrenzung der Einflussfaktoren und Optimierung der Ergebnisse.

Die Effekte von tACS werden auf eine, meist als frequenzspezifisch charakterisierte, Beeinflussung neuronaler Oszillationen zurückgeführt. Hypothesen bezüglich der Wirkungsmechanismen dieser ACS-Synchronisationseffekte umfassen neuronales Entrainment, eine Verschiebung der Erregungsschwelle sowie Rebound-Effekte. Darüber hinaus gibt es Belege dafür, dass manche Auswirkungen von tACS zumindest teilweise auf eine gewollte/ungewollte Erregung der Retina zurückzuführen sind (16).

Die fortlaufende Eingrenzung möglicher Wirkmechanismen ermöglicht immer effizientere und effektivere Stimulationstechniken. Eine Untersuchung der Effekte transkranieller Stimulation auf Oszillationen im visuellen System trägt zur Adressierung beider Herausforderungen bei. Über die Anwendung retinofugaler Stimulation, speziell retinofugaler Wechselstromstimulation (retinofugal Alternating Current Stimulation, rACS)(17) bietet sich zudem eine einzigartige Möglichkeit zur Stimulation eines klar definierten Systems über einen anatomisch und funktionell gut definierten afferenten retinokortikalen Zugang.

3.3 Wirkmechanismen der transkraniellen elektrischen Stimulation

Zwei Erklärungsmodelle für die Wirkung von tACS sind Entrainment- und Reboundeffekte. Während Entrainment die Synchronisation eines Oszillators mit einem anderen beschreibt (18), ist Rebound als eine Steigerung der Erregbarkeit definiert, die typischerweise nach einer Periode der Inhibition auftritt (19).

Bekannte frequenzspezifische Auswirkungen von tACS auf perzeptive und kognitive Leistungen werden oft auf ein potenzielles Frequenz-Entrainment während oder kurz nach einer einzelnen rhythmischen Stimulationsperiode zurückgeführt (9, 20-22). Entrainment ist in diesem Kontext definiert als eine Erhöhung der spektralen Power sowie eine Phasen- und Frequenzkopplung der neuronalen Oszillation im EEG an einen äußeren Stimulus (23).

Hinsichtlich Phasenkopplung während ACS gibt es einige Hinweise (24, 25), wobei elektrische Stimulationsartefakte die Erfassung zuverlässiger neurophysiologischer Daten erschweren (24,

26). Daten von Noury et al. deuten weiterhin darauf hin, dass solche Artefakte möglicherweise mit Entrainmenteffekten verwechselt werden können (27).

Aufgrund dieser Einschränkungen zeigen die meisten Studien spektrale Power-Steigerung erst nach Beendigung der Stimulation (17, 22-24). Dies eröffnet die Hypothese eines Rebound-Effekts als alternatives Erklärungsmodell.

Der klassische post-inhibitorische Rebound ist eine Steigerung der Erregbarkeit nach einer Phase der Inhibition. Diese Steigerung rangiert von der Senkung der Erregbarkeitsschwelle bis hin zu einem Burst-Firing zahlreicher Neuronen (19). Vergleichbare Reaktionen von den für den kortikalen α-Rhythmus essentiellen α-pacemaker-Neuronen würden eine Erhöhung der spektralen Power nach Stimulationsende erklären. Diese Erhöhung würde folglich bei der neuronalen Eigenfrequenz und ohne Phasenkopplung auftreten (19). Vergleichbare neurale Rebound-Effekte nach Stimulation wurden bereits in Tiermodellen (28-30) sowie im menschlichen Gehirn (31-33) nachgewiesen.

3.4 Transkranielle Rauschstromstimulation – eine Perspektive

Eine Erregungsschwellensenkung durch ACS würde eine Erklärung für ähnliche Effekte auf Oszillationen liefern, die nach transkranieller Rauschstimulation (Random Noise Stimulation, RNS) berichtet wurden (34).

Neuronales Rauschen erfüllt wahrscheinlich wichtige Funktionen bei Informationsverarbeitung und –weiterleitung. Stochastische Signale sind imstande, Oszillationszustände abhängig von der Intensität des Rauschens und der Oszillationen zu modulieren (35), vermutlich über stochastische Resonanz (36, 37). Dieses Phänomen beschreibt die intensitätsabhängige Verstärkung unterschwelliger Signale durch systemisches Rauschen bis zu einem Optimum, nach dem eine Störung des Signals eintritt.

Durch eine solche, abhängig von intrinsischen Oszillationen wirksame Stimulation, ließe sich die für tES-Effekte beschriebene Varianz als Abhängigkeit von Hirnzuständen nicht nur erklären, sondern auch potentiell zur Optimierung der Stimulation nutzen.

3.5 Hypothesen

Das primäre Ziel meiner Studien war es, die Wirkmechanismen nicht-invasiver oszillatorischer Hirnstimulation im Modell des visuellen Systems zu charakterisieren und die Erkenntnisse in der Anwendung transkranieller Stimulation zu überprüfen.

Hierbei ergaben sich folgende Hypothesen:

1. Niederspannungs-Wechselstromstimulation ist ein sicheres und effektives Verfahren zur Modulation von neuronaler Aktivität entlang der Sehbahn (Studie 1): Zur Überprüfung dieser Hypothese führte ich eine Erfassung von Nebenwirkungen und Verträglichkeit von retinofugaler Wechselstromstimulation (rACS) sowie ein Finite-Element-Modeling des Stromflusses während der Stimulation durch (38).
2. Elektrische Hirnstimulation moduliert intrinsische neuronale Oszillationen durch Rebound- oder Schwellenhebungseffekte (Studie 2): Hierzu untersuchte ich die Effekte von 10 Hz rACS auf spektrale Power und Frequenz occipitaler α-Oszillationen (39).
3. Elektrische Hirnstimulation moduliert Hirnfunktionen abhängig von dem intrinsischen neuronalen Gesamterregbarkeitszustand (*brain state*), konkordant mit einem Schwellenmodulationseffekt (Studie 3): Hierfür wurde transkranielle Rauschstromstimulation (tRNS) über dem primär-motorischen Kortex während zweier motorischer Aufgaben mit grundlegend verschiedenen kortikalen Erregbarkeitszuständen appliziert (40).

4. Material und Methodik

4.1 Probanden

In Studie 1 stimulierten wir 20 gesunde Proband*innen retinofugal (10 weiblich, 25.9 ± 5,0 Jahre).

In Studie 2 wurden jeweils 15 gesunde Proband*innen der Stimulationsgruppe (acht weiblich; 23,9 ± 2,5 Jahre) und der Sham-Stimulationsgruppe (vier weiblich; 25,8 ± 5,3 Jahre) untersucht.

An Studie 3 nahmen 30 gesunde Proband*innen (18 weiblich; 22,8 ± 2,8 Jahre) teil. Alle Proband*innen dieser Studie waren nach dem Edinburgh Handedness Inventory rechtshändig.

4.2 Ausschlusskriterien

Ausschlusskriterien aller Studien waren das Vorhandensein neurologischer, psychiatrischer oder internistischer Vorerkrankungen, die Einnahme von zentral wirksamen Medikamenten, das Vorhandensein implantierter oder nicht entfernbarer Metallteile oder medizinischer Geräte und epileptische Anfälle in der Vorgeschichte bzw. eine bekannte Epilepsie.

Spezifische zusätzliche Ausschlusskriterien für die Anwendung von rACS in Studie 1 und 2 waren Photophobie und photosensitive Epilepsie.

4.3 Ethik

Alle Teilnehmer gaben nach ausführlicher Aufklärung ihre mündliche und schriftliche Einwilligung. Die Studien wurde durch die Ethikkommission der Charité - Universitätsmedizin Berlin begutachtet und genehmigt (EA 1/173/12) und richteten sich sowohl nach den Grundsätzen der Charité - Universitätsmedizin Berlin zur Sicherung der guten wissenschaftlichen Praxis (good clinical practice, GCP) als auch den Prinzipien der Deklaration von Helsinki von 1954 in ihrer aktualisierten Version.

4.4 Stimulation

Alle Stimulationstechniken wurde über das Stimulations-System NextWave (EBS Technologies GmbH, Kleinmachnow, Deutschland) gesteuert und appliziert.

RACS (Studie 1 und 2) erfolgte über vier Elektroden von jeweils 0,35 cm^2, oberhalb und unterhalb der Orbita (periorbital). Die passive Elektrode (3 x 3 cm) wurde im Nackenbereich mittig angebracht. Durch schrittweisen Anstieg der Stromstärke wurde die individuelle Intensitätsschwelle für Lichtwahrnehmungen (Phosphenschwelle) bestimmt. Nachfolgend wurde in der rACS-Gruppe ein Wechselstrom mit einer Frequenz von 10 Hz und einer Amplitude von 120% der Phosphenschwelle appliziert.

Photische Stimulation (Studie 1) wurde über zwei 3 × 5 cm große RGB-LEDs appliziert. Um mit rACS vergleichbare Stimulationsintensitäten zu erreichen, wurden sinusförmige Weißlichtimpulse mit einer Intensität von 120 % der Lichtschwelle und einer Frequenz von 10 Hz appliziert.

In der Sham-Stimulationsgruppe (Studie 2) wurde ein initialer Gleichstrom appliziert (5 s ansteigend und 5 s absteigend), um typische Hautempfindungen elektrischer Stimulation zu simulieren. Beide Stimulationsgruppen erhielten sechs Stimulationsblöcke von je 30 s Dauer, jeweils gefolgt von 30 s Pause zur Messung des EEGs.

TRNS (Studie 3) erfolgte mittels zwei Elektroden (NeuroConn GmbH, Ilmenau, Deutschland). Eine Elektrode (kreisförmig, 12,5 cm^2) befand sich über dem dominanten (linken) motorischen Kortex an der C3-EEG-Elektrodenposition, die andere Elektrode (rechteckig, 30 cm^2) wurde über dem kontralateralen frontopolaren Kortex platziert. tRNS wurde mit einer Spitzenintensität von 1,51 mA für 10 min appliziert. Das Random-Noise-Signal wurde aus einer konstanten Wahrscheinlichkeitsdichte mit einer Abtastrate von 1280 Hz generiert und digital gefiltert, um ein Frequenzspektrum von 100 - 640 Hz zu gewährleisten. Zur Sham-Stimulation wurde ein 15 s ansteigender, und 15 s absteigender Gleichstrom gemäß den Empfehlungen für die transkranielle Gleichstromstimulation appliziert.

4.5 Elektroenzephalogramm

Elektroenzephalogramm (EEG)-Messungen zwischen den Stimulationsblöcken erfolgten nach dem 10-20 System mit einer 32-Elektroden-EEG-Kappe (Waveguard EEG caps, ANTBV, Enschede, Netherlands). Vor der Stimulation wurde ein Ruhe-EEG als Referenz aufgenommen.

4.6 Finite-Elemente-Modeling

Für Studie 1 wurde ein über die IT'IS Foundation verfügbares ultrahochauflösendes Kopf- und Halsmodell (MIDA: Multimodal Imaging-Based Detailed Anatomical Model) verwendet (41). Die nifti (.nii)-Farbmasken aus dem MIDA-Modell wurden zunächst in MATLAB (The MathWorks Inc, Natick, MA, USA) verarbeitet, um Segmentierungsmasken basierend auf Intensitätswerten zu erstellen. Diese Masken wurden dann in Simpleware (Synopsys Ltd., CA, Vereinigte Staaten) importiert. Fehler in der Kontinuität und anatomischen Details wurden manuell nach Datta (42) korrigiert.

Die Elektroden wurden interaktiv in den Bilddaten positioniert und simulierten die für rACS verwendete Elektrodenmontage. In der Folge wurden die aus den Segmentierungsmasken abgeleiteten adaptiven Netze in COMSOL Multiphysics (Burlington, MA, USA) zur Finite-Elemente-Berechnung importiert. Das endgültige Modell umfasste >10 Millionen Elemente mit >15 Millionen Freiheitsgraden.

Die Laplace-Gleichung wurde gelöst und Stromdichten, die einem Gesamtstrom von 350 µA entsprechen, wurden an der Anode oder aktiven Elektrode (s) angelegt. An der Gegenelektrode wurde Masse angelegt, alle anderen Außenflächen wurden als isoliert behandelt.

Zuletzt wurden sowohl Oberflächen- als auch Querschnitts-EF-Magnitudenkarten auf der grauen Substanz, der Retina und dem Sehnerv generiert. Für die Kopfhaut wurde das Oberflächen-Stromdichte-Magnituden-Diagramm erstellt.

4.7 Datenauswertung und Statistik

Der Vergleich der unerwünschten Effekte zwischen rACS und PS (Studie 1) erfolgte mittels Wilcoxon Signed Rank Tests und Fisher's Exact Tests.

Die EEG-Signale (Studie 2) wurden in MATLAB (The MathWorks Inc, Natick, MA, USA) unter Nutzung der Fieldtrip-Toolbox (43) artefaktbereinigt und gegen einen gemeinsamen Mittelwert referenziert, gefiltert (Bandpassfilter von 2 – 70 Hz) und mittels Fouriertransformation in den Frequenzbereich konvertiert. Für jeden Kanal und Stimulationsblock wurde die mittlere und maximale Power im Alpha- Frequenzspektrum (8-12 Hz) berechnet. Nach ln+1-Transformation der Daten wurden die Alpha-Power-Differenzen der Sham- und rACS-Gruppe in einer einfaktoriellen Varianzanalyse (ANOVA) mit Messwiederholungen verglichen. Frequenzveränderungen der maximalen Alpha-Power (individuelle Alpha- Frequenz, IAF) der beiden Gruppen wurden ebenfalls per ANOVA verglichen. Der Zusammenhang zwischen IAF-Änderung und Änderungen der mittleren EEG-Power im Alpha-Bereich wurde durch Berechnung des Rangkorrelationskoeffizienten von Spearman überprüft.

Die kortikale Erregbarkeit (Studie 3) wurde als Mittelwert von 20 Einzelpulsen definiert und in MATLAB normalisiert. Die statistische Analyse erfolgte per Mixed-Model-ANOVA. Die Messwerte der psychophysischen Aufgaben wurden in MATLAB z- transformiert und per Mixed-Model-ANOVA mit Messwiederholungen analysiert. Post-hoc-Tests wurden nach Bonferroni korrigiert.

Alle statistischen Analysen erfolgten mit SPSS (IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp.).

5. Ergebnisse

5.1 Studie 1 – Sicherheit und Verträglichkeit retinofugaler Wechselstromstimulation

5.1.1 Stimulationsparameter

Wir bestimmten eine durchschnittliche 10-Hz-Phosphenschwelle bei 290,50 µV (SD 45,36), Impedanzen bei 12,05 kΩ (SD 2,89) und eine durchschnittliche Amplitude von 351,69 µA (SD 63,95). Aus der Peak-to-Peak-Amplitude berechnet ergab sich auf Elektrodenebene ein Mittelwert von 1,00 mA/cm² (SD 0,28) für die Stromdichte und 0,60 C/cm² (SD 0,11) für die Ladungsdichte. Da Sinuswellenimpulse verwendet wurden, berechneten wir zusätzlich die effektive Amplitude, resultierend in einem Mittelwert von 248,68 µA (SD 47,0). Bei Verwendung der effektiven Amplitude betrug die Stromdichte im Mittel 0,71 mA/cm² (SD 0,13) und die Ladungsdichte 0,42 C/cm² (SD 0,08).

RACS lag somit innerhalb der Sicherheitsgrenzen(44), und die Ergebnisse waren mit anderen ähnlichen Stimulationsmethoden vergleichbar. Hinsichtlich der Stimulationsamplitude war rACS (0,35 mA) mit den meisten TCES- und tES-Montagen (im Bereich von 0,08-1,2 mA) vergleichbar. Die Ladungsdichte von rACS zeigte sich knapp über der TCES von Ma et al. (45) und weit unter der von Liebetanz et al. (46) veröffentlichten Sicherheitsgrenze. Hinsichtlich der Stromdichte liegt rACS (1 mA/cm²) unter Ma (1,2 mA/cm²) (40) und weit unter der von Agnew & McCreery vorgeschlagenen Sicherheitsgrenze (25 mA/cm²) (47). Diese Befunde sind bei Verwendung der effektiven Amplitude noch ausgeprägter.

5.1.2 Finite-Element-Modellierung

Das von rACS generierte elektrische Feld zeigte im Bereich der Augen die größte Intensität, wobei die höchste Stromdichte auf der Netzhaut geschätzt wurde. Als weitere Areale erhöhter Stromdichten konnten Nervus opticus und Kortex identifiziert werden.

Die berechnete maximale Stromdichte an der Netzhaut betrug maximal 1,24 A/m², während Sehnerv (0,33 A/m²) und Kortex (0,13 A/m²) beide einem geringeren Stromfluss ausgesetzt waren. Bezüglich des elektrischen Feldes schätzten wir ein Maximum von 2,6 V/m im Sehnerv, gefolgt von 1,99 V/ m für die Netzhaut und 0,47 V/m für den Kortex. Schließlich betrug die Stromdichte auf Hautniveau unterhalb der aktiven Elektrode einen maximal induzierten Wert von 14,79 A/m², wobei das elektrische Feld auf 31,80 V/m geschätzt wurde.

5.1.3 Unerwünschte Ereignisse

Keiner der Probanden erbat einen Abbruch der Stimulation oder benötigte medizinische Hilfe. In subjektiven Berichten waren rACS-assoziierte unerwünschte Ereignisse unter Stimulation im Vordergrund, während die mit der photischen Stimulation (PS) assoziierten unerwünschten Ereignisse nach der Stimulation überwogen. So trat bei 70% der Testpersonen unter, aber nicht nach rACS ein Kribbeln auf. Ein Juckreiz unter den Elektroden wurde von 30 % der Testpersonen unter rACS und 25 % nach rACS berichtet. Ein brennendes Gefühl wurde von 30 % der Teilnehmer unter, aber nicht nach rACS empfunden. Müdigkeit trat sowohl unter als auch nach der Stimulation bei 35 % der rACS- und 20 % der PS-Gruppe auf. Kopfschmerzen während Stimulation wurden nur von PS-Teilnehmern (15 %) berichtet. Nach der Stimulation wurden Kopfschmerzen sowohl bei PS- als auch bei rACS-Teilnehmern mit 5 % angegeben. Konzentrationsschwierigkeiten wurden von 10 % der Teilnehmer nach PS, nicht aber nach rACS berichtet. Es gab keine Fälle von akuten Stimmungsschwankungen, Übelkeit oder visuellen Wahrnehmungsveränderungen, oder anhaltenden unerwünschten Ereignissen drei Monate nach der Stimulation.

5.1.4 Schmerz

Unter Stimulation kam es zu unangenehmen Missempfindungen, welche gesammelt als Schmerzempfinden beschrieben wurden. 40% der Probanden berichteten über derartige Empfindungen unter rACS (mittlere Intensität 2,5, SD 1,73) und 20% unter PS (2,75, SD 0,83). Schmerzempfinden nach der Stimulation trat bei rACS nicht auf, wurde allerdings von 10% der Probanden nach PS angegeben (1,5, SD 0,5).

5.1.5 rACS vs. Photische Stimulation

In den statistischen Analysen zeigten rACS und PS keinen signifikanten Effekt des Stimulationstyps (rACS versus PS) auf Schmerzintensität, Müdigkeit, Kopfschmerzen und Konzentrationsschwierigkeiten sowohl unter als auch nach der Stimulation. PS und rACS unterschieden sich signifikant hinsichtlich der kutanen Empfindungen Kribbeln, Juckreiz und Brennen ($p < 0,05$), welche alle ausschließlich bei rACS auftraten.

5.2 Studie 2 – Einfluss von Wechselstromstimulation auf individuelles Alpha

5.2.1. Stimulationsparameter

Die durchschnittliche Phosphenschwelle betrug 290,24 µA (± 44,16) für die rACS, und 243,33 µA (± 57,07) für die Sham-Stimulationsgruppe. Entsprechend wurde mit 120% der Phosphenschwelle mit einer Amplitude von 354,15 µA ± 50,6 und einer Stromdichte von 0,71 mA/cm² ± 0,13 für rACS, bzw. 292,00 ± 68,5 µA und 0,48 mA/cm² ± 0,11 für die Sham-Stimulation stimuliert.

5.2.2. Alpha-Power-Steigerung nach retinofugaler Wechselstromstimulation (rACS)

Die einfaktorielle Varianzanalyse (ANOVA) mit Messwiederholungen zeigte einen signifikanten Haupteffekt der spektralen Power im Alpha-Frequenzspektrum (8-12 Hz) ($F(2,27) = 8,99$; $p = 0,001$), sowie einen signifikanten Interaktionseffekt zwischen Stimulationsart und Alpha-Power ($F(2,27) = 4,02$; $p = 0,03$) über den okzipitalen Elektroden. Post-hoc Bonferroni- korrigierte, paarweise Vergleiche zeigten post-Stimulation eine signifikant höhere Alpha-Power der rACS-Gruppe im Vergleich zur Sham-Gruppe (1,21 ± 0,14 vs. 0,83 ± 0,09; $p = 0,036$), sowie im Vergleich zur Grundaktivität bei offenen Augen (0,99 ± 0,13; $p = 0,01$). Der Anstieg der α-Power war vergleichbar mit dem physiologischen Anstieg bei Lidschluss (rACS-Gruppe: 1,26 (± 0,17) $p= 0,02$, Sham-Gruppe: 1,11 (± 0,16) $p = 0,05$) und signifikant stärker im Vergleich zur Sham-Gruppe (+0,28 ±0,09 vs. -0,02 ±0,07; $F(1,28) = 7,139$; $p = 0,01$).

5.1.3. Verschiebung der individuellen Alpha-Frequenz (IAF)

Es zeigte sich keine signifikante Verschiebung der IAF nach rACS: Vor Stimulation lag die mittlere IAF bei 10,22 Hz (±1,29), nach rACS bei 10,26 Hz (±1,17). Eine ANOVA zeigte keinen signifikanten Effekt von rACS auf die IAF ($F(1,28) = 0,009$; $p = 0,93$). Darüber hinaus korrelierte die Nähe der IAF zur rACS-Frequenz von 10 Hz und Stärke der Frequenzverschiebung nicht signifikant (Rangkorrelationskoeffizient von Spearman, $r = 0,04$; $p = 0,88$). Es zeigte sich ein signifikanter Anstieg der occipitalen α-Power nach 10 Hz rACS, mit einem signifikant höheren Anstieg gegenüber Sham (ANOVA: $F(1,28) = 7.139$, $p = 0.01$).

5.3 Studie 3 – Hirnzustandsabhängigkeit transkranieller Rauschstromstimulation

5.3.1. Kortikospinale Erregbarkeit (CSE)

In einer mixed-model-ANOVA zeigte sich ein signifikanter Interaktionseffekt zwischen Aufgaben- und Stimulationsart ($F(1,26) = 5,474$; $p = 0,027$, $\eta p2 = 0,17$). Post-hoc paarweise Vergleiche zeigten

einen signifikanten Anstieg der CSE in der FT-Gruppe nach tRNS gegenüber Shamstimulation (381 ± 146 µV vs. 14 ± 133 µV; p = 0,018, ηp2 = 0,2). In der GNG-Gruppe zeigte sich kein vergleichbarer Effekt (-36 ± 97 µV vs. -63 ± 93 µV; p = 0,473, ηp2 = 0,02). Zu einem Anstieg der CSE unter tRNS kam es nur nach Durchführung der FT-Aufgabe (p = 0,022, ηp2 = 0,19).

5.3.2. FT: Intertap-Intervalle (ITI) und Anzahl an Fingertaps

Ein lineares gemischtes Modell für Messwiederholungen zeigte signifikante Interaktionseffekte zwischen Stimulationsart und Zeitpunkt der Stichprobe (ITI rechts: F(20) = 3,03; p < 0,001; ITI links: F(20) = 3,29; p < 0,001; Fingertaps rechts: F(20) = 3,39; p < 0,001; Fingertaps links: F(20) = 2,63; p < 0,001) mit unspezifischen Verschlechterungen im Verlauf der Stimulationsbedingung, sowie Funktionsverbesserungen post-Stimulation. Der Haupteffekt zeigte sich nicht signifikant. In Bonferroni-korrigierten paarweisen Vergleichen zeigten sich keine signifikanten Unterschiede zwischen tRNS und Sham.

5.3.3. GNG: Reaktionszeit (RT) und Präzision

In einem erneuten linear gemischten Modell für Messwiederholungen zeigten sich signifikante Haupteffekte der Stimulation auf Reaktionszeit und Präzision (RT, F(2) = 11,69; p < 0,001; Präzision F(2) = 18,01; p< 0,001) sowie Interaktionseffekte zwischen Stimulation und Zeitpunkt der Stichprobe (RT, F(14) = 2,57; p = 0,002, Präzision, F(14) = 2,78; p = 0,001). Unter tRNS zeigten sich langsamere Reaktionszeiten und eine gesteigerte Präzision im Vergleich zu Sham (RT 0,21 ± 0,045 vs. 0,06 ± 0,045; t(160) = ± 2,35; p = 0,019 und Präzision 1,89 ± 0,32 vs. 0,29 ± 0,33; t(173) = ± 3,49; p < 0,001). In Post-hoc-Analysen zeigte sich eine spezifische Verlangsamung der Reaktionszeit unter tRNS in den Blöcken 7-8 der post-Stimulationsphase (0,24 ± 0,085 vs. -0,14 ± 0,085; t(44) = - 3,19; p = 0,002). Eine Präzisionsverbesserung durch tRNS im Vergleich zu Shamstimulation zeigte sich sowohl während der Stimulationsphase in Blöcken 2-6 (1,74 ± 0,36 vs.

0,37 ± 0,38; t(123) = -2,62; p = 0,009), als auch in der Post-Stimulationsphase (2,27 ± 0,66 vs. 0,083 ± 0,68; t(48) = -2,30; p = 0,023).

6. Diskussion

6.1 Hypothese 1: Niederspannungs-Wechselstromstimulation ist ein sicheres und effektives Verfahren zur Modulation von neuronaler Aktivität entlang der Sehbahn

Um das Sicherheitsprofil von rACS zu analysieren, wurden theoretische Sicherheitsgrenzen sowie Finite-Element-Modellierungsdaten bewertet und die unerwünschten Ereignisse für rACS und PS verglichen.

Es zeigt sich, dass rACS basierend auf den folgenden Beobachtungen sicher ist:

(I) Stimulationsparameter (Strom- und Ladungsdichten an der Elektrode) liegen innerhalb der theoretischen Sicherheitsgrenzen,

(II) Finite-Elemente-Modellierungsdaten zeigen dasselbe für EF-Schätzungen und Stromdichten an Auge, Netzhaut und Kortex und

(III) unerwünschte Ereignisse sind im direkten experimentellen Vergleich mit PS vergleichbar und die Rate sowie die Schwere der unerwünschten Ereignisse überstiegen nicht die anderer etablierter Hirnstimulationsmethoden.

Die Analysen von Studie 1 zeigten (I) Stimulationsparameter innerhalb der Sicherheitsgrenzen sowie vergleichbar mit etablierten Stimulationsarten, (I) vergleichbare Ergebnisse für Modellierungsdaten, (III) keine schweren Nebenwirkungen unter rACS, mit hautbezogenen Nebenwirkungen wie Kribbeln, Brennen und Juckreiz auf dem Niveau vergleichbarer Stimulationsarten(38).

Anhand der Daten unserer Studie schlussfolgern wir daher, dass rACS für die wissenschaftliche Anwendung als sicher zu betrachten ist.

6.2 Hypothese 2: Elektrische Hirnstimulation moduliert intrinsische neuronale Oszillationen durch Rebound- oder Schwellenhebungseffekte

Die Effekte von 10-Hz-rACS auf neuronale Oszillationen in Studie 2 lassen sich wie folgt charakterisieren: (I) 10-Hz-Stimulation führte zu einer Verbesserung der α-Leistung; (II) der α-Peak nach der Stimulation unterschied sich nicht signifikant von der IAF-Grundlinie; und (III) es zeigte sich keine signifikante Korrelation zwischen der α-Power und der Nähe der IAF zur Stimulationsfrequenz(39). Diese Beobachtungen sind diskordant zu den Charakteristika hypothetischer Entrainmenteffekte, stehen allerdings im Einklang mit mehreren Berichten über eine tACS-α-Verstärkung nach Stimulation (22-24), in denen sich ebenfalls weder Frequenzkopplung noch Korrelation zwischen Stimulationseffekten und Nähe von IAF zur Stimulationsfrequenz gezeigt hatten.

Es bleibt zu berücksichtigen, dass Entrainment während der Stimulation sowie in den ersten 300 ms nach Ende der Stimulation artefaktbedingt nicht sicher auszuschließen ist. Es gibt Berichte, die ein solches Entrainment während der Stimulation unter Verwendung von Artefaktbereinigungstechniken darstellen (24, 26, 48), die noch immer kontrovers diskutiert werden (27).

Dennoch eröffnet sich die Alternative eines Rebound- oder Burst-Firing-Effektes (49), insbesondere nach Beendigung der Stimulation (50).

Mehrere Studien präsentierten Rebound-Firing in thalamokortikalen Netzwerken und Neuronen als Reaktion auf oszillatorische elektrische Stimuli (51-53). Diese Netzwerke sind ebenfalls entscheidend an der Erzeugung des α-Rhythmus beteiligt (54).

Darüber hinaus wurden auch bestimmte TMS-Effekte auf neuronale Oszillationen einem Rebound-Firing zugeschrieben, wobei Studien eine α- und/oder β-Verstärkung nach einzelnen TMS-Impulsen fanden (31, 32, 55). Paus et al. (55) erklären dies mit einer Kombination aus rebound-artigem Feuern und der Rekrutierung von "untätigen" Neuronen. Fuggetta et al. (31) sowie Brignani et al.

(32) diskutieren α-Rebound als möglichen Effekt von kortikothalamischen Feedback-Mechanismen. Nach dem thalamischen Input sind die Neuronen der primären visuellen Kortexschicht 6 in der Lage, antiphasisches Feedback an den lateralen genikulären Nucleus zu liefern (56), was wiederum zu einem Burst-Firing führen kann, welches α- oder θ-Rhythmen induziert (54). Dies ist speziell relevant, da kortikales tACS auch Layer-6-Neuronen aktivieren sollte und ähnliche Reboundmechanismen auslösen könnte.

Unter Anwendung dieser Überlegungen können die Ergebnisse von Studie 2 wie folgt interpretiert werden: Ein mögliches anfängliches Entrainment von Schicht 4- und 6-Neuronen könnte zu einer kortikothalamischen Rückkopplung führen, die ein thalamisches Rebound-Firing auslöst, welches den kortikal beobachteten α-Rhythmus antreibt und zu einer weiteren polysynaptischen Rekrutierung kortikaler Neuronen führt. Der Rebound-Effekt könnte eine Verstärkung und Stabilisierung bei neuronaler Eigenfrequenz nach weder phasen- noch frequenzgekoppelter Stimulation erklären (19). Diese Hypothese würde auch die fehlende Korrelation zwischen der Nähe der Stimulationsfrequenz zur IAF und der ebenfalls von Helfrich et al. (24) berichteten α-Power-Steigerung hinreichend erklären. Aufgrund der stimulationsartefaktbedingt eingeschränkten Aussagekraft von EEG-Messungen während der Stimulation können wir jedoch nicht über einen Zeitraum der Inhibition vor einem Rebound berichten (19).

Im Allgemeinen bestehen folgende Herausforderungen für eine Rebound-Hypothese: I) Es wurden mehrfach frequenzspezifische psychophysische Effekte von ACS konkordant mit einer erhöhten spektralen Power der stimulierten Frequenz während der Stimulation berichtet (57, 58), was gegen eine inhibitorische Periode vor einem Rebound spricht. II) Das Phänomen des photic driving (59), welches mit der bekannten Erhöhung der okzipitalen α Power während der Stimulation ebenfalls gegen eine Inhibition spricht. Dies gilt insbesondere für unsere Studie, da wir die Netzhaut und den Sehnerv stimulieren.

Eine weitere mögliche Hypothese stellt daher die im Rattenmodell beschriebene unterschwellige Modulation dar (60). Diese würde zu einer niedrigeren Schwelle für netzwerkinduzierte Membranspannungsfluktuationen führen, um in einem Teil der neuronalen Population Spikes zu erzeugen. Die Effekte transkranieller Stimulation, speziell tES, werden meist auf eine solche unterschwellige Modulation zurückgeführt (14). Im Gegensatz zur kortikalen Stimulation der tES stimuliert rACS die stärker erregbare Retina (61) mit Amplituden oberhalb der Phosphene-Schwelle. Wir können daher von der Erzeugung von Aktionspotentialen zumindest in der Photorezeptorzelle ausgehen (62, 63). Nichtsdestotrotz würde ein schwellensenkender Effekt von rACS eine Erklärung für ähnliche Effekte auf Oszillationen liefern, die nach retinaler RNS beschrieben wurden (34).

6.3 Hypothese 3 – Elektrische Hirnstimulation moduliert Hirnfunktionen abhängig von dem intrinsischen *brain state*, konkordant mit einem Schwellenmodulationseffekt

Da sich nach den Ergebnissen von Studie 2 eine Erregungsschwellensenkung durch Rebound-Effekte und unterschwellige Modulation als mögliche Wirkmechanismen von NiBS-Techniken darstellen, bietet sich eine Perspektive für tRNS als modulierende Stimulationsart. Über den Mechanismus der stochastischen Resonanz (36, 37) könnte tRNS ermöglichen, intrinsische Oszillationen entsprechend des aktuellen Bedarfs zu modulieren. Somit könnte also beispielsweise pathologische oder suboptimale Synchronizität reduziert und konkordant physiologische Synchronizität gefördert werden.

In Studie 3 konnte gezeigt werden, dass tRNS zu einer Erhöhung der kortikalen Erregbarkeit während der Ausführung einer exzitatorischen Aufgabe (FT) führt(40). Dies deutet darauf hin, dass tRNS den zugrundliegenden, aktivierenden Erregbarkeitszustand der FT-Aufgabe verstärkt, möglicherweise über größere, aufgabenspezifische Muskelrepräsentation (64). In der inhibitorischen GNG-Aufgabe zeigte sich ein gegenteiliger Effekt: tRNS verstärkte inhibitorische Kontrolle und verlängerte Reaktionszeiten.

Zusammenfassend wird in Studie 3 gezeigt, dass tRNS den aufgabenabhängig vorherrschenden Hirnzustand verstärkt. Somit können wir psychophysische Effekte einer erregungsschwellenmodulierenden Stimulation nachweisen, die potentiell in weiteren Studien zur Verbesserung motorischer Leistungen genutzt werden können, so beispielsweise in der Schlaganfallrehabilitation (65). Eine weitere Anwendungsmöglichkeit besteht im Bereich des kognitiven Trainings (66).

6.4 Fazit

Im Hinblick auf Wirkmechanismen nicht-invasiver oszillatorischer Hirnstimulation im visuellen System konnte ich im Rahmen meiner Studien feststellen, dass die Effekte von ACS auf intrinsische neuronale Oszillationen potentiell durch erregungsschwellenmodulierende Effekte im Sinne eines Rebound-Mechanismus oder einer unterschwelligen Modulation zu erklären sind.

Diese Erkenntnis ließ sich von dem visuellen System auf das motorische System übertragen. Hier zeigte sich, dass tRNS motorische Aufgaben abhängig von dem intrinsischen Hirnzustand (brain state) moduliert. Dies ist vereinbar mit den Effekten eines Schwellenmodulationseffektes.

Generell bedarf es weiterer Studien der Effekte von NiBS auf neuronale Oszillationen, um die unterschiedlichen Wirkmechanismen verschiedener Stimulationstypen auf neuronale Zielfrequenzen zu charakterisieren und entsprechend der Ergebnisse die Stimulationsmethoden und -techniken zu optimieren. Eine Kombination aus Entrainment- und Rebound-Hypothesen, wie sie in dieser Arbeit vorgestellt wurden, könnte prospektiv ein integratives Modell der ACS-Effekte auf den α-Rhythmus liefern. Die Untersuchung der Closed-Loop-Phase-Locked-Stimulation (13) sowie eine bessere Kenntnis der psychophysischen Veränderungen während der Stimulation könnten zu einem tieferen Verständnis solcher Wirkmechanismen beitragen.

Aktuell besteht weiterhin die Herausforderung der nicht abschließend geklärten Wirkmechanismen eines möglichen Entrainments von neuronalen Oszillationen durch transkranielle Stimulation. Dies

gilt speziell angesichts potentiell relevanter Effekte des intrinsischen Hirnzustands auf die Wirksamkeit und den Wirkmechanismus der Stimulation. Hier birgt die frequenzunspezifische RNS großes Potential, mit hirnzustandsspezifischen schwellenmodulierenden Effekten physiologische intrinsische Prozesse zu stärken und pathologische zu schwächen.

7. Referenzen

1. Priori A, Berardelli A, Rona S, Accornero N, Manfredi M. Polarization of the human motor cortex through the scalp. Neuroreport. 1998;9(10):2257-60.
2. Lefaucheur J-P. A comprehensive database of published tDCS clinical trials (2005–2016). Neurophysiologie Clinique/Clinical Neurophysiology. 2016;46(6):319-98.
3. Horvath JC, Forte JD, Carter O. Evidence that transcranial direct current stimulation (tDCS) generates little-to-no reliable neurophysiologic effect beyond MEP amplitude modulation in healthy human subjects: A systematic review. Neuropsychologia. 2015;66:213-36.
4. Nitsche MA, Liebetanz D, Lang N, Antal A, Tergau F, Paulus W. Safety criteria for transcranial direct current stimulation (tDCS) in humans. Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology. 2003;114(11):2220-2; author reply 2-3.
5. Antal A, Paulus W. Investigating neuroplastic changes in the human brain induced by transcranial direct (tDCS) and alternating current (tACS) stimulation methods. Clinical EEG and neuroscience. 2012;43(3):175.
6. Antal A, Boros K, Poreisz C, Chaieb L, Terney D, Paulus W. Comparatively weak after-effects of transcranial alternating current stimulation (tACS) on cortical excitability in humans. Brain Stimul. 2008;1(2):97-105.
7. Kanai R, Paulus W, Walsh V. Transcranial alternating current stimulation (tACS) modulates cortical excitability as assessed by TMS-induced phosphene thresholds. Clin Neurophysiol. 2010;121(9):1551-4.
8. Wach C, Krause V, Moliadze V, Paulus W, Schnitzler A, Pollok B. The effect of 10 Hz transcranial alternating current stimulation (tACS) on corticomuscular coherence. Front Hum Neurosci. 2013;7.
9. Marshall L, Helgadottir H, Molle M, Born J. Boosting slow oscillations during sleep potentiates memory. Nature. 2006;444(7119):610-3.
10. Kuo MF, Nitsche MA. Effects of transcranial electrical stimulation on cognition. Clin EEG Neurosci. 2012;43(3):192-9.
11. Santarnecchi E, Polizzotto NR, Godone M, Giovannelli F, Feurra M, Matzen L, Rossi A, Rossi S. Frequency-dependent enhancement of fluid intelligence induced by transcranial oscillatory potentials. Curr Biol. 2013;23(15):1449-53.

12. Kirson ED, Dbaly V, Tovarys F, Vymazal J, Soustiel JF, Itzhaki A, Mordechovich D, Steinberg-Shapira S, Gurvich Z, Schneiderman R, Wasserman Y, Salzberg M, Ryffel B, Goldsher D, Dekel E, Palti Y. Alternating electric fields arrest cell proliferation in animal tumor models and human brain tumors. Proc Natl Acad Sci U S A. 2007;104(24):10152-7.
13. Brittain JS, Probert-Smith P, Aziz TZ, Brown P. Tremor suppression by rhythmic transcranial current stimulation. Curr Biol. 2013;23(5):436-40.
14. Zaghi S, Acar M, Hultgren B, Boggio PS, Fregni F. Noninvasive brain stimulation with low-intensity electrical currents: putative mechanisms of action for direct and alternating current stimulation. Neuroscientist. 2010;16(3):285-307.
15. Feurra M, Pasqualetti P, Bianco G, Santarnecchi E, Rossi A, Rossi S. State-Dependent Effects of Transcranial Oscillatory Currents on the Motor System: What You Think Matters. J Neurosci. 2013;33(44):17483-9.
16. Schutter DJ. Cutaneous retinal activation and neural entrainment in transcranial alternating current stimulation: A systematic review. Neuroimage. 2016;140:83-8.
17. Schmidt S, Mante A, Ronnefarth M, Fleischmann R, Gall C, Brandt SA. Progressive enhancement of alpha activity and visual function in patients with optic neuropathy: a two-week repeated session alternating current stimulation study. Brain Stimul. 2013;6(1):87-93.
18. Ermentrout GB, Rinzel J. Beyond a pacemaker's entrainment limit: phase walk-through. Am J Physiol. 1984;246(1 Pt 2):R102-6.
19. Perkel DH, Mulloney B. Motor pattern production in reciprocally inhibitory neurons exhibiting postinhibitory rebound. Science. 1974;185(4146):181-3.
20. Klimesch W, Sauseng P, Gerloff C. Enhancing cognitive performance with repetitive transcranial magnetic stimulation at human individual alpha frequency. Eur J Neurosci. 2003;17(5):1129-33.
21. Kanai R, Chaieb L, Antal A, Walsh V, Paulus W. Frequency-dependent electrical stimulation of the visual cortex. Curr Biol. 2008;18(23):1839-43.
22. Zaehle T, Rach S, Herrmann CS. Transcranial alternating current stimulation enhances individual alpha activity in human EEG. PLoS One. 2010;5(11).
23. Vossen A, Gross J, Thut G. Alpha power increase after transcranial alternating current stimulation at alpha frequency (alpha-tACS) reflects plastic changes rather than entrainment. Brain Stimul. 2015;8(3):499-508.

24. Helfrich RF, Schneider TR, Rach S, Trautmann-Lengsfeld SA, Engel AK, Herrmann CS. Entrainment of brain oscillations by transcranial alternating current stimulation. Curr Biol. 2014;24(3):333-9.
25. Ruhnau P, Neuling T, Fusca M, Herrmann CS, Demarchi G, Weisz N. Eyes wide shut: Transcranial alternating current stimulation drives alpha rhythm in a state dependent manner. Sci Rep. 2016;6:27138.
26. Soekadar SR, Witkowski M, Cossio EG, Birbaumer N, Robinson SE, Cohen LG. In vivo assessment of human brain oscillations during application of transcranial electric currents. Nature Communications. 2013;4.
27. Noury N, Hipp JF, Siegel M. Physiological processes non-linearly affect electrophysiological recordings during transcranial electric stimulation. Neuroimage. 2016.
28. Pape HC, McCormick DA. Electrophysiological and pharmacological properties of interneurons in the cat dorsal lateral geniculate nucleus. Neuroscience. 1995;68(4):1105-25.
29. Tong ZY, Overton PG, Clark D. Stimulation of the prefrontal cortex in the rat induces patterns of activity in midbrain dopaminergic neurons which resemble natural burst events. Synapse. 1996;22(3):195-208.
30. Huang X, Levine S, Paradiso MA. Rebounding V1 activity and a new visual aftereffect. J Vis. 2008;8(3):25 1-10.
31. Fuggetta G, Fiaschi A, Manganotti P. Modulation of cortical oscillatory activities induced by varying single-pulse transcranial magnetic stimulation intensity over the left primary motor area: a combined EEG and TMS study. Neuroimage. 2005;27(4):896-908.
32. Brignani D, Manganotti P, Rossini PM, Miniussi C. Modulation of cortical oscillatory activity during transcranial magnetic stimulation. Hum Brain Mapp. 2008;29(5):603-12.
33. Manganotti P, Formaggio E, Storti SF, De Massari D, Zamboni A, Bertoldo A, Fiaschi A, Toffolo GM. Time-frequency analysis of short-lasting modulation of EEG induced by intracortical and transcallosal paired TMS over motor areas. J Neurophysiol. 2012;107(9):2475-84.
34. Jooß A, Schmidt S, Haberbosch L, Köhn A, Scholz M, Brandt SA. Investigating the effects of noisy stimulation on the retinofugal pathway. Brain Stimulation. 2015;8(2):402.
35. Schmidt S, Scholz M, Obermayer K, Brandt SA. Patterned brain stimulation, what a framework with rhythmic and noisy components might tell us about recovery maximization. Front Hum Neurosci. 2013;7:325.
36. Wiesenfeld K, Moss F. Stochastic resonance and the benefits of noise: from ice ages to crayfish and SQUIDs. Nature. 1995;373(6509):33-6.

37. Gluckman BJ, Netoff TI, Neel EJ, Ditto WL, Spano ML, Schiff SJ. Stochastic resonance in a neuronal network from mammalian brain. Phys Rev Lett. 1996;77(19):4098-101.
38. Haberbosch L, Datta A, Thomas C, Jooß A, Köhn A, Rönnefarth M, Scholz M, Brandt SA, Schmidt S. Safety aspects, tolerability and modeling of retinofugal alternating current stimulation. Front Neurosci. 2019;13:783.
39. Haberbosch L, Schmidt S, Jooss A, Köhn A, Kozarzewski L, Rönnefarth M, Scholz M, Brandt SA. Rebound or entrainment? The influence of alternating current stimulation on individual alpha. Front Hum Neurosci. 2019;13:43.
40. Jooss A, Haberbosch L, Köhn A, Rönnefarth M, Bathe-Peters R, Kozarzewski L, Fleischmann R, Scholz M, Schmidt S, Brandt SA. Motor task-dependent dissociated effects of transcranial random noise stimulation in a finger-tapping task versus a Go/No-Go task on corticospinal excitability and task performance. Front Neurosci. 2019;13:161.
41. Iacono MI, Neufeld E, Akinnagbe E, Bower K, Wolf J, Vogiatzis Oikonomidis I, Sharma D, Lloyd B, Wilm BJ, Wyss M, Pruessmann KP, Jakab A, Makris N, Cohen ED, Kuster N, Kainz W, Angelone LM. MIDA: A Multimodal Imaging-Based Detailed Anatomical Model of the Human Head and Neck. PLoS One. 2015;10(4):e0124126.
42. Datta A. Inter-individual variation during transcranial direct current stimulation and normalization of dose using MRI-derived computational models. Frontiers in psychiatry. 2012;3:91.
43. Oostenveld R, Fries P, Maris E, Schoffelen JM. FieldTrip: Open source software for advanced analysis of MEG, EEG, and invasive electrophysiological data. Comput Intell Neurosci. 2011;2011:156869.
44. Bikson M, Grossman P, Thomas C, Zannou AL, Jiang J, Adnan T, Mourdoukoutas AP, Kronberg G, Truong D, Boggio P. Safety of transcranial direct current stimulation: evidence based update 2016. Brain stimulation. 2016;9(5):641-61.
45. Ma Z, Cao P, Sun P, Li L, Lu Y, Yan Y, Chen Y, Chai X. Optical imaging of visual cortical responses evoked by transcorneal electrical stimulation with different parameters. Invest Ophthalmol Vis Sci. 2014;55(8):5320-31.
46. Liebetanz D, Koch R, Mayenfels S, König F, Paulus W, Nitsche MA. Safety limits of cathodal transcranial direct current stimulation in rats. Clin Neurophysiol. 2009;120(6):1161-7.
47. Agnew WF, McCreery DB. Considerations for safety in the use of extracranial stimulation for motor evoked potentials. Neurosurgery. 1987;20(1):143.

48. Neuling T, Ruhnau P, Weisz N, Herrmann CS, Demarchi G. Faith and oscillations recovered: On analyzing EEG/MEG signals during tACS. Neuroimage. 2016.
49. Radman T, Ramos RL, Brumberg JC, Bikson M. Role of cortical cell type and morphology in subthreshold and suprathreshold uniform electric field stimulation in vitro. Brain Stimul. 2009;2(4):215-28, 28 e1-3.
50. Reato D, Rahman A, Bikson M, Parra LC. Low-intensity electrical stimulation affects network dynamics by modulating population rate and spike timing. J Neurosci. 2010;30(45):15067-79.
51. Destexhe A, Bal T, McCormick DA, Sejnowski TJ. Ionic mechanisms underlying synchronized oscillations and propagating waves in a model of ferret thalamic slices. J Neurophysiol. 1996;76(3):2049-70.
52. Birdno MJ, Tang W, Dostrovsky JO, Hutchison WD, Grill WM. Response of human thalamic neurons to high-frequency stimulation. PLoS One. 2014;9(5):e96026.
53. Sakata S. State-dependent and cell type-specific temporal processing in auditory thalamocortical circuit. Sci Rep. 2016;6:18873.
54. Hughes SW, Crunelli V. Thalamic mechanisms of EEG alpha rhythms and their pathological implications. Neuroscientist. 2005;11(4):357-72.
55. Paus T, Sipila PK, Strafella AP. Synchronization of neuronal activity in the human primary motor cortex by transcranial magnetic stimulation: an EEG study. J Neurophysiol. 2001;86(4):1983-90.
56. Yousif N, Denham M. The role of cortical feedback in the generation of the temporal receptive field responses of lateral geniculate nucleus neurons: a computational modelling study. Biol Cybern. 2007;97(4):269-77.
57. Pogosyan A, Gaynor LD, Eusebio A, Brown P. Boosting cortical activity at Beta-band frequencies slows movement in humans. Curr Biol. 2009;19(19):1637-41.
58. Joundi RA, Jenkinson N, Brittain JS, Aziz TZ, Brown P. Driving oscillatory activity in the human cortex enhances motor performance. Curr Biol. 2012;22(5):403-7.
59. Walker A, Woolf JI, Halstead WC, Case TJ. Photic driving. Arch Neurol Psychiatry. 1944;52(2):117.
60. Ozen S, Sirota A, Belluscio MA, Anastassiou CA, Stark E, Koch C, Buzsaki G. Transcranial electric stimulation entrains cortical neuronal populations in rats. J Neurosci. 2010;30(34):11476-85.

61. Lindenblatt G, Silny J. Electrical phosphenes: on the influence of conductivity inhomogeneities and small-scale structures of the orbita on the current density threshold of excitation. Med Biol Eng Comput. 2002;40(3):354-9.
62. Grützner A, Grüsser O-J, Baumgartner G. Reaktionen einzelner Neurone im optischen Cortex der Katze nach elektrischer Reizung des Nervus opticus. Arch Psychiatr Nervenkr. 1958;197(4):377-404.
63. Schutter DJ, Hortensius R. Retinal origin of phosphenes to transcranial alternating current stimulation. Clin Neurophysiol. 2010;121(7):1080-4.
64. Koeneke S, Lutz K, Herwig U, Ziemann U, Jäncke L. Extensive training of elementary finger tapping movements changes the pattern of motor cortex excitability. Exp Brain Res. 2006;174(2):199-209.
65. Hayward KS, Brauer SG, Ruddy KL, Lloyd D, Carson RG. Repetitive reaching training combined with transcranial Random Noise Stimulation in stroke survivors with chronic and severe arm paresis is feasible: a pilot, triple-blind, randomised case series. J Neuroeng Rehabil. 2017;14(1):1-9.
66. Looi CY, Lim J, Sella F, Lolliot S, Duta M, Avramenko AA, Kadosh RC. Transcranial random noise stimulation and cognitive training to improve learning and cognition of the atypically developing brain: a pilot study. Sci Rep. 2017;7(1):1-10.

8. Eidesstattliche Versicherung/Anteilserklärung

„Ich, Linus Haberbosch, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: „Schwellenmodulation als Mechanismus transkranieller elektrischer Hirnstimulation/Threshold modulation as a mechanism of transcranial electrical brain stimulation“ selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.
Alle Stellen, die wörtlich oder dem Sinne nach auf Publikationen oder Vorträgen anderer Autoren/innen beruhen, sind als solche in korrekter Zitierung kenntlich gemacht. Die Abschnitte zu Methodik (insbesondere praktische Arbeiten, Laborbestimmungen, statistische Aufarbeitung) und Resultaten (insbesondere Abbildungen, Graphiken und Tabellen) werden von mir verantwortet.

Ich versichere ferner, dass ich die in Zusammenarbeit mit anderen Personen generierten Daten, Datenauswertungen und Schlussfolgerungen korrekt gekennzeichnet und meinen eigenen Beitrag sowie die Beiträge anderer Personen korrekt kenntlich gemacht habe (siehe Anteilserklärung). Texte oder

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Meine Anteile an etwaigen Publikationen zu dieser Dissertation entsprechen denen, die in der untenstehenden gemeinsamen Erklärung mit dem Erstbetreuer, angegeben sind. Für sämtliche im Rahmen der Dissertation entstandenen Publikationen wurden die Richtlinien des ICMJE (International Committee of Medical Journal Editors; www.icmje.og) zur Autorenschaft eingehalten. Ich erkläre ferner, dass ich mich zur Einhaltung der Satzung der Charité – Universitätsmedizin Berlin zur Sicherung Guter Wissenschaftlicher Praxis verpflichte.

Weiterhin versichere ich, dass ich diese Dissertation weder in gleicher noch in ähnlicher Form bereits an einer anderen Fakultät eingereicht habe.

Die Bedeutung dieser eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unwahren eidesstattlichen Versicherung (§§156, 161 des Strafgesetzbuches) sind mir bekannt und bewusst.“

Datum Unterschrift

Anteilserklärung an den erfolgten Publikationen

Linus Haberbosch hatte folgenden Anteil an den folgenden Publikationen:

Publikation 1: Haberbosch, L, Datta, A, Thomas, C, Jooß, A, Köhn, A, Rönnefarth, M, Scholz M, Brandt SA & Schmidt, S. Safety Aspects, Tolerability and Modeling of Retinofugal Alternating Current Stimulation. *Frontiers in Neuroscience 2019*, *13*:783.

Beitrag im Einzelnen: Die Studienidee und das Studiendesign wurden durch L. Haberbosch, Dr. A. Datta, Dr. S. Schmidt, Dr. M. Scholz und Prof. Dr. S.A. Brandt ausgearbeitet. Die Studiendurchführung mit Probandenrekrutierung und -untersuchung, sowie die Datenverarbeitung und -analyse erfolgten durch L. Haberbosch, Dr. S. Schmidt, A. Jooss und A. Köhn. Die finale Datenauswertung sowie statistische Analyse erfolgte durch L. Haberbosch. Die Berechnung des Finite-Elemente-Modelings erfolgte durch Dr. A. Datta und C. Thomas. Die Erstellung von Figure 1 und Table 1 erfolgte durch L. Haberbosch, Dr. A. Datta und C. Thomas. Alle weiteren Graphiken und Tabellen wurden durch L. Haberbosch generiert. Die Interpretation, Diskussion und Einordnung der Ergebnisse erfolgten gemeinsam mit allen Koautoren. Ein erster Entwurf des Manuskripts wurde durch L. Haberbosch und Dr. S. Schmidt verfasst. Revisionen des Manuskripts erfolgten durch Dr. A. Datta, A. Jooss, A. Köhn, Dr. M. Rönnefarth, Dr. M. Scholz und Prof. Dr. S.A. Brandt. Die finale Version des Manuskripts wurde vor Einreichung durch alle Autoren revidiert und in der publizierten Form akzeptiert.

Publikation 2: Haberbosch L, Schmidt S, Jooss A, Kohn A, Kozarzewski L, Ronnefarth M, Scholz M, Brandt SA. Rebound or Entrainment? The Influence of Alternating Current Stimulation on Individual Alpha. Frontiers in Human Neuroscience 2019;13:43.

Beitrag im Einzelnen: Die Studienidee und das Studiendesign wurden durch L. Haberbosch, Dr. S. Schmidt, Dr. M. Scholz und Prof. Dr. S.A. Brandt ausgearbeitet. Die Studiendurchführung mit Probandenrekrutierung und -untersuchung, sowie die Datenverarbeitung und initiale Analyse erfolgten durch L. Haberbosch, Dr. S. Schmidt, A. Jooß und L. Kozarzewski. Alle Graphiken wurden durch L. Haberbosch erstellt. Die Interpretation, Diskussion und Einordnung der Ergebnisse erfolgten gemeinsam mit allen Koautoren. Ein erster Entwurf des Manuskripts wurde durch L. Haberbosch und Dr. S. Schmidt verfasst. Revisionen des Manuskripts erfolgten durch A. Jooß, A. Köhn, L. Kozarzewski, M. Rönnefarth, Dr. M. Scholz und Prof. Dr. S.A. Brandt. Die finale Version des Manuskripts wurde vor Einreichung durch alle Autoren revidiert und in der publizierten Form akzeptiert.

Publikation 3: Jooss A, Haberbosch L, Köhn A, Rönnefarth M, Bathe-Peters R, Kozarzewski L, Fleischmann R, Scholz M, Schmidt S, Brandt SA. Motor Task-Dependent Dissociated Effects of Transcranial Random Noise Stimulation in a Finger-Tapping Task Versus a Go/No-Go Task on Corticospinal Excitability and Task Performance. Frontiers in Neuroscience. 2019;13:161.

Beitrag im Einzelnen: Die Umsetzung der Studienidee und Erarbeitung des Studiendesigns erfolgte führend durch A. Jooß mit Unterstützung von L. Haberbosch, A. Köhn, Dr. R. Fleischmann und M. Rönnefarth nach Konzeption durch Dr. S. Schmidt, Dr. M. Scholz und Prof. Dr. S.A. Brandt. Die Durchführung der Studie oblag A. Jooß und L. Haberbosch und inkludierte Probandenrekrutierung und -untersuchung, sowie eine erste Datenverarbeitung. Die weitere Datenverwaltung und -auswertung erfolgte durch A. Jooß durch Programmierung einer MATLAB-basierten Software. Die statistische Auswertung erfolgte durch A. Jooß in der Statistiksoftware SPSS mit nachfolgender Validierung durch das Institut für Biometrie und Klinische Epidemiologie der Charité. Die Interpretation, Diskussion und Einordnung der Ergebnisse erfolgten gemeinsam

mit allen Koautoren. Das Manuskript und die darin enthaltenen Graphiken wurden von A. Jooß erstellt und durch die Koautoren auf Konsistenz, Verständlichkeit und Fehlerfreiheit überprüft. Die Korrespondenz mit der Fachzeitschrift und Überarbeitungen im Rahmen des Review-Prozesses erfolgten durch A. Jooß nach kritischer Diskussion mit Dr. Sein Schmidt und Prof. Dr. S.A. Brandt. Alle Autoren revidierten und akzeptierten das Manuskript in der publizierten Form.

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9. Druckexemplare der ausgewählten Publikationen

Publikation 1: Haberbosch, L, Datta, A, Thomas, C, Jooß, A, Köhn, A, Rönnefarth, M, Scholz M, Brandt SA & Schmidt, S. Safety Aspects, Tolerability and Modeling of Retinofugal Alternating Current Stimulation. *Frontiers in Neuroscience 2019*, *13*:783.

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Safety Aspects, Tolerability and Modeling of Retinofugal Alternating Current Stimulation

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Background: While alternating current stimulation (ACS) is gaining relevance as a tool in research and approaching clinical applications, its mechanisms of action remain unclear. A review by Schutter and colleagues argues for a retinal origin of transcranial ACS' neuromodulatory effects. Interestingly, there is an alternative application form of ACS specifically targeting α-oscillations in the visual cortex via periorbital electrodes (retinofugal alternating current stimulation, rACS). To further compare these two methods and investigate retinal effects of ACS, we first aim to establish the safety and tolerability of rACS.

Objective: The goal of our research was to evaluate the safety of rACS via finite-element modeling, theoretical safety limits and subjective report.

Methods: 20 healthy subjects were stimulated with rACS as well as photic stimulation and reported adverse events following stimulation. We analyzed stimulation parameters at electrode level as well as distributed metric estimates from an ultra-high spatial resolution magnetic resonance imaging (MRI)-derived finite element human head model and compared them to existing safety limits.

Results: Topographical modeling revealed the highest current densities in the anterior visual pathway, particularly retina and optic nerve. Stimulation parameters and finite element modeling estimates of rACS were found to be well below existing safety limits. No serious adverse events occurred.

Conclusion: Our findings are in line with existing safety guidelines for retinal and neural damage and establish the tolerability and feasibility of rACS. In comparison to tACS, retinofugal stimulation of the visual cortex provides an anatomically circumscribed model to systematically study the mechanisms of action of ACS.

Keywords: retinofugal alternating current stimulation, electrical stimulation, feasibility, tolerability, safety, adverse events, finite element modeling

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Abbreviations: EF, electrical field; NiBS: non-invasive brain stimulation; NRS, numeric rating scale; PS, photic stimulation; rACS: retinofugal alternating current stimulation; RPE, retinal pigment epithelium; tACS, transcranial alternating current stimulation.

INTRODUCTION

Non-invasive brain stimulation (NiBS) is an effective method for research, as well as a promising tool for therapy in cognitive and clinical neuroscience (Paulus, 2003; Hallett, 2007; Liew et al., 2014). Its effects range from direct brief modification of neural activity to long lasting recovery maximization following neural injury (Hallett, 2005; Talelli and Rothwell, 2006; Hummel et al., 2008; Sandrini and Cohen, 2013). Recently, transcranial alternating current stimulation (tACS), characterized by oscillatory low-voltage stimulation, showed promising effects on the motor system (Feurra et al., 2011, 2013), motor performance (Pogosyan et al., 2009; Joundi et al., 2012), memory (Marshall et al., 2006; Polania et al., 2012), higher order cognition (Santarnecchi et al., 2013, 2016) and tremor (Brittain et al., 2013). Despite these encouraging results, tACS' mechanisms of action remain unclear (Zaghi et al., 2010) and a retinal contribution to its effects on neural synchrony is still being discussed (Schutter, 2016).

Retinofugal alternating current stimulation (rACS) is a comparably novel form of alternating current stimulation (ACS). In contrast to tACS, rACS is characterized by transmission along retinofugal tracts terminating predominantly in cortical visual areas and neuromodulation of central rhythms (Gall et al., 2011; Schmidt et al., 2013a). While differing from other forms of NiBS in regard to stimulation site, rACS shares its use of alternating current and effects on the intrinsic frequencies of the visual system with tACS (Schmidt et al., 2013a; Haberbosch et al., 2019). Moreover, in comparison to other forms of NiBS (namely, most types of tES) with diffusely induced electric fields (EF) throughout large parts of the brain (Peterchev et al., 2012), rACS affects the well-defined retinofugal pathway (Rager and Singer, 1998) for stimulation confined to the visual system. Thus, rACS renders a unique means to study mechanisms underlying NiBS as it physiologically affects the circumscribed primary visual cortex with separate input from each eye.

Before any novel method can be employed to its full potential or compared with other methodologies, establishing its safety and tolerability is critically important (Bath et al., 2014). The lack of knowledge of safety parameters could culminate in ineffective or even hazardous use (Antal et al., 2008; Bath et al., 2014). While ineffective stimulation could lead to incoherent findings regarding stimulation effects, effective but hazardous use could possibly result in severe adverse events and lasting damages in stimulation subjects. As rACS is used for research purposes, its safety as well as tolerability has to be determined rigorously.

Refraining from potentially dangerous invasive measures, the safety of a novel NiBS montage should be assessed in several different ways.

Firstly, stimulation parameters can be compared to theoretical safety limits as established for NiBS and neural tissue damage in animal studies (Agnew and McCreery, 1987; Liebetanz et al., 2009; Jackson et al., 2017), which have since been used to assess NiBS safety in human studies (Poreisz et al., 2007; Bikson et al., 2009, 2016). The primarily employed metrics include current density (A/m^2) and charge density (C/m^2), although other parameters such as charge per phase (C/ph) have been proposed to account for the shifting polarity of AC stimulation (Nitsche et al., 2003; Merrill et al., 2005).

Secondly, these safety metrics can be modeled onto CNS structures (Datta et al., 2011; Bikson et al., 2016), to determine the possibility of damage at critical locations (Bikson et al., 2016) while accounting for anatomy and electrode position (Bikson et al., 2009; Bikson and Datta, 2012; Peterchev et al., 2012; Saturnino et al., 2015).

Finally, experimental validation of theoretical results by subjective reports of adverse events with validated questionnaires can be acquired (Brunoni et al., 2011). These reports are also instrumental in assessing the tolerability of the novel method.

In this study we hypothesized that rACS is to be considered safe if: (1) Stimulation parameters (current and charge densities at the electrode) are within theoretical safety limits, (2) finite element modeling data shows the same for EF estimates and current densities at eye, retina and cortex, and (3) adverse events do not exceed that of other established stimulation methods in rate as well as severity.

To address the primary hypothesis, the stimulation parameters of rACS were recorded during stimulation and employed for the calculation of safety limits. Ultra-high resolution topographical finite element modeling was performed to identify regions of critical interest and to calculate theoretical safety parameters. Adverse events were identified with an extended adverse events questionnaire developed for tDCS (Brunoni et al., 2011). For direct experimental comparison, we employed simple and safe photic stimulation (PS) (Walker et al., 1944) as the gold-standard method for stimulation of the retinofugal pathway regarding safety and clinical experience (Cobb, 1947; Trenite et al., 1999).

MATERIALS AND METHODS

To address the safety profile of rACS, we observed and questioned 20 test subjects during rACS and PS sessions. We assessed cutaneous, retinal and central adverse events and drew a comparison between PS and rACS.

Participants

We stimulated 20 healthy volunteers (10 men), mean age 25.9 ± 4.95, as part of a study investigating a common framework of action for NiBS. The subjects were interviewed prior to experimentation regarding their state of health. We applied established exclusion criteria for NiBS (Brunoni et al., 2012) and added evidence for photophobia and photosensitive epilepsy. Written informed consent was obtained from all individual participants included in the study. The subjects received financial compensation for their participation. All procedures were performed in accordance with the ethical standards of the Ethics Committee of the Charité – Universitätsmedizin Berlin ("Ethikkommission der Charité – Universitätsmedizin Berlin") and with the 1964 Declaration of Helsinki and its later amendments. This study adheresto the principles of good scientific practice of the Charité – Universitätsmedizin Berlin ("Grundsätze der Charité zur Sicherung guter wissenschaftlicher Praxis").

Retinofugal Alternating Current Stimulation (rACS)

Retinofugal alternating current stimulation was applied via a multi-channel low-voltage stimulation device certified for clinical use, which delivered weak oscillatory current sinus-pulses over four individual periorbital electrodes, respectively (NextWave, Eyetronic, Germany). The four superficial active stimulating electrodes (Grass SAFELEAD^{TM} gold electrodes, Astro-Med, Inc., RI, United States) were contained in foam-padded stimulation goggles and bilaterally made skin contact via small felt buffers (0.35 cm^2) superior and inferior to the eye. The return electrode (rectangular electrode, 30 × 30 mm polished stainless steel) was fastened on the back of the neck at the midline.

Alternating current was applied at 10 Hz, as ACS has shown robust effects at this frequency (Kanai et al., 2008; Helfrich et al., 2014; Vossen et al., 2015) and gold standard PS typically also employs 10 Hz stimulation (Photic driving) (Walker et al., 1944). Stimulation amplitude was set to 120% phosphene threshold (resulting in 351.69 μA (SD 63.95) peak-to-peak amplitude). The phosphene threshold was determined employing an ascending method of limits (Herrick, 1967) provided by the NextWave software. rACS was delivered in 30 s blocks followed by 30 s pauses over 10 min. The subjects were told to keep their eyes open and focus a fixed point on a white surface in 1 m distance for the duration of the experiment.

To assess the safety parameters of stimulation we additionally calculated the effective amplitude. The effective amplitude of the applied current is defined as the time normed integral of the signal, which simplifies to its mean value for discrete signals as is the case here, since the used stimulator receives an equidistant sampled discrete function as input. In the simplest case of a pure sine wave this simplifies to the following formula:

$$a(eff) = \frac{a_{\max}}{\sqrt{2}}$$

In the case of more complex stimuli such as noise + sine wave or signals with an additional amplitude modulation, the use of peak- to-peak "a(max)" values to describe the resulting electrical powerof an electric current stimulation would be misleading.

Regarding charge, we decided to refrain from more complex line integral calculations, and instead used the following simple formula:

$$Q = I * t$$

This was done to ensure straightforward comparability of resulting values. It also adds to the rigidity of our safety considerations by rather over-than underestimating the injected charge.

Photic Stimulation

Photic Stimulation was applied via two 3 × 5 cm multi-color white LEDs contained in the stimulation goggles, which work via red, green and blue LEDs mixing their emissions to form white light. To be able to compare stimulation intensities with rACS, sinusoidal pulses of white light were applied at an intensity of 120% light threshold and with a frequency of 10 Hz. This threshold was also determined by an ascending method of limits and resulted in an average luminous intensity of 1.24 cd (±0.44) for stimulation.

The stimulation was also delivered in 30 s blocks followed by 30 s pauses over 10 min, and the subjects received the same instructions as for rACS.

Modeling

The ultra-high resolution head and neck model (MIDA: Multimodal Imaging-Based Detailed Anatomical Model) available through the IT'IS Foundation was used in this study (Iacono et al., 2015). The nifti (.nii) color masks from the MIDA model were first processed in MATLAB to re-create segmentation masks based on intensity values. These masks were then imported into Simpleware (SynopsysLtd., CA, United States) and any errors in continuity andanatomical details were manually corrected for Datta et al. (2012). Masks with similar electrical conductivities were then merged to a single compartment barring the regions of interest (eye structures) in order to perform individual current flow analysis through them. For instance, mandible, teeth, vertebra, skull dipole, skull inner table, skull outer table, hyoid bone were combined with the skull mask but eye retina, choroid, and sclera were treated as individual masks.

The stimulation electrodes were created as CAD files mimicking the exact physical geometry and dimensions of the electrodes used in the experiments. The electrodes were positioned interactively within the image data simulating the electrode montage used for rACS (see **Figure 1C**). The adaptive meshes derived from the segmentation masks were then imported into COMSOL Multiphysics (Burlington, MA, United States) for finite element computation. The final model comprised >10 million elements with >15 million degrees of freedom.

The representative isotropic average electrical conductivities assigned to the different tissue compartments and the electrode materials (in S/m) are listed in **Table 1**.

The Laplace equation was solved and current densities corresponding to 350 μA total current were applied at the anode or active electrode (s). Ground was applied at the return electrode and all other external surfaces were treated as insulated. The linear iterative solver of conjugate gradients with a relative tolerance of 1E-6 was used.

Surface as well as cross-sectional EF magnitude maps on the gray matter, retina, and the optic nerve were obtained. For the scalp, the surface current density magnitude plot was obtained.

Questionnaire

The questionnaire we employed is based on the one proposed by Brunoni et al. (2011) and investigated the presence of headaches, difficulties in concentrating, acute mood changes, visual perceptual changes, fatigue and discomforting sensations tingling, itching and/or burning under the electrodes duringand after rACS, as well as PS. The item "Difficulties in concentrating" was defined in accordance with Montgomery and Asberg (1979), while the item "Fatigue" was defined in accordance with Chaudhuri and Behan (2004).

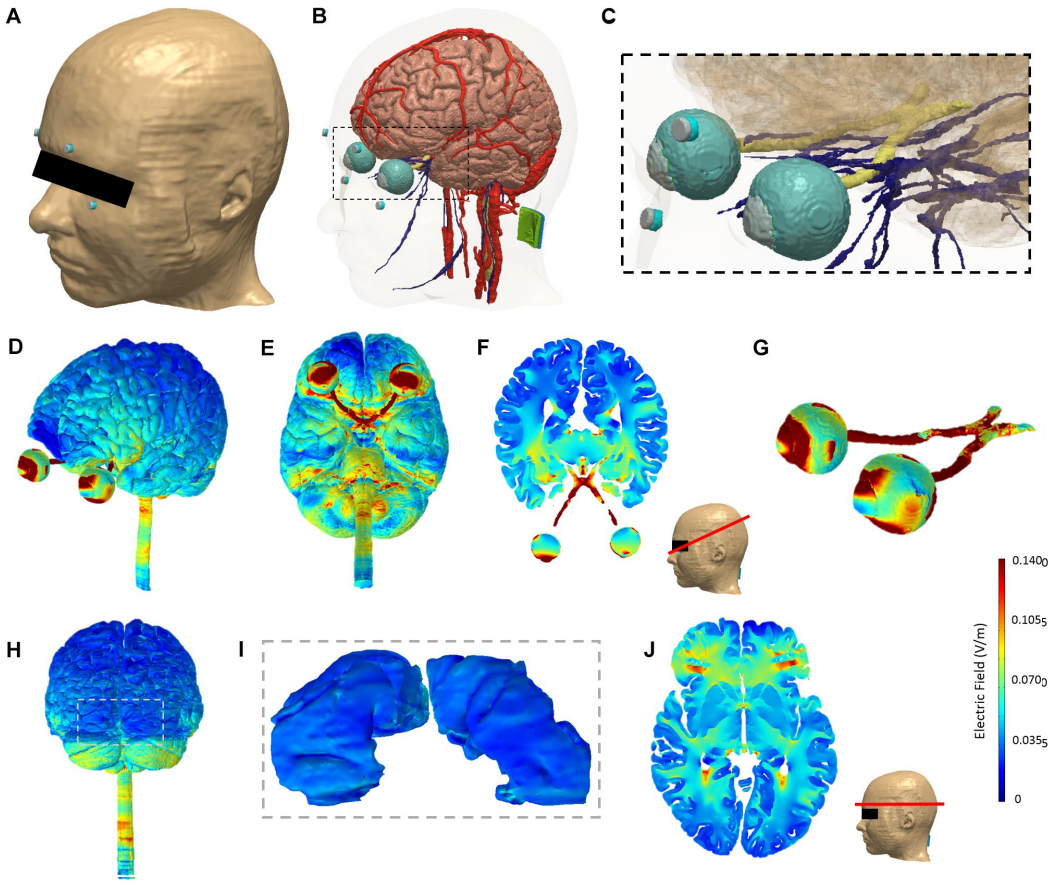

FIGURE 1 | Model segmentation and finite element analysis. The ultra-high resolution MIDA model was adapted for analysis in this study. (A) Skin tissue mask with periorbital electrodes (gray: electrode; blue: sponge). (B) The modeled brain, cranial nerves, blood vessels, eye structure, optic nerves, and electrodes (both active periorbital and the return inion electrode shown). (C) Zoomed view corresponding to the dashed section in panel (B) highlighting segmentation detail in the region of interest. Finite element analysis of current flow produced by rACS: Induced electric field magnitude plots on the cortical and eye level perspective (D) and bottom view (E). A representative axial 2D cross-section view of electric field magnitude following the retinofugal tract was chosen and plotted (F). Panel (G) shows the induced electric field on the eyes and optic nerve. Panel (H) shows the rear view. Panel (I) shows the primary visual cortex (V1) corresponding to the dashed section in panel (H). A representative 2D axial cross-section view of electric field magnitude taken at the level of half of the visual cortex along the superior–inferior plane is shown in panel (J).

We modified the questionnaire by adding a description of phosphenes. Furthermore, to assess the overall tolerability, we defined the broad category of "Pain" as a summary of all discomforting sensations mentioned above and added a Numeric Rating Scale (NRS-11, 11 stages from 0 to 10, 10 being the strongest imaginable pain and 0 the absence of pain) (Farrar et al., 2001) as a more in-depth and reliable measurement (Downie et al., 1978). We discarded the four-point intensity rating for the other categories to avoid a "halo effect" bias (Streiner and Norman, 2008). We assumed that the foreign body feeling reported for physiologically similar transcorneal electrical stimulation (TCES) came from the electrode lying directly on the cornea (Gekeler et al., 2006) and therefore decided not to include it.

Three months after stimulation, the subjects received a second questionnaire to identify late and longer lasting after-effects.

As the data is not normally distributed and equal variance of residuals cannot be assumed, the severity of pain was analyzed in Wilcoxon Signed Ranks Tests for paired samples. The nominally scaled side effects were analyzed in Fishers Exact Tests, as expected values in several of the cells of a contingency table are below the recommended threshold for a classical Chi-Squared Test (Larntz, 1978). *P*-values of <0.05 were considered significant. All analyses were performed using IBM SPSS Statistics, Version 19.0.0.1 (IBM, United States).

RESULTS

Stimulation Parameters

An average 10 Hz phosphene threshold at 290.50 μV (SD 45.36), impedances at 12.05 k□ (SD 2.89), and an average amplitude of 351.69 μA (SD 63.95) were noted. Calculated from peak-to-peak amplitude, the current density at electrode level amounted to a mean 1.00 mA/cm^2 (SD 0.28), and the charge density to 0.60 C/cm^2 (SD 0.11). As sine waves pulses were employed,

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TABLE 1 | Assigned electrical conductivities.

Tissue compartment/Electrode material	Electrical conductivity (S/m)
Scalp	0.465
Muscle	0.35
Skull	0.01
CSF	1.65
Gray Matter	0.276
White Matter	0.126
Fat	0.04
Blood vessels	0.7
Eye Lens	0.32
Eye Retina/Choroid/Sclera	0.623
Eye Vitreous	1.55
Eye Cornea	0.5
Eye Aqueous	1.5
Optic Tract/Optic Chiasm/Cranial Nerve II	0.126
Air	1.00E−0
Sponge (felt buffer)	7
Periorbital electrode (gold electrodes)	4.10E+0
Inion electrode (stainless steel)	7 1.45E+06

The representative isotropic average electrical conductivities assigned to the different tissue compartments and the electrode materials (in S/m).

we additionally calculated the effective amplitude, resulting in a mean 248.68 μA (SD 47.0). Using effective amplitude, current density amounted to a mean 0.71 mA/cm^2 (SD 0.13), and the charge density to 0.42 C/cm^2 (SD 0.08). RACS was found to be well within safety limits and the findings comparable to other similar stimulation methods (see **Table 2**). Regarding stimulation amplitude, rACS (0.35 mA) was comparable to most TCES and tES montages (ranging from 0.08 to 1.2 mA). Electrosleep and the maximum intensity stimulation employed by Gekeler et al. (2006) were found to employ higher amplitudes (3–25 mA). The stimulated area (0.35 cm^2) is smaller than most tES montages (16–35 cm^2), comparable only to Electrosleep and TCES (0.35–1.25 cm^2). Regarding stimulation frequencies, rACS was compared to studies using similar frequencies (10–20 Hz), with the exceptions of Electrosleep, which is set at higher frequencies (100 Hz) as well as the non-oscillating tDCS and Gekeler's TCES. The calculations following these observations place the charge density of rACS just above the TCES of Ma et al. (2014) and far below the safety limit published by Liebetanz et al. (2009). This is consistent for charge per phase and charge density per phase. Regarding current density, rACS (1 mA/cm^2) ranks below Ma (1.2 mA/cm^2), well below the maximum intensity employed by Gekeler (8.57 mA/cm^2) and far below the safety limit proposed by McCreery (25 mA/cm^2). These findings are even more pronounced when using effective amplitude.

Finite Element Modeling

The EF distributed by rACS is strongest at the eye level,with the highest current density estimates at the retina. Further areas of elevated current densities are optic nerve and cortex (**Figures 1A,B**).

The calculated maximum current density at the retina amounted to a maximum of 1.24 A/m^2, while optic nerve (0.33 A/m^2) and cortex (0.13 A/m^2) were both subjected to less current flow. Regarding the EF, we estimated a maximum of 2.6 V/m in the optic nerve, followed by 1.99 V/m for the retina and 0.47 V/m for the cortex. Finally, current density at skin level underneath the active electrode amounted to a maximum induced value of 14.79 A/m^2 (**Figure 1C**), with the EF estimated at 31.80 V/m. It should be noted that due to edge effects, the observed values are higher than the current density toward the middle of the electrode which is simply the current injected over the contact area. For a detailed view, see **Table 3**.

TABLE 2 | Comparison of stimulation parameters.

	Amplitud	Area	Duratio	Frequency	Current density	Charge density	Charge per phase	CD per phase
	(mA)	(cm^2)	(min	(Hz)	(mA/cm^2)	(C/cm^2)	(C/ph)	(C/(cm^2∗ph))
Safety limits (Agnew and McCreery, 1987)	–	–	–	–	25	–	–	0.000400
Safety limits (Liebetanz et al., 2009)	0.5	0.035	10	–	14.29	85.71	–	–
rACS	0.35	0.35	10	10	1	4 (52.400	0.000035	0.0001
rACS (effective amplitude)	0.25	0.35	10	10	0.71	)	0.000025	0.000071
Electrosleep (Sergeev, 1963)	25	1.25	60	100	20	0.599	0.00025	0.0002
TCES (Ma et al., 2014)	1.2	1	5	20	1.2	0.423	0.00006	0.00006
TCES (Delbeke et al., 2001)	0.28	1.25	7	10	0.22	72	0.000028	0.000022
TCES (Gekeler et al., 2006) max	3	0.35	7	–	8.57	0.36	–	–
TCES (Gekeler et al., 2006) optimal	0.08	0.35	7	–	0.22	0.094	–	–
tACS (Antal et al., 2008)	0.4	16	5	1	0.03	–	0.0000	0.000003
tSDCS (Paulus et al., 2013)	0.25	16	4	0	0.02	–	4	0.000002
tDCS (Nitsche et al., 2003)	1	35	9	10	0.03	0.00	0.000025	–

Current density measures at electrode level employed for stimulation types employing continuous current (TCES (Gekeler) and tDCS), charge density, charge per phase and charge density (CD) per phase for oscillatory stimulation (rACS, Electrosleep, TCES (Ma and Delbeke), tACS and tSDCS).

TABLE 3 | Modeling data and comparison to safety limits.

		Current density	Electric field
		(mA/cm^2)	(V/m)
Safety limits	Liebetanz et al., 2009	14.29	42
rACS (retina)	Max	0.124	1.99
	Mean	0.007	
	Median	0.005	0.11
rACS (optic nerve)	Max	0.033	0.08
	Mean	0.003	
	Median	0.002	2.6
rACS (cortex)	Max	0.013	0.2
	Mean	0.001	0.14
	Median	0.001	
rACS (V1)	Max	0.003	0.47
	Mean	0.001	
	Median	0.001	0.05

This table presents the finite element modeling results for rACS: Current density and electric field estimates for retina, optic nerve, cortex and specifically the primary visual cortex (V1) in comparison to reported safety limits.

TABLE 4 | Adverse events for rACS and PS.

		rtACS		Photic Stim	
		n	%	*n*	%
Pain (overall)	During	8	40	4	20
	After	0	0	2	10
Fatigue	During	7	35	4	20
	After	7	35	4	20
Tingling	During	14	70	0	0
	After	0	0	0	0
Headache	During	0	0	3	15
	After	1	5	1	5
Itching	During	6	30	0	0
	After	5	25	0	0
Burning	During	6	30	0	0
	After	0	0	0	0
Difficulties in Concentrating	During	0	0	0	0
	After	0	0	2	10
Metallic Taste	During	3	15	0	0
	After	0	0	0	0
Muscle twitches	During	3	15	0	0
	After	0	0	0	0
Acute mood changes	During	0	0	0	0
	After	0	0	0	0
Nausea	During	0	0	0	0
	After	0	0	0	0

Reports of adverse events during and after stimulation in subjects and percent (data rounded to integers).

Adverse Events

Table 4 summarizes the adverse events in the 20 rACS and PS sessions in healthy participants. None of the subjects requested the stimulation to be terminated or required medical attention. In their subjective reports, rACS associated adverse events were predominant during stimulation, and PS associated adverse events were predominant following stimulation. More explicitly, a tingling sensation occurred in 70% of the subjects during but not after rACS. An itching sensation under the electrodes was reported by 30% of the subjects during rACS and 25% after rACS. A burning sensation was felt by 30% of the participants during but not after rACS. Fatigue occurred during, as well as after, stimulation in 35 and 20% of the rACS and PS group, respectively. Headaches were reported only by PS participants during stimulation (15%). After stimulation, it was reported by 5% for both PS, as well as rACS participants. Difficulties in concentrating were reported by 10% of the participants after PS, but not after rACS. There were no cases of acute mood changes, nausea and visual perceptual changes or lasting adverse events 3 months after stimulation.

Pain

Forty-percent of the subjects reported pain (**Figure 2A**) during rACS (mean intensity 2.5, SD 1.73) and 20% during PS (2.75, SD 0.83). While none of the participants reported pain after rACS, this was the case for 10% after PS (1.5, SD 0.5).

rACS vs. Photic Stimulation

In the statistical analyses, rACS and PS showed no significant effect of stimulation type (rACS versus PS) on pain intensity as assessed by Wilcoxon Signed Ranks Tests (**Figure 2A**), fatigue, headache and difficulties in concentrating as assessed by Fisher's Exact Tests (**Figure 2B**) during as well as after stimulation. PS and rACS significantly differed regarding skin sensations of tingling, itching and burning ($P < 0.05$, Fisher's Exact Tests), which all occurred exclusively in rACS. For a more detailed view, see **Tables 5**, **6**. The full dataset behind this comparison is available as **Supplementary Material**.

DISCUSSION

To address the safety profile of rACS, we assessed theoretical safety limits as well as finite-element modeling data and compared the reported adverse events for rACS and simple PS.

The primary findings are that rACS is safe based on the following observations: (1) stimulation parameters (current and charge densities at the electrode) are within theoretical safety limits, (2) finite element modeling data shows the same for EF estimates and current densities at eye, retina and cortex, and (3) adverse events are comparable to PS in direct experimental comparison (see **Tables 3**, **4**) and rate as well as severity of adverse events did not exceed that of other established brain stimulation methods (see **Table 2**).

Stimulation Parameters

To be efficacious and safe, a stimulation system must stimulate neural tissue without damaging tissue or electrode. Tissue damage is caused when excitable tissue is overstimulatedand electrode damage ensues as metal oxidation occurs

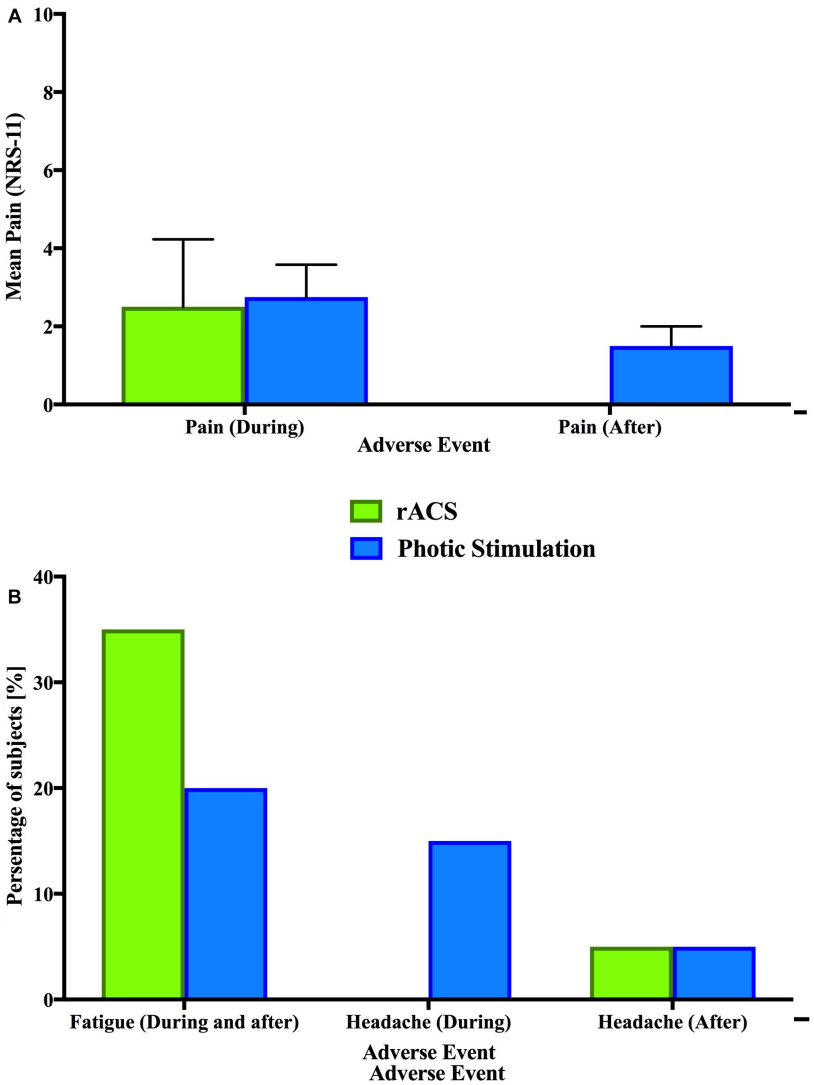

FIGURE 2 | Adverse events. A comparison of adverse events between rACS (green) and PS (blue). None of the depicted differences were significant in Bonferroni-corrected multiple comparisons. (A) Depicted is the mean rating (NRS-11) of overall pain and discomfort in affected subjects during and after stimulation. Error bars represent the standard deviation. (B) Comparison of shared adverse events (fatigue and headache) in percentage of subjects.

(Peterchev et al., 2012). Current density and charge density have been proposed as predictors for such damage (Bikson et al., 2016).

Current Density

Current density is the proposed optimal safety parameter for a constant current stimulation (Nitsche et al., 2003) and can be derived from the effective amplitude and compared to safety limits (Agnew and McCreery, 1987) as well as other similar stimulation paradigms (Gekeler et al., 2006; Ma et al., 2014).

We find that rACS current densities are within reported safety limits for tissue damage (Yuen et al., 1981; Lindenblatt and Silny, 2002; Liebetanz et al., 2009; Gellner et al., 2016).

TABLE 5 | Results of the Wilcoxon-signed ranks tests.

Source			Z	Asymp. Sig. (2-tailed)
Stimtype (rACS vs. PS)	Pain (overall)	During	–0.987[a]	0.323
		After	–1.342[b]	0.180

The results of the statistical analysis on overall pain during and after stimulation. [a]*Based on positive ranks.* [b]*Based on negative ranks.*

Charge Density and Charge per Phase

While current density is a well-established safety parameter, it is best suited for assessing the safety of constant current stimulation. ACS injects less charge than constant current stimulation of the same amplitude (Liebetanz et al., 2009; Schmidt et al., 2013a), dependent on stimulation frequency and duty cycle (Chaieb et al., 2014). The safety limits of charge balanced ACS, such as rACS, are therefore more precisely

TABLE 6 | Results of the Fisher's exact tests.

Source			Exact Sig. (2-tailed)
Stimtype (rACS vs. PS)	Fatigue	During	0.480
		After	0.480
	Difficulties in Concentrating	During	–
		After	0.487
	Headache	During	0.231
		After	1.000
	Itching	During	0.020 *
		After	
	Burning	During	0.047 *
		After	
	Tingling	During	0.020 *
		After	–

The results of the statistical analysis on the nominally scaled adverse events presented in Table 4. The asterisk (*) marks significant results.

determined by charge density and charge per phase (Nitsche et al., 2003; Merrill et al., 2005).

We find that rACS charge densities are also within reported safety limits for tissue damage (Yuen et al., 1981; Lindenblatt and Silny, 2002; Liebetanz et al., 2009; Gellner et al., 2016; Jackson et al., 2017).

Comparison to Other Stimulation Types

While stimulating at higher current and charge densities than most forms of tES, rACS stimulation parameters proved comparable to dose parameters reported for TCES using up to 10 mA per pulse to establish safety guidelines (Gekeler et al., 2011), well below early montage parameters for both stronger and longer stimulation used in early studies addressing Electrosleep therapy (Robinovitch, 1914; Knutson, 1967; Peterchev et al., 2012), and well below current densities reported for stimulation via implanted self-sizing spiral cuff electrodes in blind patients over the course of several years (Delbeke, 2011) (see **Table 2**).

Despite arguable differences between different stimulation techniques, there are remarkable similarities, e.g., comparably distant periorbital montage of electrodes, as well as modeling results for the serial resistance of the skin and eyelid (Delbeke et al., 2000; Merrill et al., 2005; Gekeler et al., 2006) to motivate this comparison.

This leads to the conclusion that the employed charge injection was safe with regards to possible tissue as well as electrode damage. In the future, studies addressing the calculation of rheobase and chronaxie and stimulation with variable pulse parameters might help to further reduce charge injection to the minimum necessary to efficaciously achieve a neuronal response (Irnich, 1980, 2010; Delbeke et al., 2001).

Finite Element Modeling

Electric Field Distribution

Expectedly, the EF distribution shows a clear focus on retina and optic nerve, while the cortical electric current flow is much weaker. Due to the electrode montage being superior–inferior, we see stronger EFs in the temporal regions and at the return electrode. While there is increased flow through the subcortical structures, brain stem and cerebellum, there appears to be no strong current flow to occipital areas, with a maximum current density of 0.033 A/m^2 and a maximum EF of 0.1208 V/m (**Table 3** and **Figure 1**).

This confirms the retinofugal pathway as the primary target of rACS. Still, stimulation intensity should be closely monitored, as strong over-threshold stimulation might lead to unwanted effects on subcortical structures.

Current Densities and EF Estimates

Evidence from relevant animal models indicates that brain injury by tDCS occurs at predicted brain current densities (14.9 A/m^2) (Liebetanz et al., 2009; Gellner et al., 2016; Jackson et al., 2017). Considering the well-established threshold proposed by Liebetanz et al. (2009), rACS maximum current densities rank two orders of magnitude (OOM) below lesion threshold for retina and optic nerve and three OOM below for the cortex.

Additionally, all of the EF estimates are at least one OOM below the safety threshold of 42 V/m (Liebetanz et al., 2009; Gellner et al., 2016; Jackson et al., 2017). It should be noted that, as mentioned above, ACS injects less charge than constant current stimulation of the same amplitude (Liebetanz et al., 2009; Schmidt et al., 2013a), and we calculated the current densities from peak-to-peak amplitude instead of effectiveamplitude. The risk of damage will consequently rather be over-than underestimated. We therefore conclude the rACS employed in this study should be safe from a modeling standpoint as well.

Adverse Events

No fatal or serious adverse events (Wester et al., 2008) were observed for rACS. The most notable adverse events in the present study were tingling, burning, itching and fatigue. The hazard rate for these adverse events is to be considered "very common" (>1/10 cases). This is comparable to results from other forms of tES (Brunoni et al., 2011), suggesting for tDCS that the type of adverse event is mild and their frequency of occurrence is "common." Direct experimental evidence shows significantly more cutaneous adverse events, but significantly less concentration deficits after stimulation for rACS as compared to PS (**Table 5**).

As the modeling results showed high maximum current densities and EF estimates at skin level, the presence of cutaneous adverse events during and after rACS comes as no surprise. Comparing rACS and PS regarding the summary category of pain, we have to note the complete lack of cutaneous sensations in PS and that multiple aversive sensations may be clustered and perceived in sum total as painful (Tuckett, 1982).

Skin Rashes and Damage

None of the subjects reported skin rashes or damage. Whereas the applied charge density is clearly strong enough to stimulate

C-nociceptors, it is too low and the duration is too short to induce skin damage (Dzhokic et al., 2008). For direct current stimulation, it has been shown that 1 mA via two 7 × 5 cm rubber electrodes in over 2000 stimulation sessions (Loo et al., 2011) can be applied for 20 min with no skin damage. Again, ACS is less likely than direct current stimulation to induce tissue or electrode damage. Although rACS is unlikely to induce skin damage, this study adhered to previous suggestions for avoiding cutaneous adverse events (Loo et al., 2011).

Tingling, Itching and Burning

Electrical stimulation of skin nociceptors is known to produce itching, burning and tingling sensations in the animal model, as well as in human subjects (Jarvis and Voita, 1971; Tuckett, 1982; Kellogg et al., 1989; Ledger, 1992). While even persisting shortly after stimulation due to central processes, these sensations are not necessarily indicative of local damage induced by stimulation (Tuckett, 1982).

Pain

One third of the subjects reported pain with a median strength of 2.5 NRS. The sensation of pain during and after electric stimulation is understood to be a combination of several factors, with the terminal branches of C-nociceptors of the stimulated skin acting as the primary central conductor (Magerl et al., 1987; Garnsworthy et al., 1988; Hakkinen et al., 1995). This matches subject descriptions of deep and spread pain associated with itch and burning sensations in this study (six cases) as well as anecdotal reports of painful perceptions that could not be attenuated by topical anesthetic and the lack of radiating pain sensations reported elsewhere (Hakkinen et al., 1995). Due to the common occurrence of cutaneous sensations, topical anesthesia might be preferential especially for placebo control or rACS versus PS studies. This study did not use topical anesthesia, as it might mask development of skin damage.

While the feeling of pain and discomfort should be monitored closely in future studies, it should be noted that we found no significant difference between rACS and well-established and tolerable PS regarding overall discomfort/pain (**Table 5**).

This pain during and after PS is most likely a form of "discomfort glare" associated with visual discomfort, annoyance, irritability or distraction without affecting the ability to see, but leading to symptoms of visual fatigue (Ticleanu and Littlefair, 2015).

Phosphenes

As we stimulated our subjects at 120% phosphene threshold, all subjects experienced phosphenes. These phosphenes induced by rACS were typically described as flickering at the edges of the field of view and not experienced as painful.

Historically, phosphenes induced by alternating current have been seen as a purely retinal phenomenon (Rohracher, 1935) resulting from the high susceptibility of the retina to electricity (Ziemssen, 1864). For rACS and other forms of tES the amount of confounding retinal or cortical stimulation following low-voltage stimulation is unknown or a matter of controversy (Paulus, 2010).

Yet, due to the respective montages there should be a magnitude of difference between methods (Peterchev et al., 2012) with TCES inducing the most, rACS with periorbital-occipital montages intermediate, and tES the least retinal stimulation (Delbeke et al., 2001; Thil et al., 2007; Paulus, 2010).

A previous tACS modeling effort indicated why transcranial stimulation may induce retinal phosphenes (Laakso and Hirata, 2013) by virtue of current density induced in the eyes exceeding phosphene thresholds. As different electrode montages result in different current flow patterns, whether a particular montage would result in retinal phosphenes would naturally depend on the montage being studied. Specifically they show thatthe threshold for retinal phosphenes for commonly used tACS montages is exceeded with stimulation current of 500– 1000 μA (depending on the montage considered). Another prior tACS/tDCS modeling effort demonstrated that bilateral montages result in not only more focused current flow but higher current intensities than midline montages (Neulinget al., 2012). While no detailed analysis is performed onthe eye regions, the authors state that the closer one of the stimulation electrodes is to the eye regions, the easier it is to perceive phosphenes.

Where exactly rACS phosphenes are generated remains subject to further investigation. While we find the highest EF estimates in the optic nerve, other authors (Brindley, 1955; Ma et al., 2014) suggested bipolar cells, or the parts of rod and cone cells lying inside the external limiting membrane as the main site of stimulation. In line with the flickering at the edgesof the field of view as reported by our subjects for rACS at 120% phosphene threshold, it can be argued that inner retinal neurons are the most probable site at which an electrical stimulus exerts its primary effect, with predominant activation of the peripheral retina (Ma et al., 2014). This adds further support to previous findings suggesting that the primary location of the majority of retinal damage (the retinal pigment epithelium, RPE) induced by photochemical noxae is bypassed by electrical stimulation (Grützner et al., 1958). Besides fatigue and cutaneous effects, the participants described more phosphene or light related adverse events in association with well-known and safe PS applied at 120% light threshold than with rACS applied at 120% phosphene threshold.

Fatigue

Fatigue, reported by one third of the subjects after rACS, has been suggested in previous research to be an unspecific effect of tES. Similar to rACS, the early approaches to tES involved two "active" electrodes placed directly over the eyes, presumably to facilitate active current delivery through the optic foramina. These montages were first used in Electrosleep research initiated in Robinovitch (1914), with extensive research following (Obrosow, 1959; Sergeev, 1963; Brown, 1975). The consensus after about 60 years was that Electrosleep induces unspecific sleepiness and fatigue related to stimulation (Guleyupoglu et al., 2013).

The findings in this study, that rACS produces more fatigue than PS, support the notion of an indirect and unspecific central

(adverse) effect specific to electrical stimulation. This notion is in line with previous findings showing that action potentials induced by electrical stimulation of the retina can propagate directly to the visual cortex (Grützner et al., 1958), produce different evoked potentials (Potts et al., 1968) and modulate central rhythms (Schmidt et al., 2013a) as well as large scale networks of the brain (Bola et al., 2014).

CNS Damage and Seizure Risk

Beyond fatigue, the possibility of direct structural damage to central nervous structures by rACS seems low considering the distance between charge injection and brain tissue as well as stimulation strength. Yet, for rhythmic PS the danger of inducing an epileptic seizure is well established. Although not found in this study, for electrical stimulation the danger must also be assumed to be high due to neurophysiological similarities with intermittent photic stimulation (IPS) (Brindley, 1955) and proven effects on central processes and neural synchrony (Parra et al., 2003). Additionally, although no reports of seizures after comparable electrical stimulation sessions exist (Brunoni et al., 2012), we will continue to employ photosensitivity and epilepsy as exclusion criteria for future rACS studies.

CONCLUSION AND OUTLOOK

Having theoretically and experimentally characterized the relative safety profile of rACS, we believe future studies can further investigate retinal mechanisms of action for ACS effects, especially in comparison with tACS. Additionally, rACS allows for studies addressing the interaction of different signal types entering the visual system through two separate input channels (left and right eye) and converging at the level of the primary visual cortex. This provides an promising tool for studies aiming to address a common framework of action for NiBS with more than one input-signal, e.g., noise and oscillation (Schmidt et al., 2013b).

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

All protocols conformed to the Declaration of Helsinki, and were approved by the Ethics Committee of the Charité – Universitätsmedizin Berlin ("Ethikkommission der Charité – Universitätsmedizin Berlin"). Informed consent was obtained from all individual participants included in the study. This study adheres to the principles of good scientific practice of the Charité – Universitätsmedizin Berlin ("Grundsätze der Charité zur Sicherung guter wissenschaftlicher Praxis").

AUTHOR CONTRIBUTIONS

LH, AD, SS, MS, and SB conceived and designed the study. LH, AD, CT, AJ, and SS carried out data acquisition and analysis. LH, AD, and SS drafted the manuscript. CT, AJ, AK, MR, MS, and SB critically revised the manuscript. All authors participated in the interpretation of the data.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2019.00783/full#supplementary-material

REFERENCES

Agnew, W. F., and McCreery, D. B. (1987). Considerations for safety in theuse of extracranial stimulation for motor evoked potentials. *Neurosurgery* 20,143–147.

Antal, A., Boros, K., Poreisz, C., Chaieb, L., Terney, D., and Paulus, W. (2008). Comparatively weak after-effects of transcranial alternating current stimulation(tACS) on cortical excitability in humans. *Brain Stimul.* 1, 97–105. doi: 10.1016/j.brs.2007.10.001

Bath, P. M., Brainin, M., Brown, C., Campbell, B., Davis, S. M., Donnan, G. A., et al. (2014). Testing devices for the prevention and treatment of stroke and its complications. *Int. J. Stroke* 9, 683–695. doi: 10.1111/ijs.12302

Bikson, M., and Datta, A. (2012). Guidelines for precise and accurate computational models of tDCS. *Brain Stimul.* 5, 430–431. doi: 10.1016/j.brs.2011.06.001

Bikson, M., Datta, A., and Elwassif, M. (2009). Establishing safety limits for transcranial direct current stimulation. *Clin. Neurophysiol.* 120, 1033–1034. doi: 10.1016/j.clinph.2009.03.018

Bikson, M., Grossman, P., Thomas, C., Zannou, A. L., Jiang, J., Adnan, T., et al.(2016). Safety of transcranial direct current stimulation: evidence based update 2016. *Brain Stimul.* 9, 641–661. doi: 10.1016/j.brs.2016.06.004

Bola, M., Gall, C., Moewes, C., Fedorov, A., Hinrichs, H., and Sabel, B. A. (2014).Brain functional connectivity network breakdown and restoration in blindness.*Neurology* 83, 542–551. doi: 10.1212/WNL.0000000000000672

Brindley, G. S. (1955). The site of electrical excitation of the human eye. *J. Physiol.* 127, 189–200. doi: 10.1113/jphysiol.1955.sp005248

Brittain, J. S., Probert-Smith, P., Aziz, T. Z., and Brown, P. (2013). Tremor suppression by rhythmic transcranial current stimulation. *Curr. Biol.* 23, 436– 440. doi: 10.1016/j.cub.2013.01.068

Brown, C. C. (1975). Electroanesthesia and electrosleep. *Am. Psychol.* 30, 402–410. doi: 10.1037/0003-066x.30.3.402

Brunoni, A. R., Amadera, J., Berbel, B., Volz, M. S., Rizzerio, B. G., and Fregni, F. (2011). A systematic review on reporting and assessmentof adverse effects associated with transcranial direct current stimulation. *Int. J. Neuropsychopharmacol.* 14, 1133–1145. doi: 10.1017/S146114571000 1690

Brunoni, A. R., Nitsche, M. A., Bolognini, N., Bikson, M., Wagner, T., Merabet,L., et al. (2012). Clinical research with transcranial direct current stimulation (tDCS): challenges and future directions. *Brain Stimul.* 5, 175–195. doi: 10.1016/j.brs.2011.03.002

Chaieb, L., Antal, A., Pisoni, A., Saiote, C., Opitz, A., Ambrus, G. G., et al. (2014). Safety of 5 kHz tACS. *Brain Stimul.* 7, 92–96. doi: 10.1016/j.brs.2013.08.004

Chaudhuri, A., and Behan, P. O. (2004). Fatigue in neurological disorders. *Lancet* 363, 978–988. doi: 10.1016/S0140-6736(04)15794-2

Cobb, S. (1947). Photic driving as a cause of clinical seizures in epileptic patients. *Arch. Neurol. Psychiatry* 58:70. doi: 10.1001/archneurpsyc.1947.02300300080008

Datta, A., Baker, J. M., Bikson, M., and Fridriksson, J. (2011). Individualized model predicts brain current flow during transcranial direct-current stimulation treatment in responsive stroke patient. *Brain Stimul.* 4, 169–174. doi: 10.1016/j. brs.2010.11.001

Datta, A., Truong, D., Minhas, P., Parra, L., and Bikson, M. (2012). Inter-individual variation during transcranial direct current stimulation and normalization of dose using MRI-derived computational models. *Front. Psychiatry* 3:91. doi: 10.3389/fpsyt.2012.00091

Delbeke, J. (2011). Electrodes and chronic optic nerve stimulation. *Biocybern. Biomed. Eng.* 31, 81–94. doi: 10.1016/s0208-5216(11)70021-3

Delbeke, J., Parrini, S., Andrien, A., Oozer, M., Legat, V., and Veraart, C. (2000). Modelling activation of visual structures through eyelid surface electrodes:preliminary result. *Pflügers Archiv. Eur. J. Physiol.* 440:R4.

Delbeke, J., Pins, D., Michaux, G., Wanet-Defalque, M. -C., Parrini, S., and Veraart, C. (2001). Electrical stimulation of anterior visual pathways in retinitis pigmentosa. *Invest. Ophthalmol. Vis. Sci.* 42, 291–297.

Downie, W., Leatham, P., Rhind, V., Wright, V., Branco, J., and Anderson, J. (1978). Studies with pain rating scales. *Ann. Rheum. Dis.* 37, 378–381.

Dzhokic, G., Jovchevska, J., and Dika, A. (2008). Electrical injuries: etiology, pathophysiology and mechanism of injury. *Macedonian J. Med. Sci.* 1, 54–58.doi: 10.3889/mjms.1857-5773.2008.0019

Farrar, J. T., Young J. P. Jr., LaMoreaux, L., Werth, J. L., and Poole, R. M. (2001). Clinical importance of changes in chronic pain intensity measured on an 11-point numerical pain rating scale. *Pain* 94, 149–158. doi: 10.1016/s0304-3959(01)00349-9

Feurra, M., Bianco, G., Santarnecchi, E., Del Testa, M., Rossi, A., and Rossi, S. (2011). Frequency-dependent tuning of the human motor system induced by transcranial oscillatory potentials. *J. Neurosci.* 31, 12165–12170. doi: 10.1523/JNEUROSCI.0978-11.2011

Feurra, M., Pasqualetti, P., Bianco, G., Santarnecchi, E., Rossi, A., and Rossi, S. (2013). State-dependent effects of transcranial oscillatory currents on the motor system: what you think matters. *J. Neurosci.* 33, 17483–17489. doi: 10.1523/JNEUROSCI.1414-13.2013

Gall, C., Sgorzaly, S., Schmidt, S., Brandt, S., Fedorov, A., and Sabel, B. A. (2011). Noninvasive transorbital alternating current stimulation improves subjective visual functioning and vision-related quality of life in optic neuropathy. *Brain Stimul.* 4, 175–188. doi: 10.1016/j.brs.2011.07.003

Garnsworthy, R. K., Gully, R. L., Kenins, P., and Westerman, R. A. (1988). Transcutaneous electrical stimulation and the sensation of prickle.*J. Neurophysiol.* 59, 1116–1127. doi: 10.1152/jn.1988.59.4.1116

Gekeler, F., Messias, A., Ottinger, M., Bartz-Schmidt, K. U., and Zrenner, E. (2006). Phosphenes electrically evoked with DTL electrodes: a study in patients with retinitis pigmentosa, glaucoma, and homonymous visual field loss and normal subjects. *Invest. Ophthalmol. Vis. Sci.* 47, 4966–4974.

Gekeler, F., Wrobel, W.G., and Messias, A. (2011). Method for treating an eye. United States patent application US 13/199,904. Washington, DC: U.S. Patent and Trademark Office.

Gellner, A. K., Reis, J., and Fritsch, B. (2016). Glia: a neglected player in non- invasive direct current brain stimulation. *Front. Cell. Neurosci.* 10:188. doi: 10.3389/fncel.2016.00188

Grützner, A., Grüsser, O. -J., and Baumgartner, G. (1958). Reaktionen einzelnerNeurone im optischen Cortex der Katze nach elektrischer Reizung des Nervus opticus. *Arch. Psychiatrie Nervenkrankheiten* 197, 377–404. doi: 10.1007/ bf00345845

Guleyupoglu, B., Schestatsky, P., Edwards, D., Fregni, F., and Bikson, M. (2013). Classification of methods in transcranial electrical stimulation (tES) and evolving strategy from historical approaches to contemporary innovations. *J. Neurosci. Methods* 219, 297–311. doi: 10.1016/j.jneumeth.2013.07.016

Haberbosch, L., Schmidt, S., Jooß, A., Köhn, A., Kozarzewski, L., Rönnefarth, M.,et al. (2019). Rebound or entrainment? The influence of alternating current stimulation on individual alpha. *Front. Hum. Neurosci.* 13:43. doi: 10.3389/fnhum.2019.00043

Hakkinen, V., Eskola, H., Yli-Hankala, A., Nurmikko, T., and Kolehmainen, S. (1995). Which structures are sensitive to painful transcranial electricstimulation? *Electromyogr. Clin. Neurophysiol.* 35, 377–383.

Hallett, M. (2005). Neuroplasticity and rehabilitation. *J. Rehabil. Res. Dev.* 42, 17–22.

Hallett, M. (2007). Transcranial magnetic stimulation: a primer. *Neuron* 55, 187–199. doi: 10.1016/j.neuron.2007.06.026

Helfrich, R. F., Schneider, T. R., Rach, S., Trautmann-Lengsfeld, S. A., Engel, A. K., and Herrmann, C. S. (2014). Entrainment of brain oscillations by transcranialalternating current stimulation. *Curr. Biol.* 24, 333–339. doi: 10.1016/j.cub.2013. 12.041

Herrick, R. M. (1967). Psychophysical methodology: comparison of thresholds of the method of limits and of the method of constant stimuli. *Percept. Mot. Skills* 24, 915–922. doi: 10.2466/pms.1967.24.3.915

Hummel, F. C., Celnik, P., Pascual-Leone, A., Fregni, F., Byblow, W. D., Buetefisch, C. M., et al. (2008). Controversy: noninvasive and invasive cortical stimulation show efficacy in treating stroke patients. *Brain Stimul.* 1, 370–382. doi: 10.1016/j.brs.2008.09.003

Iacono, M. I., Neufeld, E., Akinnagbe, E., Bower, K., Wolf, J., Vogiatzis Oikonomidis, I., et al. (2015). MIDA: a multimodal imaging-based detailed anatomical model of the human head and neck. *PloS One* 10:e0124126. doi: 10.1371/journal.pone.0124126

Irnich, W. (1980). The chronaxie time and its practical importance. *Pacing Clin. Electrophysiol.* 3, 292–301. doi: 10.1111/j.1540-8159.1980.tb05236.x

Irnich, W. (2010). The terms "chronaxie" and "rheobase" are 100 years old. *PacingClin. Electrophysiol.* 33, 491–496. doi: 10.1111/j.1540-8159.2009.02666.x

Jackson, M. P., Truong, D., Brownlow, M. L., Wagner, J. A., McKinley, R. A., Bikson, M., et al. (2017). Safety parameter considerations of anodal transcranial Direct Current Stimulation in rats. *Brain Behav. Immun.* 64, 152–161. doi: 10.1016/j.bbi.2017.04.008

Jarvis, C. W., and Voita, D. A. (1971). Low voltage skin burns. *Pediatrics* 48, 831–832.

Joundi, R. A., Jenkinson, N., Brittain, J. S., Aziz, T. Z., and Brown, P. (2012). Driving oscillatory activity in the human cortex enhances motor performance. *Curr.Biol.* 22, 403–407. doi: 10.1016/j.cub.2012.01.024

Kanai, R., Chaieb, L., Antal, A., Walsh, V., and Paulus, W. (2008). Frequency-dependent electrical stimulation of the visual cortex. *Curr. Biol.* 18, 1839–1843. doi: 10.1016/j.cub.2008.10.027

Kellogg, D. L. Jr., Johnson, J. M., and Kosiba, W. A. (1989). Selective abolition of adrenergic vasoconstrictor responses in skin by local iontophoresis of bretylium. *Am. J. Physiol.* 257(5 Pt 2), H1599-H1606.

Knutson, R.C. (1967). First international symposium on electrosleep therapy and electroanesthesia: a report. *Anesth. Analg.* 46, 333–339.

Laakso, I., and Hirata, A. (2013). Computational analysis shows why transcranial alternating current stimulation induces retinal phosphenes. *J. Neural. Eng.* 10:046009. doi: 10.1088/1741-2560/10/4/046009

Larntz, K. (1978). Small-sample comparisons of exact levels for chi-squared goodness-of-fit statistics. *J. Am. Stat. Assoc.* 73, 253–263. doi: 10.2307/2286650 Ledger, P. W. (1992). Skin biological issues in electrically enhanced transdermaldelivery. *Adv. Drug Delivery Rev.* 9, 289–307. doi: 10.1016/0169-409X(92)90027-N

Liebetanz, D., Koch, R., Mayenfels, S., Konig, F., Paulus, W., and Nitsche, M. A.(2009). Safety limits of cathodal transcranial direct current stimulation in rats. *Clin. Neurophysiol.* 120, 1161–1167. doi: 10.1016/j.clinph.2009.01.022

Liew, S. L., Santarnecchi, E., Buch, E. R., and Cohen, L. G. (2014). Non-invasivebrain stimulation in neurorehabilitation: local and distant effects for motor recovery. *Front. Hum. Neurosci.* 8:378. doi: 10.3389/fnhum.2014.00378

Lindenblatt, G., and Silny, J. (2002). Electrical phosphenes: on the influence of conductivity inhomogeneities and small-scale structures of the orbita on the current density threshold of excitation. *Med. Biol. Eng. Comput.* 40, 354–359. doi: 10.1007/bf02344219

Loo, C., Martin, D., Alonzo, A., Gandevia, S., Mitchell, P., and Sachdev, P. (2011). Avoiding skin burns with transcranial direct current stimulation: preliminary considerations. *Int. J. Neuropsychopharmacol.* 14, 425–426. doi: 10.1017/s1461145710001197

Ma, Z., Cao, P., Sun, P., Li, L., Lu, Y., Yan, Y., et al. (2014). Optical imaging of visual cortical responses evoked by transcorneal electrical stimulation with different

parameters. *Invest. Ophthalmol. Vis. Sci.* 55, 5320–5331. doi: 10.1167/iovs.14-14600

Magerl, W., Szolcsanyi, J., Westerman, R. A., and Handwerker, H. O. (1987). Laser Doppler measurements of skin vasodilation elicited by percutaneous electrical stimulation of nociceptors in humans. *Neurosci. Lett.* 82, 349–354. doi: 10.1016/0304-3940(87)90281-3

Marshall, L., Helgadottir, H., Molle, M., and Born, J. (2006). Boosting slow oscillations during sleep potentiates memory. *Nature* 444, 610–613. doi: 10. 1038/nature05278

Merrill, D. R., Bikson, M., and Jefferys, J. G. (2005). Electrical stimulation of excitable tissue: design of efficacious and safe protocols. *J. Neurosci. Methods*141, 171–198. doi: 10.1016/j.jneumeth.2004.10.020

Montgomery, S. A., and Asberg, M. (1979). A new depression scale designed tobe sensitive to change. *Br. J. Psychiatry* 134, 382–389. doi: 10.1192/bjp.134. 4.382

Neuling, T., Wagner, S., Wolters, C. H., Zaehle, T., and Herrmann, C. S. (2012). Finite-element model predicts current density distribution for clinical applications of tDCS and tACS. *Front. Psychiatry* 3:83. doi: 10.3389/fpsyt.2012. 00083

Nitsche, M. A., Liebetanz, D., Lang, N., Antal, A., Tergau, F., and Paulus, W. (2003).Safety criteria for transcranial direct current stimulation (tDCS) in humans. *Clin. Neurophysiol.* 114, 2220–2222. doi: 10.1016/s1388-2457(03)00235-9

Obrosow, A. E. (1959). "Electrosleep therapy," in *Therapeutic Electricity and Ultraviolet Radiation*, ed. S. Licht (New Haven, CT: Imprint unknown), 169.

Parra, J., Kalitzin, S., Iriarte, J., Blanes, W., Velis, D., and Da Silva, F. L. (2003). Gamma-band phase clustering and photosensitivity: is there an underlying mechanism common to photosensitive epilepsy and visual perception? *Brain* 126, 1164–1172. doi: 10.1093/brain/awg109

Paulus, W. (2003). Transcranial direct current stimulation (tDCS). *Suppl. Clin. Neurophysiol.* 56, 249–254. doi: 10.1016/s1567-424x(09)70229-6

Paulus, W. (2010). On the difficulties of separating retinal from cortical originsof phosphenes when using transcranial alternating current stimulation (tACS). *Clin. Neurophysiol.* 121, 987–991. doi: 10.1016/j.clinph.2010.01.029

Paulus, W., Peterchev, A. V., and Ridding, M. (2013). Transcranial electric and magnetic stimulation: technique and paradigms. *Handbook Clin. Neurol.* 116,329–342. doi: 10.1016/B978-0-444-53497-2.00027-9

Peterchev, A. V., Wagner, T. A., Miranda, P. C., Nitsche, M. A., Paulus, W., Lisanby, S. H., et al. (2012). Fundamentals of transcranial electric and magnetic stimulation dose: definition, selection, and reporting practices. *Brain Stimul.* 5, 435–453. doi: 10.1016/j.brs.2011.10.001

Pogosyan, A., Gaynor, L. D., Eusebio, A., and Brown, P. (2009). Boosting cortical activity at Beta-band frequencies slows movement in humans. *Curr. Biol.* 19,1637–1641. doi: 10.1016/j.cub.2009.07.074

Polania, R., Paulus, W., and Nitsche, M. A. (2012). Noninvasively decoding thecontents of visual working memory in the human prefrontal cortex within high-gamma oscillatory patterns. *J. Cogn. Neurosci.* 24, 304–314. doi: 10.1162/jocn_a_00151

Poreisz, C., Boros, K., Antal, A., and Paulus, W. (2007). Safety aspects of transcranial direct current stimulation concerning healthy subjects and patients. *Brain Res. Bull.* 72, 208–214. doi: 10.1016/j.brainresbull.2007.01.004

Potts, A. M., Inoue, J., and Buffum, D. (1968). The electrically evoked response of the visual system (EER). *Invest. Ophthalmol. Vis. Sci.* 7, 269–278.

Rager, G., and Singer, W. (1998). The response of cat visual cortex to flicker stimuliof variable frequency. *Eur. J. Neurosci.* 10, 1856–1877. doi: 10.1046/j.1460-9568.1998.00197.x

Robinovitch, L. (1914). "Electrical analgesia, sleep and resuscitation," in *Anesthesia*,ed. J.T. Gwathmey (New York, NY: D. Appleton and Company), 628–643.

Rohracher, H. (1935). Die gehirnelektrischen Erscheinungen bei geistiger Arbeit. *Zeitschrift für Psychologie und Charakterkunde* 136, 308–324.

Sandrini, M., and Cohen, L.G. (2013). "Noninvasive brain stimulation in neurorehabilitation," in *Handbook of Clinical Neurology*, eds A.M. Lozano & M. Hallett (Amsterdam: Elsevier), 499–524. doi: 10.1016/b978-0-444-53497-2.00040-1

Santarnecchi, E., Muller, T., Rossi, S., Sarkar, A., Polizzotto, N. R., Rossi, A., et al. (2016). Individual differences and specificity of prefrontal gamma frequency- tACS on fluid intelligence capabilities. *Cortex* 75, 33–43. doi: 10.1016/j.cortex. 2015.11.003

Santarnecchi, E., Polizzotto, N. R., Godone, M., Giovannelli, F., Feurra, M., Matzen,L., et al. (2013). Frequency-dependent enhancement of fluid intelligence induced by transcranial oscillatory potentials. *Curr. Biol.* 23, 1449–1453. doi: 10.1016/j.cub.2013.06.022

Saturnino, G. B., Antunes, A., and Thielscher, A. (2015). On the importance of electrode parameters for shaping electric field patterns generated by tDCS. *Neuroimage* 120, 25–35. doi: 10.1016/j.neuroimage.2015.06.067

Schmidt, S., Mante, A., Ronnefarth, M., Fleischmann, R., Gall, C., and Brandt, S. A. (2013a). Progressive enhancement of alpha activity and visual function in patients with optic neuropathy: a two-week repeated session alternating currentstimulation study. *Brain Stimul.* 6, 87–93. doi: 10.1016/j.brs.2012.03.008

Schmidt, S., Scholz, M., Obermayer, K., and Brandt, S. A. (2013b). Patterned brain stimulation, what a framework with rhythmic and noisy components might tellus about recovery maximization. *Front. Hum. Neurosci.* 7:325. doi: 10.3389/fnhum.2013.00325

Schutter, D. J. (2016). Cutaneous retinal activation and neural entrainment in transcranial alternating current stimulation: a systematic review. *Neuroimage* 140, 83–88. doi: 10.1016/j.neuroimage.2015.09.067

Sergeev, G. V. (1963). Electrosleep as a method of neurotropic therapy of patients with hypertensive disease. *Am. Heart J.* 66, 138–139. doi: 10.1016/0002-8703(63)90081-4

Streiner, D. L., and Norman, G. R. (2008). *Health Measurement Scales: A Practical Guide to their Development and Use*. Oxford: Oxford university press.

Talelli, P., and Rothwell, J. (2006). Does brain stimulation after stroke have a future? *Curr. Opin. Neurobiol.* 19, 543–550. doi: 10.1097/WCO.0b013e32801080d1 Thil,

M.-A., Duy, D. T., Colin, I. M., and Delbeke, J. (2007). Time course of tissue remodelling and electrophysiology in the rat sciatic nerve after spiralcuff electrode implantation. *J. Neuroimmunol.* 185, 103–114. doi: 10.1016/j.jneuroim.2007.01.021

Ticleanu, C., and Littlefair, P. (2015). A summary of LED lighting impacts on health. *Int. J. Sustain. Light.* 17, 5–11. doi: 10.26607/ijsl.v17i0.11

Trenite, D. G., Binnie, C. D., Harding, G. F., Wilkins, A., Covanis, T., Eeg-Olofsson, O., et al. (1999). Medical technology assessment photic stimulation–standardization of screening methods. *Neurophysiol. Clin.* 29, 318–324. doi: 10.1016/s0987-7053(99)90045-x

Tuckett, R. P. (1982). Itch evoked by electrical stimulation of the skin. *J. Invest. Dermatol.* 79, 368–373. doi: 10.1111/1523-1747.ep12529734

Vossen, A., Gross, J., and Thut, G. (2015). Alpha power increase after transcranial alternating current stimulation at alpha frequency (alpha-tACS) reflects plastic changes rather than entrainment. *Brain Stimul.* 8, 499–508. doi: 10.1016/j.brs.2014.12.004

Walker, A. E., Woolf, J. I., Halstead, W. C., and Case, T. J. (1944). Photic driving. *Arch. Neurol. Psychiatry* 52, 117–125.

Wester, K., Jönsson, A. K., Spigset, O., Druid, H., and Hägg, S. (2008). Incidenceof fatal adverse drug reactions: a population based study. *Br. J. Clin. Pharmacol.* 65, 573–579. doi: 10.1111/j.1365-2125.2007.03064.x

Yuen, T. G., Agnew, W. F., Bullara, L. A., Jacques, S., and McCreery, D. B. (1981). Histological evaluation of neural damage from electrical stimulation: considerations for the selection of parameters for clinical application. *Neurosurgery* 9, 292–299. doi: 10.1227/00006123-198109000-00013

Zaghi, S., Acar, M., Hultgren, B., Boggio, P. S., and Fregni, F. (2010). Noninvasive brain stimulation with low-intensity electrical currents: putative mechanismsof action for direct and alternating current stimulation. *Neuroscientist* 16, 285–307. doi: 10.1177/1073858409336227

Ziemssen, H. (1864). *Die Electricität in der Medicin*. Berlin: August Hirschwald.

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Rebound or Entrainment? The Influence of Alternating Current Stimulation on Individual Alpha

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Alternating current stimulation (ACS) is an established means to manipulate intrinsic cortical oscillations. While working towards clinical impact, ACS mechanisms of action remain unclear. For ACS's well-documented influence on occipital alpha, hypotheses include neuronal entrainment as well as rebound phenomena. As a retinal origin is also discussed, we employed a novel form of ACS with the advantage that it specifically targets occipital alpha-oscillations *via* retinofugal pathways retinofugal ACS (rACS). We aimed to confirm alpha-enhancement outlasting the duration of stimulation with 10 Hz rACS. To distinguish entrainment from rebound effects, we investigated the correlation between alpha peak frequency change and alpha-enhancement strength. We quantified the alpha band power before and after 10 Hz rACS in 15 healthy subjects. Alpha power enhancement and alpha peak frequency change were assessed over the occipital electrodes and compared to sham stimulation. RACS significantly enhanced occipital alpha power in comparison to sham stimulation ($p < 0.05$). Alpha peak frequency changed by a mean 0.02 Hz ($\pm$ 0.04). A greater change in alpha peak frequency did not correlate with greater effects on alpha power. Our findings show an alpha-enhancement consistent with studies conducted for transcranial ACS (tACS) and contribute evidence for a retinal involvement in tACS effects on occipital alpha. Furthermore, the lack of correlation between alpha peak frequency change and alpha-enhancement strength provides an argument against entrainment effects and in favor of a rebound phenomenon.

Keywords: alternating current stimulation, neuromodulation, alpha rhythm, rebound, entrainment, phosphenes

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INTRODUCTION

Non-invasive brain stimulation (NIBS), including transcranial electric stimulation (tES; Paulus, 2011), has shown impressive effects ranging from short changes in neural activity to long lasting recovery maximization following neural injury (Hallett, 2005; Talelli and Rothwell, 2006; Hummel et al., 2008; Sandrini and Cohen, 2013). A novel form of NIBS is transcranial alternating current stimulation (tACS), an oscillatory stimulation technique associated with psychophysical changes (Antal et al., 2008; Kanai et al., 2010; Wach et al., 2013), enhancement of working memory,

learning, and long-term memory formation (Marshall et al., 2006; Kuo and Nitsche, 2012; Santarnecchi et al., 2013), as well as clinical improvements including tumor growth slowing (Kirson et al., 2007) and tremor suppression in patients with Parkinson's disease (Brittain et al., 2013). Despite these encouraging results, little is known about the mechanism of action (Zaghi et al., 2010). Due to this lack of knowledge tACS may consequently be in danger of facing, similar to transcranial direct current stimulation (tDCS; Horvath et al., 2015), the challenges of large effect variability and poor result reproducibility (Zaghiet al., 2010; Feurra et al., 2013; Wach et al., 2013). Thereis also evidence that the effects of tACS are at least in part due to retinal stimulation (Schutter, 2016). To efficiently and efficaciously apply tACS, especially in the visual system, it critically important for its users to further investigate the retinal as well as cortical mechanisms of action behind the observed effects.

In general, the effects of AC stimulation have been mainly attributed to synchronization of neural oscillations (Antal et al., 2008; Paulus, 2011; Herrmann et al., 2013), with most effects reported on alpha (α) oscillations (8–12 Hz; Antal et al., 2008; Helfrich et al., 2014; Vossen et al., 2015), the dominant frequency of the visual cortex (Klimesch, 1999). As the mechanism of action is unknown and effects of photic stimulation (Photic Driving; Walker et al., 1944) as well as AC stimulation on α oscillations *via* the retinofugal (visual) pathway are well-known (Brindley, 1955; Grützner et al., 1958; Sakamoto et al., 1993; Rager and Singer, 1998; Schmidt et al., 2013a), a better understanding of the underlying neurophysiology of alternating current stimulation (ACS) effects on oscillations should help address these issues.

Hypotheses concerning the mechanism of action for ACS synchronization effects include *entrainment* of neural oscillations as well as *rebound* effects. While entrainment describes the synchronization of one oscillator to another (Ermentrout and Rinzel, 1984), rebound is defined as an increase in excitability typically following inhibition (Perkel and Mulloney, 1974).

Several studies have shown frequency-specific effects on perceptual or cognitive task performance, ascribed to a potential frequency entrainment during or shortly after a single train of rhythmic stimulation (Klimesch et al., 2003; Marshall et al., 2006; Kanai et al., 2008; Zaehle et al., 2010). Entrainment requires an increase of spectral power as well as phase- and frequency-lock of a neural oscillation to an external stimulus (Vossen et al., 2015).

Phase-lock has been reported during stimulation (Helfrich et al., 2014; Ruhnau et al., 2016), although electrical stimulation artifacts in EEG and MEG aggravate the acquisition of reliable neurophysiological data (Soekadar et al., 2013; Helfrich et al., 2014). Newly presented data by Noury et al. suggests that these artifacts may be mistaken for entrainment effects (Noury et al., 2016).

Consequently, most reports present spectral power enhancement only after the cessation of ACS (Zaehle et al., 2010; Schmidt et al., 2013a; Helfrich et al., 2014; Vossen et al., 2015). This offers the hypothesis of a rebound effect as an alternative explanation. Classical post-inhibitory rebound is an increase in excitability following inhibition, generating responses ranging from threshold lowering up to a train of impulses (Perkel and Mulloney, 1974). This, considered for α-pacemaker neurons, would also result in an increase of spectral power after stimulation. Such an increase would occur at an intrinsic frequency and without a phase-lock (Perkel and Mulloney, 1974). Neural rebound effects after stimulation have been found in animal models (Pape and McCormick, 1995; Tong et al., 1996; Huang et al., 2008) as well as in the human brain (Fuggetta et al., 2005; Brignani et al., 2008; Manganotti et al., 2012).

The primary goal of this study was to examine the mechanisms of action behind ACS effects on occipital α oscillations. We therefore investigated a possible contribution of retinal stimulation to the observed α enhancement by employing a periorbital montage. Furthermore, to address the differentiation between neural entrainment and rebound effects after AC stimulation, any frequency peak shift in endogenous α rhythms towards the stimulation frequency was investigated. The presence of such a frequency peak shift would indicate an entrainment, while its absence would provide evidence in favor of a rebound phenomenon.

We decided on the α frequency band as a target, as ACS has shown robust effects in this frequency range (Kanai et al., 2010; Helfrich et al., 2014; Vossen et al., 2015). Cortical α is associated with numerous perceptional processes (Surwillo, 1961; VanRullen and Koch, 2003; Mathewson et al., 2009; Ai and Ro, 2014; Lange et al., 2014; Cecere et al., 2015; Samaha and Postle, 2015) as well as cognitive performance (Klimesch, 1999; Klimesch et al., 2003; Hanslmayr et al., 2005; Zoefel et al., 2011) and characterized by a peak in spectral analysis, the individualα frequency (IAF; Klimesch et al., 2007), which presents an optimal opportunity to investigate synchronization to external stimulation. The Berger effect, describing the repression of the highest physiological occipital α in a subject *via* opening of the eyes (Berger, 1929), presents further opportunities. Firstly, we can employ our stimulation in an eyes open condition and expect little interference from intrinsic α. Furthermore, the measurement of high occipital α during an eyes closed baseline condition can serve as a reference for effect size, since it allows us to compare exogenous stimulation effects to the highest intrinsically generated α power.

Retinal contribution to tACS effects is part of an ongoing discussion (Schwiedrzik, 2009; Paulus, 2010; Schutter and Hortensius, 2010). A recent review by Schutter (2016) suggested that phosphenes might be involved in tACS α-enhancement in the visual system.

To further investigate this and effectively target α oscillations in the visual cortex, we employed a periorbital application type of ACS termed retinofugal ACS (rACS). This technique has been applied aiming for vision restoration in a therapeutic regimen called repetitive transorbital ACS (rtACS; Gall et al., 2010). It utilizes signal transmission along well-defined retinofugal tracts terminating predominantly in cortical visual areas (Gray and Singer, 1989) and offers a comparatively focal (Peterchev et al., 2012) method to investigate ACS effects on

intrinsic frequencies in the well-circumscribed visual system (Gall et al., 2011; Schmidt et al., 2013a).

MATERIALS AND METHODS

Participants

We stimulated 15 healthy volunteers in the rACS group (eight female, seven male, mean age 23.9 ± 2.5) as well as in the sham group (four female, 11 male, mean age 25.8 ± 5.3). The subjects were interviewed prior to experimentation regarding their state of health and gave written informed consent. We applied established exclusion criteria for NIBS (Brunoni et al., 2012), and added evidence for photophobia and photosensitive epilepsy. All protocols conformed to the Declaration of Helsinki, and were approved by the Ethics Committee of the Charité—Universitätsmedizin Berlin (''Ethikkommission der Charité—Universitätsmedizin Berlin''). This study adheres to the principles of good scientific practice of the Charité—Universitätsmedizin Berlin (''Grundsätze der Charité zur Sicherung guter wissenschaftlicher Praxis'').

Stimulation

RACS was applied by a multi-channel low-voltage stimulation device certified for clinical use, which delivered voltage-controlled weak periorbital sinusoidal current over four individual periorbital electrodes respectively (NextWave, Eyetronic, Germany). The four superficial active stimulating electrodes (sintered Ag/AgCl ring electrode, Easycap, Germany) were contained in foam-padded stimulation goggles and bilaterally made skin contact *via* small felt buffers (0.35 cm^2) superior and inferior to the eye (''periorbital''). The passive electrode (rectangular electrode, 30 × 30 mm polished stainless steel) was fastened on the back of the neck at the midline relative to the occipital poles.

The electrical impedance of the four stimulating electrodes was measured for four different frequencies (5 Hz, 10 Hz, 15 Hz and 20 Hz) and held below 15 k□. This measurement at different frequencies was done as part of the innate safety procedure of the CE-certified stimulation device. Phosphene thresholds, defined as the current intensity when participants first subjectively perceived phosphenes, were determined employing an ascending method of limits (Herrick, 1967) provided by the NextWave software.

In the rACS group, stimulation was applied with bandwidth restricted to 10 Hz at an amplitude of 120% phosphene threshold (resulting in a mean 354.15 μA ± 50.6 peak-to-peak amplitude) and delivered in six 30 s blocks followed by 30 s pauses each. Solely 10 Hz as stimulation frequency was chosen to stay close to the critical α-eigenfrequencies of our subjects.

In the sham group, six blocks of a 5 s ramp-up/ramp-down DC stimulus followed by 25 s signal silence was applied at 120% phosphene threshold (resulting in a mean 292.10 μA ± 68.9 peak-to-peak amplitude), again followed by 30 s pauses each. During the signal silence, the stimulator remained switched on while no current was delivered. The sham signal was designed to match initial skin sensations known from other forms of NIBS (Siebner et al., 2004) as well as rACS phosphene perception.

EEG

EEG measurements were performed during the sessions using a 32-electrode EEG cap (Waveguard EEG caps, ANT BV, Enschede, Netherlands), according to the 10–20-system, with impedances kept below 10 kΩ. Data acquisition was carried out in a shielded room without natural light in the electrophysiological unit of the neurological department.

Two minutes of baseline EEG with four conditions were recorded prior to stimulation: 30 s eyes open, 30 s eyes closed and 30 s blinking followed by 30 s of muscle artifact production. To avoid artifacts, the subjects were told to focus a fixed point on a white surface in 1 m distance for the duration of the experiment. Moreover, the experimenter controlled the eyes open/closed conditions and reminded the subjects to follow the instructions and keep alert if necessary.

The EEG data was imported into MATLAB R2014a (The MathWorks Inc, Natick, MA, USA) and analyzed using the FieldTrip toolbox (Oostenveld et al., 2011). We segmented the EEG signals in 25 s-intervals for the eyes-open (EO) baseline, the eyes-closed (EC) baseline and the EO post-stimulation (Post-Stim) condition, including data from 2.5 s after stimulation cessation to 2.5 s before the beginning of a new stimulation block. Remaining artifacts, especially blinking and muscle twitches, were excluded *via* visual artifact rejection for each trial and channel. The blinking and muscle artifact conditions run prior to baseline EEG served as a decision support in borderline cases. EEG signals were referenced against the common average and filtered (Band-Pass Filter from 2 to 70 Hz with filter-slope 24 dB/oct). Following resampling of the data from originally 2,000–500 Hz to streamline and accelerate data analysis, a fast fourier transformation (FFT) with a discrete prolate spheroidal sequences (DPSS) multitaper determined the bandwidth-specific power (mV^2). The sliding time window was set to 0.5 s, while the frequency analysis window was set to 0.04 Hz. The frequency with the highest resulting α power was defined as the IAF/α peak. For illustration, we calculated the EEG-power spectra grand average during EC and post-stimulation and baseline-corrected them by dividing it by the EO baseline grand average. Topographical plots of these values were then generated with FieldTripand smoothed using a moving average filter with a spanof two.

After this, the mean α power over the spectrum 8–12 Hz for each electrode and stimulation block was calculated. We then confirmed that there was no significant correlation between stimulation block number and α power increase in either the sham (r = 0.02, p = 0.83) or the rACS condition (r = p = 0.45) *via* Spearman's rho to ensure the absence of an alpha power ramp-up effect over consecutive trains of stimulation. Consecutively, we calculated the mean α power and peak frequency over all six stimulation blocks for each electrode, preparing the data for statistical analysis.

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Statistics

To statistically investigate the effect of rACS on occipital alpha, we compared the mean spectral α (8–12 Hz) α power for rACS and Sham. Due to the lack of normal distribution determined by the Shapiro-Wilk test, we ln+1-transformed the data. Normal distribution and homoscedasticity of the data were then confirmed *via* the Shapiro-Wilk and Levene's test respectively. We then calculated a repeated measure analyses of variance (ANOVA) with the dependent variable ''α power'', consisting of the levels (EO Baseline, EC Baseline and EO Post-Stim) as well as the fixed factor ''group.''

For our analysis of α power change, we baseline-corrected the ln-transformed EO Post-Stim α power data by dividingit by the EO Baseline and subtracting 1. After confirming normal distribution and homoscedasticity, we performed an ANOVA with ''group'' as fixed factor and baseline-corrected ln-transformed ''alpha power'' as dependent variable.

To gain insight on a possible frequency shift after rACS, the effect of rACS of the distance of α peak frequency to stimulation frequency was analyzed with an univariate ANOVA after confirming normal distribution of the data *via* Shapiro-Wilk test. A possible correlation between distance of individual α to stimulation frequency and α-power change was also assessed, again using the Spearman correlation coefficient.

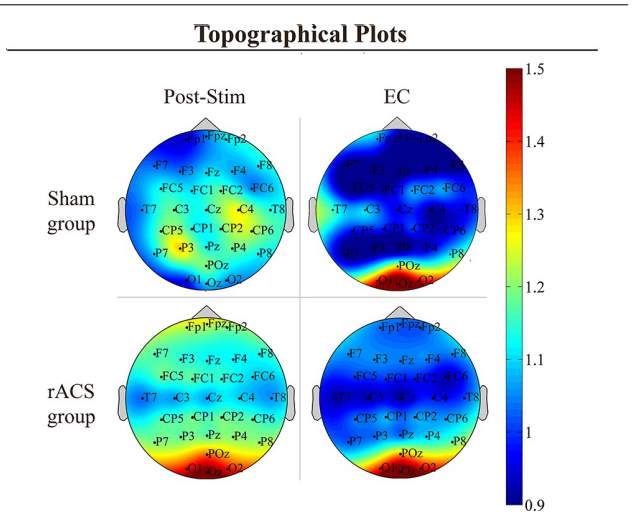

FIGURE 1 | Topographical plots. Topographical plots of the α power change [divided by eyes open (EO) Baseline] in the conditions post-stimulation (Post-Stim) and eyes closed (EC) Baseline as well as the retinofugal alternating current stimulation (rACS)- and Sham group. Color spectrum ranges from 0.9 to 1.5. In the EC and rACS conditions, α increase is most prominent across the occipital electrodes O1, Oz and O2.

P-values of ≤ 0.05 were considered significant. All analyses were performed using IBM SPSS Statistics, Version 19.0.0.1 (IBM, Armonk, NY, USA). Spectral power bar plots as well as spectral α peak box- and scatter plots were created using GraphPad Prism version 7.02 for Windows (GraphPad Software, La Jolla, CA, USA[1]).

RESULTS

Stimulation Parameters

An average phosphene threshold at 290.24 μA (± 44.16) for the rACS and 243.33 μA (± 57.07) for the sham group, impedances at 12.57 kΩ (± 1.8) and 11.73 kΩ (± 2.7), as well as an average peak-to-peak amplitude of 354.15 μA (± 50.6) for rACS and 292.00 (± 68.5) μA for sham were noted. We additionally calculated the effective (root mean square) amplitude, resulting in a mean 250.41 μA (± 47.7) for rACS and 168.58 μA (± 39.5) for sham. The current density amounted to a mean 0.71 mA/cm^2 (± 0.13) for rACS and a mean 0.48 mA/cm^2 (± 0.11) for sham.

Alpha Power Enhancement After rACS

We found a spectral α power enhancement over the occipital scalp area after rACS, with subjects showing significantly greater α power increase after rACS as compared to sham. The enhancement was comparable in size to the one found in the EC condition.

Topographical plots of the frequency grand average (**Figure 1**) showed a strong focus of the overall α-power enhancement after rACS and during EC around the occipital scalp area. Additionally, a diffuse increase in 8–12 Hz spectral power can be observed after sham and rACS, with foci in the frontal and centroparietal scalp area after rACS and in the central scalp area after sham.

Mean ln+1-transformed EO Baseline α power (**Figure** amounted to 0.99 (± 0.13) for the rACS group and 0.88 (± 0.10) for the Sham group. In the EC Baseline condition, we found a mean α power of 1.26 (± 0.17) in the rACS and 1.11 (± 0.16) in the Sham group. Alpha power in the EO Post-Stim condition amounted to a mean 1.21 (± 0.14) in the rACS and a mean 0.83 (± 0.09) in the Sham group. The repeated measure ANOVA resulted in a significant main effect for mean α power ($F_{(2,27)}$ = 8.99, p = 0.001) as well as interaction the stimulation type and mean alpha power ($F_{(2,27}$ = p = 0.03). Bonferroni-corrected pairwise comparisons showed a significantly higher alpha power in the rACS Post-Stim condition compared to Sham (p = 0.036), but no significant differences in the baseline (p = 0.48) and EC (p = 0.52) conditions. The rACS group also showed significantly higher EO Post-Stim α power compared to EO Baseline (p = 0.01), whereas the Sham group showed no significant difference in this regard (p = 0.99). Furthermore, EC Baseline α power was significantly higher than the EO Baseline in both groups (rACS: 0.02, Sham: 0.05), replicating the Berger-effect. There was no significant difference between rACS group EO Post-Stim α and EC Baseline (p = 1.00), indicating a physiologically plausible α increase.

Baseline-corrected α power (change; **Figure 2B**) amounted to a mean −0.02 (± 0.07) for EO Post-Stim Sham and (± 0.09) for EO Post-Stim rACS. The ANOVA showed a significantly higher alpha power change after rACS compared to Sham ($F_{(1,28)}$ = 7.139, p = 0.01).

Individual α Frequency Shift

We found no significant shift of the individual α peak frequency after rACS compared to baseline. The spectral peak of the α-band

[1]http://www.graphpad.com

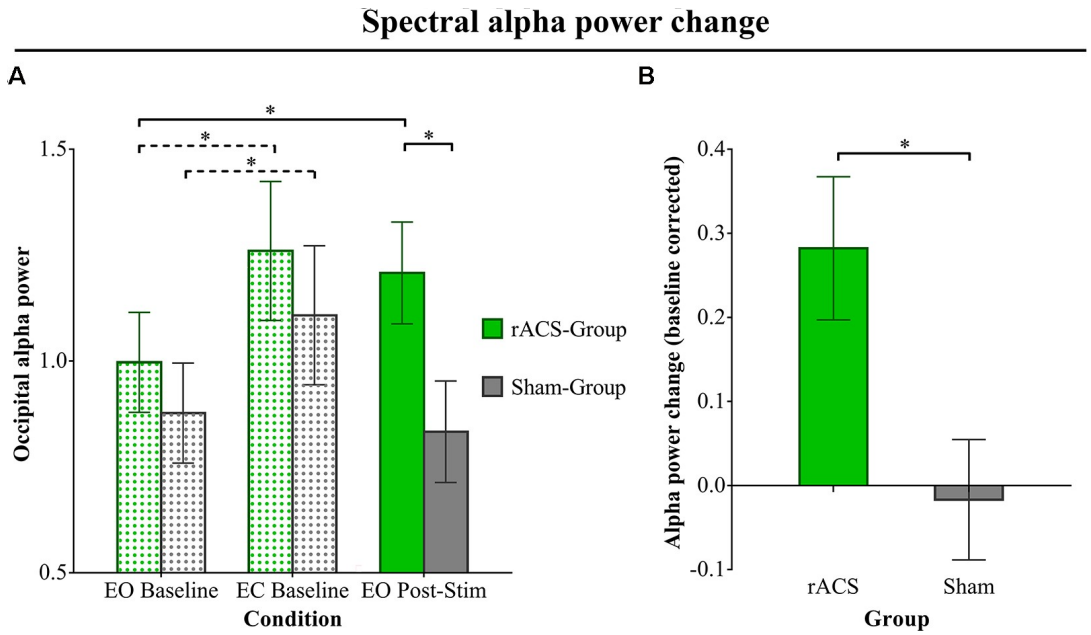

FIGURE 2 | Spectral alpha power change. (A) Ln+1-transformed spectral α power over the occipital electrodes O1, Oz and O2 for the rACS group (green) and the Sham group (gray) in three conditions: eyes open (EO) Baseline, eyes closed (EC) Baseline and EO Post-Stim. Baseline condition bars are dot patterned, while the Post-Stim bars are plain. Error bars depict standard error of the mean. The EC Baseline shows a significant increase in α power compared to EO Baseline in both groups, replicating the Berger effect (indicated by dashed lines). The EO Post-Stim condition α in the rACS group is significantly higher than the Sham EO Post-Stim α, as well as significantly higher than its (rACS Group) EO Baseline (indicated by solid lines), showing a clear stimulation effect of rACS on occipital α. (B) Mean baseline-corrected α power change (EO Post-Stim/EO Baseline −1) over the occipital electrodes O1, Oz and O2 for the sham and rACS groups. Post-stimulation rACS is depicted as green, post-stimulation sham as gray. A value of 0 represents no change from baseline α power. Error bars depict standard error of the mean. The rACS group shows a significantly stronger increase in α power compared to the sham group. Significant differences ($p < 0.05$) are marked with an asterisk *.

amounted to a mean 10.22 Hz (± 1.29) prior to rACS and a mean 10.26 Hz (± 1.17) after rACS (**Figure 3**). The mean absolute distance to stimulation frequency (10 Hz) amounted to 1.05 Hz (± 0.73) before and 0.97 Hz (± 0.67) after application of rACS. There was no significant effect of rACS on the distance of α peak frequency to stimulation frequency ($F_{(1,28)} = 0.009$, $p = 0.93$) as assessed by an univariate ANOVA. This indicates the lack of an overall frequency lock of intrinsic α to stimulation frequency.

Moreover, the proximity of IAF to the stimulation frequency of 10 Hz did not correlate to a greater increase in α-power ($r = 0.04$, $p = 0.88$) employing Spearman's rho. This provides evidence against accidental frequency-locked stimulation leading to enhanced occipital α.

DISCUSSION

The effects of 10 Hz rACS on neural oscillations showed the following characteristics: (1) 10 Hz stimulation resulted in an enhancement of α-power; (2) the post-stimulation α-peak did not significantly differ from baseline IAF; and (3) there was no significant correlation between α-power and proximity to stimulation frequency. These findings are not consistent with the hypothesized entrainment effects. Since entrainment of neural oscillations is the most proposed mechanism of action for tACS effects (Helfrich et al., 2014), an in-depth discussion of these conflicting findings is required.

Enhancement

The specific enhancement of α-power over the occipital electrodes after 10 Hz rACS is consistent with studies conducted for tACS (Antal et al., 2008; Zaehle et al., 2010; Helfrich et al., 2014; Vossen et al., 2015). While a diffuse increase in 8–12 Hz spectral power in the frontal and centroparietal scalp can be observed after rACS, the distribution of the enhancement was comparable to that of the highest physiological α in the EC condition. Additionally, it did not significantly differ from EC regarding effect size, showing a physiologically plausible increase in synchrony. A similar α-enhancement outlasting stimulation duration has already been reported after 10 Hz audiovisual (Rosenfeld et al., 1997) and photic stimulation (Sakamoto et al., 1993; Spaak et al., 2014). This supports the hypothesis of Schutter (2016) that retinal stimulation may induce neural effects similar to tACS. Retinal phosphenes induced by tACS and their possible role in ACS effects of neural oscillations are subject of a long ongoing discussion.

Early work by Kanai et al. (2008) showed phosphene elicitation during occipital tACS in a frequency- and illumination-dependent manner. While this was interpreted as cortical stimulation, others proposed a retinal origin. The higher sensitivity of the retina and an experimentally confirmed occipital-to-frontal threshold decrease supportedthis hypothesis (Schwiedrzik, 2009; Schutter and Hortensius, 2010). Interestingly, moving the electrode from the occipital

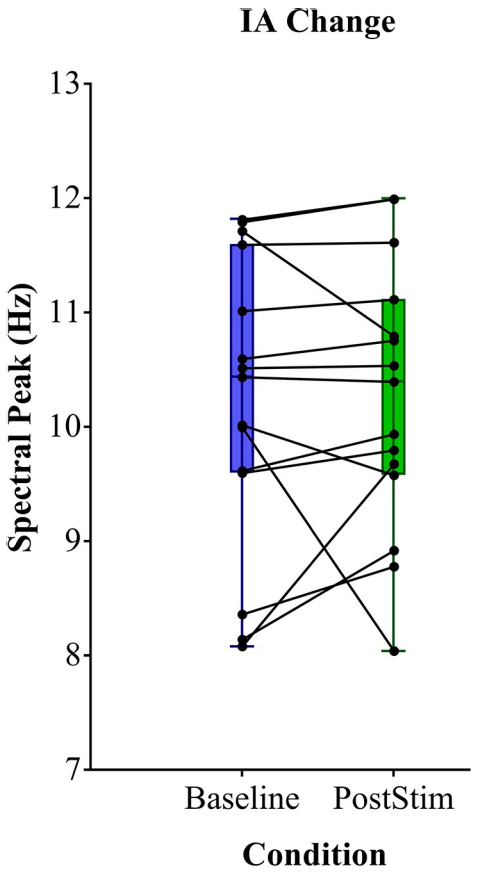

FIGURE 3 | Mean spectral peak analysis. Individual α peak frequency before (blue) and after (green) rACS. Black points and lines depict individual subjects. No consistent shift of the individual α peak to stimulation frequency can be observed.

area towards the retina did not change the latency of phosphene perception (Kar and Krekelberg, 2012). The effects of tACS over the visual cortex may therefore be a result of stimulation along the retinofugal pathway similar to rACS.

Yet, due to the respective montages there should be a magnitude of difference between methods (Peterchev et al., 2012) with rACS inducing the most, traditional occiput-vertex montages intermediate and bilateral or 4 × 1 ring electrode montages inducing the least retinal activation (Paulus, 2010; Neuling et al., 2012; Datta et al., 2013; Laakso and Hirata, 2013).

In sum, we provide further evidence for α-power-enhancement after ACS being induced *via* retinofugal pathway activation.

Entrainment

Neural oscillators, as the target of tACS, share features of relaxation oscillators and harmonic oscillators (Glass, 2001; Winfree, 2001) and therefore present a stable eigenfrequency as well as synchronization capabilities (Somers and Kopell, 1993; Buzsáki and Draguhn, 2004).

This enables entrainment to an external force resulting in effects that could outlast stimulation duration. Such entrainment effects would present themselves as phase- and frequency-locked to stimulation and have been shown *in vitro* (Fröhlich and McCormick, 2010) and in the animal model (Ali et al., 2013).

We observed no such frequency-lock after rACS.

This lack of a shift towards stimulation frequency is also consistent with several reports of tACS α-enhancement post-stimulation (Zaehle et al., 2010; Helfrich et al., 2014; Vossen et al., 2015). Vossen et al. (2015) have also presented this as strong evidence against entrainment, whereas Helfrich et al. (2014) and Zaehle et al., 2010 focused on ACS effects during stimulation *via* artifact rejection. The lack of a correlation between proximity of IAF to stimulation frequency has also been reported for tACS (Helfrich et al., 2014), even going as far as a negative correlation (Vossen et al., 2015). Both groups have reported these findings as counterintuitive. While this presents an argument against entrainment, one would need reliable recordings during stimulation to falsify this hypothesis.

Still, entrainment effects could produce the results at hand in the following ways.

Entrainment During Stimulation

The effects of stimuli on neuronal oscillators are mainly dependent on phase and stimulus amplitude (Glass, 2001). RACS was not applied at IAF and consequently not applied phase-locked as well. Therefore, the stimulus was unlikely to arrive at the opening phase of the target oscillator. This could have been compensated through a higher stimulus amplitude (Ai and Ro, 2014). Although possible for TMS (Thut andMiniussi, 2009), this is unlikely for most types of tES (including rACS), which reach comparatively low current densities at the target site (Poreisz et al., 2007). As we encountered stimulation artifacts as well as a residual stimulation cessation artifact covering the first 100–300 ms after stimulation, an entrainment effect in this time period cannot be reliably investigated and therefore remains a possibility. There are reports presenting such entrainment during stimulation utilizing artifact rejection techniques (Soekadar et al., 2013; Helfrich et al., 2014; Neuling et al., 2017), which are still controversially discussed (Noury et al., 2016).

Frequency Pulling

A stable entrainment during stimulation with an external oscillator would also not explain the immediate loss of synchronization to the external oscillator following stimulus cessation found in this study. A possible explanation is a weak coupling during stimulation, resulting in not a frequency lock, but rather a frequency pulling (Cross et al., 2004)with frequent phase walk-throughs (Ermentrout and Rinzel, 1984). Hereby, an oscillator, instead of fully synchronizing, only appeases the frequency of an external stimulus and desynchronizes quickly (Hoppensteadt and Keener, 1982). This can be the result of the external stimulus being too weak or too far removed from the oscillators eigenfrequency (Cross et al., 2004). A short period of synchronization followed by desynchronization would be the result, which has been reported for the neurophysiologically similar photic stimulation (Jin et al., 2000). A polysynaptic recruitment of further neurons after the

initial driving could then explain the observed enhancement (Ozen et al., 2010).

Subthreshold Modulation

The lack of a frequency appeasement may also be due to the applied stimulation inducing only subthreshold modulation as shown in the rat model (Ozen et al., 2010). This would result in a lower threshold for network-induced membrane voltage fluctuations to generate spikes in a fraction of the neuronal population. The effects of transcranial stimulation, specifically tES, are mostly attributed to such subthreshold modulation (Zaghi et al., 2010). In contrast to the cortical stimulation of tES, rACS stimulated the more excitable retina (Lindenblatt and Silny, 2002) with amplitudes above phosphene threshold. We can therefore assume the generation of action potentials at least in the visual cell (Grützner et al., 1958; Schutter and Hortensius, 2010). Nevertheless, a threshold-lowering effect of rACS would provide an explanation for similar effects on oscillations found after retinal random noise stimulation (RNS; Jooss et al., 2015) through the mechanism of stochastic resonance (Wiesenfeld and Moss, 1995; Gluckman et al., 1996).

Rebound

As the entrainment-hypothesis delivers no conclusive explanation for the alpha enhancement following ACS, our findings can be interpreted as evidence for a rebound effect.

Rebound firing is a long known mechanism of action for signal transmission in neurons (Creutzfeldt et al., 1962; Grenier et al., 1998). Diffuse electric fields have been shown to promote a rebound-like burst firing compared to direct somatic current injection (Radman et al., 2009), especially after cessation of stimulation (Reato et al., 2010). Several studies presented rebound firing in thalamocortical networks and neurons in response to oscillatory electric stimuli (Destexhe et al., 1996; Birdno et al., 2014; Sakata, 2016). Thalamocortical networks are well-known to be vitally involved in the generation of the α rhythm (Hughes and Crunelli, 2005).

TMS effects on neural oscillations have also been attributed to rebound firing, with studies finding an α- and/or β-enhancement following single TMS-Pulses (Paus et al., 2001; Fuggetta et al., 2005; Brignani et al., 2008). Paus et al. (2001) explained this as a combination of rebound-like firing and recruitment of ''idle'' neurons. Fuggetta et al. (2005) as well as Brignani et al., 2008 discuss α-rebound as a possible effect of corticothalamic feedback mechanisms. Following thalamic input, primary visual cortex layer 6 neurons are able to deliver antiphasic feedback to the lateral geniculate nucleus (Yousif and Denham, 2007), which in turn may result in burst firing inducing α- or θ-rhythms (Hughes and Crunelli, 2005). This is particularly interesting, as cortical tACS should activate layer 6 neurons as well and might trigger similar rebound mechanisms.

Applying these considerations, the findings reported in this article can be interpreted as follows: a possible initial entrainment of layer 4 and 6 neurons could result in a corticothalamic feedback triggering thalamic rebound firing, which would drive the cortically observed α-rhythm and lead to further polysynaptic recruitment of cortical neurons. The rebound effect could explain an enhancement and stabilization at an intrinsic frequency after neither phase- nor frequency-locked stimulation (Perkel and Mulloney, 1974). This would also sufficiently explain the lack of correlation between proximity of stimulation frequency to IAF and α-power increase also reported by Helfrich et al. (2014). However, due to the lack of recordings during stimulation, we cannot report a period of inhibition preceding a rebound (Perkel and Mulloney, 1974).

Generally, the challenges of the rebound hypothesis lie in frequency-specific psychophysical effects of ACS reported during stimulation (Pogosyan et al., 2009; Joundi et al., 2012) and the findings of photic driving (Walker et al., 1944), especially as we stimulate the retina and the optic nerve.

Outlook

A combination of entrainment and rebound hypotheses as presented above may prospectively provide an integrative model of ACS effects on the α-rhythm. Investigation of closed-loop phase-locked stimulation (Brittain et al., 2013) as well as better knowledge of psychophysical changes during stimulation could contribute to a deeper understanding of such mechanisms of action.

Users of tACS in the visual system should take note of the accumulating evidence of a retinal contribution, at least to the effects of cortical α power. Furthermore, cortical tACS should activate layer 6 neurons similar to rACS and might therefore trigger the same rebound mechanisms described in this article instead of achieving entrainment effects.

Finally, a thorough study of the effects achieved by frequency-unspecific RNS, especially in combination with tACS, could provide further insight into subthreshold modulation induced by tES. This would be an intriguing step towards the understanding of a common framework utilizing both noise and oscillation in the human brain (Schmidt et al., 2013b).

AUTHOR CONTRIBUTIONS

LH, SS, MS and SB conceived and designed the study. LH, SS, LK and AJ carried out data acquisition and analysis. All authors participated in the interpretation of the data. The manuscript was drafted by LH and SS and critically revised by AJ, AK, MR, MS, LK and SB.

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REFERENCES

Ai, L., and Ro, T. (2014). The phase of prestimulus alpha oscillations affects tactile perception. *J. Neurophysiol.* 111, 1300–1307. doi: 10.1152/jn.00125.2013

Ali, M. M., Sellers, K. K., and Fröhlich, F. (2013). Transcranial alternating current stimulation modulates large-scale cortical network activity by networkresonance. *J. Neurosci.* 33, 11262–11275. doi: 10.1523/JNEUROSCI.5867-12. 2013

Antal, A., Boros, K., Poreisz, C., Chaieb, L., Terney, D., and Paulus, W. (2008). Comparatively weak after-effects of transcranial alternating currentstimulation (tACS) on cortical excitability in humans. *Brain Stimul.* 1, 97–105. doi: 10.1016/j.brs.2007.10.001

Berger, H. (1929). Über das elektrenkephalogramm des menschen. *Eur. Arch. Psychiatry Clin. Neurosci.* 87, 527–570.

Birdno, M. J., Tang, W., Dostrovsky, J. O., Hutchison, W. D., and Grill, W. M. (2014). Response of human thalamic neurons to high-frequency stimulation. *PLoS One* 9:e96026. doi: 10.1371/journal.pone.0096026

Brignani, D., Manganotti, P., Rossini, P. M., and Miniussi, C. (2008). Modulationof cortical oscillatory activity during transcranial magnetic stimulation. *Hum. Brain Mapp.* 29, 603–612. doi: 10.1002/hbm.20423

Brindley, G. S. (1955). The site of electrical excitation of the human eye. *J. Physiol.*127, 189–200. doi: 10.1113/jphysiol.1955.sp005248

Brittain, J. S., Probert-Smith, P., Aziz, T. Z., and Brown, P. (2013). Tremor suppression by rhythmic transcranial current stimulation. *Curr. Biol.* 23, 436–440. doi: 10.1016/j.cub.2013.01.068

Brunoni, A. R., Nitsche, M. A., Bolognini, N., Bikson, M., Wagner, T., Merabet, L., et al. (2012). Clinical research with transcranial direct current stimulation (tDCS): challenges and future directions. *Brain Stimul.* 5, 175–195. doi: 10.1016/j.brs.2011.03.002

Buzsáki, G., and Draguhn, A. (2004). Neuronal oscillations in cortical networks. *Science* 304, 1926–1929. doi: 10.1126/science.1099745

Cecere, R., Rees, G., and Romei, V. (2015). Individual differences in alpha frequency drive crossmodal illusory perception. *Curr. Biol.* 25, 231–235. doi: 10.1016/j.cub.2014.11.034

Creutzfeldt, O. D., Fromm, G. H., and Kapp, H. (1962). Influence of transcortical d-c currents on cortical neuronal activity. *Exp. Neurol.* 5, 436–452. doi: 10.1016/0014-4886(62)90056-0

Cross, M. C., Zumdieck, A., Lifshitz, R., and Rogers, J. L. (2004). Synchronization by nonlinear frequency pulling. *Phys. Rev. Lett.* 93:224101. doi: 10.1103/PhysRevLett.93.224101

Datta, A., Zhou, X., Su, Y., Parra, L. C., and Bikson, M. (2013). Validation of finite element model of transcranial electrical stimulation using scalp potentials: implications for clinical dose. *J. Neural Eng.* 10:036018. doi: 10.1088/1741- 2560/10/3/036018

Destexhe, A., Bal, T., McCormick, D. A., and Sejnowski, T. J. (1996). Ionic mechanisms underlying synchronized oscillations and propagating waves in amodel of ferret thalamic slices. *J. Neurophysiol.* 76, 2049–2070. doi: 10.1152/jn. 1996.76.3.2049

Ermentrout, G. B., and Rinzel, J. (1984). Beyond a pacemaker's entrainment limit: phase walk-through. *Am. J. Physiol.* 246, R102–R106. doi: 10.1152/ajpregu. 1984.246.1.r102

Feurra, M., Pasqualetti, P., Bianco, G., Santarnecchi, E., Rossi, A., and Rossi, S. (2013). State-dependent effects of transcranial oscillatory currents on the motor system: what you think matters. *J. Neurosci.* 33, 17483–17489. doi: 10.1523/JNEUROSCI.1414-13.2013

Fröhlich, F., and McCormick, D. A. (2010). Endogenous electric fields may guide neocortical network activity. *Neuron* 67, 129–143. doi: 10.1016/j.neuron.2010. 06.005

Fuggetta, G., Fiaschi, A., and Manganotti, P. (2005). Modulation of cortical oscillatory activities induced by varying single-pulse transcranial magnetic stimulation intensity over the left primary motor area: a combined EEG and TMS study. *Neuroimage* 27, 896–908. doi: 10.1016/j.neuroimage.2005. 05.013

Gall, C., Fedorov, A. B., Ernst, L., Borrmann, A., and Sabel, B. A. (2010). Repetitive transorbital alternating current stimulation in optic neuropathy.*NeuroRehabilitation* 27, 335–341. doi: 10.3233/NRE-2010-0617

Gall, C., Sgorzaly, S., Schmidt, S., Brandt, S., Fedorov, A., and Sabel, B. A. (2011). Noninvasive transorbital alternating current stimulation improves subjective visual functioning and vision-related quality of life in optic neuropathy. *BrainStimul.* 4, 175–188. doi: 10.1016/j.brs.2011.07.003

Glass, L. (2001). Synchronization and rhythmic processes in physiology. *Nature* 410, 277–284. doi: 10.1038/35065745

Gluckman, B. J., Netoff, T. I., Neel, E. J., Ditto, W. L., Spano, M. L.,and Schiff, S. J. (1996). Stochastic resonance in a neuronal network from mammalian brain. *Phys. Rev. Lett.* 77, 4098–4101. doi: 10.1103/PhysRevLett.77.4098

Gray, C. M., and Singer, W. (1989). Stimulus-specific neuronal oscillations in orientation columns of cat visual-cortex. *Proc. Natl. Acad. Sci. U S A* 86, 1698–1702. doi: 10.1073/pnas.86.5.1698

Grenier, F., Timofeev, I., and Steriade, M. (1998). Leading role of thalamic overcortical neurons during postinhibitory rebound excitation. *Proc. Natl. Acad.Sci. U S A* 95, 13929–13934. doi: 10.1073/pnas.95.23.13929

Grützner, A., Grüsser, O.-J., and Baumgartner, G. (1958). Reaktionen einzelner neurone im optischen cortex der Katze nach elektrischer Reizung des nervus opticus. *Arch. Psychiatr. Nervenkr. Z. Gesamte. Neurol. Psychiatr.* 197, 377–404. doi: 10.1007/BF00345845

Hallett, M. (2005). Neuroplasticity and rehabilitation. *J. Rehabil. Res. Dev.* 42, xvii–xxii. doi: 10.1682/JRRD.2005.07.0126

Hanslmayr, S., Sauseng, P., Doppelmayr, M., Schabus, M., and Klimesch, W. (2005). Increasing individual upper alpha power by neurofeedback improves cognitive performance in human subjects. *Appl. Psychophysiol. Biofeedback* 30, 1–10. doi: 10.1007/s10484-005-2169-8

Helfrich, R. F., Schneider, T. R., Rach, S., Trautmann-Lengsfeld, S. A., Engel, A. K.,and Herrmann, C. S. (2014). Entrainment of brain oscillations by transcranial alternating current stimulation. *Curr. Biol.* 24, 333–339. doi: 10.1016/j.cub. 2013.12.041

Herrick, R. M. (1967). Psychophysical methodology: comparison of thresholds of the method of limits and of the method of constant stimuli. *Percept. Mot. Skills* 24, 915–922. doi: 10.2466/pms.1967.24.3.915

Herrmann, C. S., Rach, S., Neuling, T., and Struber, D. (2013). Transcranial alternating current stimulation: a review of the underlying mechanisms and modulation of cognitive processes. *Front. Hum. Neurosci.* 7:279. doi: 10.3389/fnhum.2013.00279

Hoppensteadt, F. C., and Keener, J. P. (1982). Phase locking of biological clocks. *J. Math. Biol.* 15, 339–349. doi: 10.1007/bf00275692

Horvath, J. C., Forte, J. D., and Carter, O. (2015). Evidence that transcranial direct current stimulation (tDCS) generates little-to-no reliable neurophysiologic effect beyond MEP amplitude modulation in healthy human subjects: a systematic review. *Neuropsychologia* 66, 213–236. doi: 10.1016/j. neuropsychologia.2014.11.021

Huang, X., Levine, S., and Paradiso, M. A. (2008). Rebounding V1 activity and a new visual aftereffect. *J. Vis.* 8:25. doi: 10.1167/8.3.25

Hughes, S. W., and Crunelli, V. (2005). Thalamic mechanisms of EEG alpha rhythms and their pathological implications. *Neuroscientist* 11, 357–372. doi: 10.1177/1073858405277450

Hummel, F. C., Celnik, P., Pascual-Leone, A., Fregni, F., Byblow, W. D.,Buetefisch, C. M., et al. (2008). Controversy: noninvasive and invasive cortical stimulation show efficacy in treating stroke patients. *Brain Stimul.* 1, 370–382. doi: 10.1016/j.brs.2008.09.003

Jin, Y., Castellanos, A., Solis, E. R., and Potkin, S. G. (2000). EEG resonant responses in schizophrenia: a photic driving study with improved harmonic resolution. *Schizophr. Res.* 44, 213–220. doi: 10.1016/s0920-9964(99)00211-x

Jooss, A., Schmidt, S., Haberbosch, L., Köhn, A., Scholz, M., and Brandt, S. A. (2015). Investigating the effects of noisy stimulation on the retinofugal pathway. *Brain Stimul.* 8:402. doi: 10.1016/j.brs.2015.01.282

Joundi, R. A., Jenkinson, N., Brittain, J. S., Aziz, T. Z., and Brown, P. (2012). Driving oscillatory activity in the human cortex enhances motor performance. *Curr. Biol.* 22, 403–407. doi: 10.1016/j.cub.2012.01.024

Kanai, R., Chaieb, L., Antal, A., Walsh, V., and Paulus, W. (2008). Frequency-dependent electrical stimulation of the visual cortex. *Curr. Biol.* 18, 1839–1843. doi: 10.1016/j.cub.2008.10.027

Kanai, R., Paulus, W., and Walsh, V. (2010). Transcranial alternating current stimulation (tACS) modulates cortical excitability as assessed by TMS-induced phosphene thresholds. *Clin. Neurophysiol.* 121, 1551–1554. doi: 10.1016/j. clinph.2010.03.022

Kar, K., and Krekelberg, B. (2012). Transcranial electrical stimulation over visual cortex evokes phosphenes with a retinal origin. *J. Neurophysiol.* 108, 2173–2178. doi: 10.1152/jn.00505.2012

Kirson, E. D., Dbalý, V., Tovarys, F., Vymazal, J., Soustiel, J. F., Itzhaki, A.,et al. (2007). Alternating electric fields arrest cell proliferation in animal tumor models and human brain tumors. *Proc. Natl. Acad. Sci. U S A* 104, 10152–10157. doi: 10.1073/pnas.0702916104

Klimesch, W. (1999). EEG alpha and theta oscillations reflect cognitive and memory performance: a review and analysis. *Brain Res.* 29, 169–195. doi: 10.1016/s0165-0173(98)00056-3

Klimesch, W., Sauseng, P., and Gerloff, C. (2003). Enhancing cognitive performance with repetitive transcranial magnetic stimulation at human individual alpha frequency. *Eur. J. Neurosci.* 17, 1129–1133. doi: 10.1046/j. 1460-9568.2003.02517.x

Klimesch, W., Sauseng, P., and Hanslmayr, S. (2007). EEG alpha oscillations: the inhibition-timing hypothesis. *Brain Res. Rev.* 53, 63–88. doi: 10.1016/j. brainresrev.2006.06.003

Kuo, M. F., and Nitsche, M. A. (2012). Effects of transcranial electrical stimulationon cognition. *Clin. EEG Neurosci.* 43, 192–199. doi: 10.1177/1550059412444975 Laakso, I., and Hirata, A. (2013). Computational analysis shows why transcranialalternating current stimulation induces retinal phosphenes. *J. Neural Eng.* 10:046009. doi: 10.1088/1741-2560/10/4/046009

Lange, J., Keil, J., Schnitzler, A., van Dijk, H., and Weisz, N. (2014). The roleof alpha oscillations for illusory perception. *Behav. Brain Res.* 271, 294–301. doi: 10.1016/j.bbr.2014.06.015

Lindenblatt, G., and Silny, J. (2002). Electrical phosphenes: on the influence of conductivity inhomogeneities and small-scale structures of the orbita on the current density threshold of excitation. *Med. Biol. Eng. Comput.* 40, 354–359. doi: 10.1007/bf02344219

Manganotti, P., Formaggio, E., Storti, S. F., De Massari, D., Zamboni, A., Bertoldo, A., et al. (2012). Time-frequency analysis of short-lasting modulation of EEG induced by intracortical and transcallosal paired TMS over motor areas.*J. Neurophysiol.* 107, 2475–2484. doi: 10.1152/jn.00543.2011

Marshall, L., Helgadóttir, H., Mölle, M., and Born, J. (2006). Boostingslow oscillations during sleep potentiates memory. *Nature* 444, 610–613. doi: 10.1038/nature05278

Mathewson, K. E., Gratton, G., Fabiani, M., Beck, D. M., and Ro, T. (2009). To see or not to see: prestimulus alpha phase predicts visual awareness. *J. Neurosci.* 29,2725–2732. doi: 10.1523/JNEUROSCI.3963-08.2009

Neuling, T., Ruhnau, P., Weisz, N., Herrmann, C. S., and Demarchi, G. (2017).Faith and oscillations recovered: on analyzing EEG/MEG signals during tACS. *Neuroimage* 15, 960–963. doi: 10.1016/j.neuroimage.2016.11.022

Neuling, T., Wagner, S., Wolters, C. H., Zaehle, T., and Herrmann, C. S.(2012). Finite-element model predicts current density distribution for clinical applications of tDCS and tACS. *Front. Psychiatry* 3:83. doi: 10.3389/fpsyt.2012. 00083

Noury, N., Hipp, J. F., and Siegel, M. (2016). Physiological processes non-linearly affect electrophysiological recordings during transcranial electric stimulation. *Neuroimage* 140, 99–109. doi: 10.1016/j.neuroimage.2016.03.065

Oostenveld, R., Fries, P., Maris, E., and Schoffelen, J. M. (2011). FieldTrip: open source software for advanced analysis of MEG, EEG and invasive electrophysiological data. *Comput. Intell. Neurosci.* 2011:156869. doi: 10.1155/ 2011/156869

Ozen, S., Sirota, A., Belluscio, M. A., Anastassiou, C. A., Stark, E., Koch, C., et al. (2010). Transcranial electric stimulation entrains cortical neuronal populations in rats. *J. Neurosci.* 30, 11476–11485. doi: 10.1523/JNEUROSCI.5252-09.2010

Pape, H. C., and McCormick, D. A. (1995). Electrophysiological and pharmacological properties of interneurons in the cat dorsal lateral geniculate nucleus. *Neuroscience* 68, 1105–1125. doi: 10.1016/0306-4522(95) 00205-w

Paulus, W. (2010). On the difficulties of separating retinal from cortical originsof phosphenes when using transcranial alternating current stimulation (tACS). *Clin. Neurophysiol.* 121, 987–991. doi: 10.1016/j.clinph.2010.01.029

Paulus, W. (2011). Transcranial electrical stimulation (tES - tDCS; tRNS, tACS) methods. *Neuropsychol. Rehabil.* 21, 602–617. doi: 10.1080/09602011.2011. 557292

Paus, T., Sipila, P. K., and Strafella, A. P. (2001). Synchronization of neuronal activity in the human primary motor cortex by transcranial magnetic stimulation: an EEG study. *J. Neurophysiol.* 86, 1983–1990. doi: 10.1152/jn. 2001.86.4.1983

Perkel, D. H., and Mulloney, B. (1974). Motor pattern production in reciprocally inhibitory neurons exhibiting postinhibitory rebound. *Science* 185, 181–183. doi: 10.1126/science.185.4146.181

Peterchev, A. V., Wagner, T. A., Miranda, P. C., Nitsche, M. A., Paulus, W., Lisanby, S. H., et al. (2012). Fundamentals of transcranial electric and magneticstimulation dose: definition, selection and reporting practices. *Brain Stimul.* 5, 435–453. doi: 10.1016/j.brs.2011.10.001

Pogosyan, A., Gaynor, L. D., Eusebio, A., and Brown, P. (2009). Boosting cortical activity at Beta-band frequencies slows movement in humans. *Curr. Biol.* 19, 1637–1641. doi: 10.1016/j.cub.2009.07.074

Poreisz, C., Boros, K., Antal, A., and Paulus, W. (2007). Safety aspects of transcranial direct current stimulation concerning healthy subjects andpatients. *Brain Res. Bull.* 72, 208–214. doi: 10.1016/j.brainresbull.2007.01.004

Radman, T., Ramos, R. L., Brumberg, J. C., and Bikson, M. (2009). Role of cortical cell type and morphology in subthreshold and suprathreshold uniform electric field stimulation *in vitro*. *Brain Stimul.* 2, 215–228.e3. doi: 10.1016/j.brs.2009.03.007

Rager, G., and Singer, W. (1998). The response of cat visual cortex to flicker stimuliof variable frequency. *Eur. J. Neurosci.* 10, 1856–1877. doi: 10.1046/j.1460-9568. 1998.00197.x

Reato, D., Rahman, A., Bikson, M., and Parra, L. C. (2010). Low-intensity electrical stimulation affects network dynamics by modulating population rate and spiketiming. *J. Neurosci.* 30, 15067–15079. doi: 10.1523/JNEUROSCI.2059-10.2010

Rosenfeld, J. P., Reinhart, A. M., and Srivastava, S. (1997). The effects of alpha(10-Hz) and beta (22-Hz) ''entrainment'' stimulation on the alpha and betaEEG bands: individual differences are critical to prediction of effects. *Appl. Psychophysiol. Biofeedback* 22, 3–20. doi: 10.1023/A:1026233624772

Ruhnau, P., Neuling, T., Fuscá, M., Herrmann, C. S., Demarchi, G., and Weisz, N. (2016). Eyes wide shut: transcranial alternating current stimulation drives alpharhythm in a state dependent manner. *Sci. Rep.* 6:27138. doi: 10.1038/srep 27138

Sakamoto, H., Inouye, T., and Shinosaki, K. (1993). Preservation of alpha rhythm shortly after photic driving. *Int. J. Neurosci.* 73, 227–233. doi: 10.3109/00207459308986673

Sakata, S. (2016). State-dependent and cell type-specific temporal processing in auditory thalamocortical circuit. *Sci. Rep.* 6:18873. doi: 10.1038/srep18873 Samaha, J., and Postle, B. R. (2015). The speed of alpha-band oscillations predicts the temporal resolution of visual perception. *Curr. Biol.* 25, 2985–2990.doi: 10.1016/j.cub.2015.10.007

Sandrini, M., and Cohen, L. G. (2013). Noninvasive brain stimulation in neurorehabilitation. *Handb. Clin. Neurol.* 116, 499–524. doi: 10.1016/b978-0-444-53497-2.00040-1

Santarnecchi, E., Polizzotto, N. R., Godone, M., Giovannelli, F., Feurra, M., Matzen, L., et al. (2013). Frequency-dependent enhancement of fluid intelligence induced by transcranial oscillatory potentials. *Curr. Biol.* 23, 1449–1453. doi: 10.1016/j.cub.2013.06.022

Schmidt, S., Mante, A., Rönnefarth, M., Fleischmann, R., Gall, C., and Brandt, S. A. (2013a). Progressive enhancement of alpha activity and visual function in patients with optic neuropathy: a two-week repeated session alternating current stimulation study. *Brain Stimul.* 6, 87–93. doi: 10.1016/j.brs.2012.03.008

Schmidt, S., Scholz, M., Obermayer, K., and Brandt, S. A. (2013b). Patterned brain stimulation, what a framework with rhythmic and noisy components might tell us about recovery maximization. *Front. Hum. Neurosci.* 7:325. doi: 10.3389/fnhum.2013.00325

Schutter, D. J. (2016). Cutaneous retinal activation and neural entrainment in transcranial alternating current stimulation: a systematic review. *Neuroimage* 140, 83–88. doi: 10.1016/j.neuroimage.2015.09.067

Schutter, D. J., and Hortensius, R. (2010). Retinal origin of phosphenesto transcranial alternating current stimulation. *Clin. Neurophysiol.* 121, 1080–1084. doi: 10.1016/j.clinph.2009.10.038

Schwiedrzik, C. M. (2009). Retina or visual cortex? The site of phosphene induction by transcranial alternating current stimulation. *Front. Integr. Neurosci.* 3:6. doi: 10.3389/neuro.07.006.2009

Siebner, H. R., Lang, N., Rizzo, V., Nitsche, M. A., Paulus, W., Lemon, R. N., et al. (2004). Preconditioning of low-frequency repetitive transcranial magnetic stimulation with transcranial direct current stimulation: evidence for

homeostatic plasticity in the human motor cortex. *J. Neurosci.* 24, 3379–3385.doi: 10.1523/JNEUROSCI.5316-03.2004
Soekadar, S. R., Witkowski, M., Cossio, E. G., Birbaumer, N., Robinson, S. E., and Cohen, L. G. (2013). *In vivo* assessment of human brain oscillations during application of transcranial electric currents. *Nat. Commun.* 4:2032. doi: 10.1038/ncomms3032
Somers, D., and Kopell, N. (1993). Rapid synchronization through fast threshold modulation. *Biol. Cybern.* 68, 393–407. doi: 10.1007/bf00198772
Spaak, E., de Lange, F. P., and Jensen, O. (2014). Local entrainment of alpha oscillations by visual stimuli causes cyclic modulation of perception.*J. Neurosci.* 34, 3536–3544. doi: 10.1523/JNEUROSCI.4385-13.2014
Surwillo, W. W. (1961). Frequency of alpha rhythm, reaction time and age. *Nature*191, 823–824. doi: 10.1038/191823a0
Talelli, P., and Rothwell, J. (2006). Does brain stimulation after strokehave a future? *Curr. Opin. Neurol.* 19, 543–550. doi: 10.1097/WCO. 0b013e32801080d1
Thut, G., and Miniussi, C. (2009). New insights into rhythmic brain activity from TMS-EEG studies. *Trends Cogn. Sci.* 13, 182–189. doi: 10.1016/j.tics.2009. 01.004
Tong, Z. Y., Overton, P. G., and Clark, D. (1996). Stimulation of theprefrontal cortex in the rat induces patterns of activity in midbrain dopaminergic neurons which resemble natural burst events. *Synapse* 22, 195–208. doi: 10.1002/(SICI)1098-2396(199603)22:3<195::AID-SYN1>3.0. CO;2-7
VanRullen, R., and Koch, C. (2003). Is perception discrete or continuous? *Trends Cogn. Sci.* 7, 207–213. doi: 10.1016/S1364-6613(03)00095-0
Vossen, A., Gross, J., and Thut, G. (2015). Alpha power increase after transcranial alternating current stimulation at alpha frequency (α-tACS) reflects plasticchanges rather than entrainment. *Brain Stimul.* 8, 499–508. doi: 10.1016/j.brs.2014.12.004
Wach, C., Krause, V., Moliadze, V., Paulus, W., Schnitzler, A., and Pollok, B. (2013). The effect of 10 Hz transcranial alternating current stimulation(tACS) on corticomuscular coherence. *Front. Hum. Neurosci.* 7:511. doi: 10.3389/fnhum.2013.00511
Walker, A., Woolf, J. I., Halstead, W. C., and Case, T. J. (1944). Photic driving. *Arch. Neurol. Psychiatry* 52:117. doi: 10.1001/archneurpsyc.1944. 02290320032004
Wiesenfeld, K., and Moss, F. (1995). Stochastic resonance and the benefitsof noise: from ice ages to crayfish and SQUIDs. *Nature* 373, 33–36.doi: 10.1038/373033a0
Winfree, A. T. (2001). *The Geometry of Biological Time.* New York: Springer Science and Business Media.
Yousif, N., and Denham, M. (2007). The role of cortical feedback in the generation of the temporal receptive field responses of lateral geniculate nucleus neurons: a computational modelling study. *Biol. Cybern.* 97, 269–277. doi: 10.1007/s00422-007-0171-3
Zaehle, T., Rach, S., and Herrmann, C. S. (2010). Transcranial alternating current stimulation enhances individual alpha activity in human EEG. *PLoS One* 5:e13766. doi: 10.1371/journal.pone.0013766
Zaghi, S., Acar, M., Hultgren, B., Boggio, P. S., and Fregni, F. (2010). Noninvasive brain stimulation with low-intensity electrical currents: putative mechanisms of action for direct and alternating current stimulation. *Neuroscientist* 16, 285–307. doi: 10.1177/1073858409336227
Zoefel, B., Huster, R. J., and Herrmann, C. S. (2011). Neurofeedback training of the upper alpha frequency band in EEG improves cognitive performance. *Neuroimage* 54, 1427–1431. doi: 10.1016/j.neuroimage.2010.08.078

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Motor Task-Dependent Dissociated Effects of Transcranial Random Noise Stimulation in a Finger-Tapping Task Versus a Go/No-Go Task on Corticospinal Excitability and Task Performance

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Background and Objective: Transcranial random noise stimulation (tRNS) is an emerging non-invasive brain stimulation technique to modulate brain function, with previous studies highlighting its considerable benefits in therapeutic stimulation of the motor system. However, high variability of results and bidirectional task-dependent effects limit more widespread clinical application. Task dependency largely results from a lack of understanding of the interaction between externally applied tRNS and the endogenous state of neural activity during stimulation. Hence, the aim of this study was to investigate the task dependency of tRNS-induced neuromodulation in the motor system using a finger-tapping task (FT) versus a go/no-go task (GNG). We hypothesized that the tasks would modulate tRNS' effects on corticospinal excitability (CSE) and task performance in opposite directions.

Methods: Thirty healthy subjects received 10 min of tRNS of the dominant primary motor cortex in a double-blind, sham-controlled study design. tRNS was applied during two well-established tasks tied to diverging brain states. Accordingly, participants were randomly assigned to two equally-sized groups: the first group performed a simple motor training task (FT task), known primarily to increase CSE, while the second group performed an inhibitory control task (go/no-go task) associated with inhibition of CSE. To establish task-dependent effects of tRNS, CSE was evaluated prior to- and after stimulation with navigated transcranial magnetic stimulation.

Results: In an 'activating' motor task, tRNS during FT significantly facilitated CSE. FT task performance improvements, shown by training-related reductions in intertap intervals and increased number of finger taps, were similar for both tRNS and sham stimulation. In an 'inhibitory' motor task, tRNS during GNG left CSE unchanged while

inhibitory control was enhanced as shown by slowed reaction times and enhanced task accuracy during and after stimulation.

Conclusion: We provide evidence that tRNS-induced neuromodulatory effects are task-dependent and that resulting enhancements are specific to the underlyingtask-dependent brain state. While mechanisms underlying this effect require further investigation, these findings highlight the potential of tRNS in enhancing task-dependent brain states to modulate human behavior.

Keywords: random noise stimulation, transcranial electrical stimulation, task dependency, finger-tapping task, go/no-go task, corticospinal excitability, neuroplasticity

INTRODUCTION

Transcranial electrical stimulation applied to the primary motor cortex is a non-invasive, portable, and low-cost method shown to enhance motor function in healthy subjects and maximize recovery after stroke (Talelli and Rothwell, 2006; Hummel et al., 2008). In addition to tDCS, tRNS is emerging as a promising neuromodulatory tool (Terney et al., 2008; Schmidt et al., 2013b; Prichard et al., 2014). In contrast to the constant direct current of tDCS, tRNS uses a biphasic alternating current with a random amplitude and frequency, drawn from a frequency range between 0.1–640 Hz (full spectrum) or 100–640 Hz (high-frequency). While tDCS modulates resting membrane potential, tRNS is understood to facilitate transmission of existing subthreshold neural activity to increase neuron excitability (Terney et al., 2008; Schmidt et al., 2013b).

Transcranial random noise stimulation is reported to provide considerable benefits over tDCS including polarity independence of stimulation effects (Terney et al., 2008), more pronounced effect sizes (Fertonani et al., 2011) and possibly improved reliability (Antal et al., 2010). Interestingly, tRNS has been suggested to be a vital component in a patterned, individualized stimulation algorithm aiming to maximize recovery after stroke (Schmidt et al., 2013b). Together, these findings suggest that tRNS might be more reliable, safer and better suited for therapeutic stimulation of the motor system.

However, a major and largely unresolved challenge across all transcranial electrical stimulation methods is the high variability of results, limiting more widespread clinical application. Important factors influencing interindividual variability in transcranial electrical stimulation studies are the baseline neuronal level of motor and cognitive function, psychological factors, circadian rhythm, genetics, anatomy, age, and variability in assessment methods (e.g., TMS) (Li et al., 2015). Additionally, since the state of neuron populations during stimulation is likely to play a pivotal role for the final behavioral effect, a significant part of variability is understood to be related to the brain's task dependent activity state during stimulation (Silvanto et al., 2008; Li et al., 2015). The term brain state is used to describe characteristic changes in global brain activity dynamically adjusted to task demands (Gilbert and Sigman, 2007; Lee and Dan, 2012). Task dependency is a well-established phenomenon in non-invasive brain stimulation studies (Antalet al., 2007; Silvanto et al., 2008; Terney et al., 2008). It implies thatthe neuromodulatory effects of non-invasive brain stimulation might vary strongly dependent on the endogenous brain state both prior to as well as during stimulation.

In the motor system, CSE, acquired by TMS, is an electrophysiological parameter providing a direct, temporally and spatially precise readout to monitor task-dependent activation and inhibition via MEPs. CSE quantifies state changes of the stimulated motor cortex by probing post-synaptic corticospinal projections (Bestmann and Krakauer, 2015).

Studies aiming to modulate CSE and induce behavioral changes with tRNS highlight the controversial role of task-dependent brain states. tRNS was shown to havebidirectional task-dependent effects on CSE, which is associated with motor learning and recovery. tRNS applied *offline*, i.e., in idle subjects, was shown to increase CSE (Terneyet al., 2008). Motor and cognitive tasks carried out *online*,i.e., during stimulation were shown to reduce CSE (Terneyet al., 2008). Nevertheless, motor skill learning enhancements were found to be driven primarily by online effects during stimulation (Prichard et al., 2014). Saiote and colleagues investigated functional magnetic resonance imaging changes following a visuomotor task with online tRNS and found stimulation related blood-oxygen-level dependent changes only in regions related to the task, implying direct interaction of online tRNS with task related activity (Saiote et al.,2013). Results from these and other studies conducted in the visual- and cognitive domains (Fertonani et al., 2011; Pirulli et al., 2013; Snowball et al., 2013) suggest that the neuromodulatory effects of tRNS are dependent on *whether*a task and *what type* of task is performed online during stimulation, with enhancements specific to the engaged neural population or brain state.

The aim of this study was to investigate the task dependency of tRNS-induced neuromodulation in the motor system. The hypothesis of this study was that tRNS would modulate task effects in opposite directions, dependingon the underlying brain state. Hence, for tRNS during a simple motor training task (FT task), known primarily to increase CSE, we hypothesize an increase in CSE and

Abbreviations: CSE, corticospinal excitability; FT, finger-tapping; GNG, go/no- go; ITI, intertap interval; MEP, motor evoked potential; nTMS, navigatedtranscranial magnetic stimulation; RT, reaction time; tDCS, transcranial direct current stimulation; TMS, transcranial magnetic stimulation; tRNS, transcranial random noise stimulation.

behavioral performance (Koeneke et al., 2006). For tRNS during an inhibitory control task (GNG task), associated with inhibition of CSE, we hypothesize a decrease in CSEand enhanced behavioral performance reflecting greaterinhibition (Bestmann and Duque, 2016).

For this purpose, we closely monitored online as well as offline changes of behavioral and electrophysiological parameters that are established indicators of task-dependent brain states (Schmidt et al., 2013b). As the primary electrophysiological parameter, CSE was acquired via MEPs by nTMS. Compared to conventional, non-navigated TMS, nTMS uses an optical tracking system to control the physical variance relatedto the 3D parameters of the TMS coil in space. Since small divergences in TMS coil location and orientation can lead to significant variance in CSE estimates, nTMS is an often neglected, but essential prerequisite to reliably quantify changes of task-dependent brain states (Schmidt et al., 2009). Understanding the interaction between tRNS and task-dependent brain activity is imperative for increasing reliability, repeatability, and ultimately, therapeutic usefulness of this emerging neuromodulatory technique.

MATERIALS AND METHODS

Participants

Thirty healthy, right-handed individuals (18 females, mean age 22.8 ± 2.8 years) received tRNS as well as sham stimulation to the dominant (left) primary motor cortex. All participants were right handed as assessed with the Edinburgh handedness inventory. General exclusion criteria for non-invasive brain stimulation were applied (Brunoni et al., 2011). Specifically, none of the subjects had a history of neurological disease, including movement disorders or epilepsy (Brunoni et al., 2011). All participants gave written informed consent. The study was approved by the local ethics committee and adheres to the principles of good clinical practice of the Charité – Universitätsmedizin Berlin ("Grundsätze der Charité zur Sicherung guter wissenschaftlicher Praxis"), as well as "The Code of Ethics of the World Medical Association" (Declaration of Helsinki).

Experimental Paradigm

A double-blind sham-controlled design was used in this study. The participants were randomly divided into two groups according to the task they were to perform during tRNS or sham stimulation: one group (15 participants) performed an 'activating' task (FT task) during stimulation, known primarily to increase CSE. The other 15 participants performed an 'inhibitory' task (GNG task), associated with inhibition of CSE. Behavioral and electrophysiological measurements were acquired offline in a baseline condition prior to stimulation, and apost-stimulation condition following 10 min of stimulation. Offline measurements were complemented by online behavioral assessments during stimulation as described below and in **Figure 1**. In this context, it is important to note that tasks served two functions during stimulation: they are indicators of task performance changes in response to stimulation and utilized to induce a well-established task-dependent brain state (**Figure 1**).

Finger-Tapping Task (FT Task)

The experimental timeline for the FT task is depicted in **Figure 1A**. For the FT task, subjects were instructed to use the index finger of either hand to repeatedly exert a vertical force on a standard telegraph key as quickly and regularly as possible while receiving visual feedback on a screen. Visual feedback was provided with a live graphical display of ITIs on the x-axis and the corresponding number of taps on the y-axis. For the first block, the starting hand was randomly allocated and the tapping duration for one hand was 30 s before switching to the other hand for 30 s (Schulze et al., 2002). Two blocks for each hand (i.e., 4 × 30 s = 2 min) were followed by a 120 s pause (60 s pause during stimulation) to avoid excessive build- up of fatigue (Rönnefarth et al., 2018). As another precaution, the vertical force required to complete a tapping motion was adjusted to the lowest possible setting. Preventing excessive fatigue with regular pauses served to minimize its confounding influence on CSE (Terney et al., 2008). Prior to the experiment, participants were instructed and practiced the task for two blocks for each hand, resulting in a total of 1 min practice for each hand. The baseline condition consisted of two blocks for each hand, the stimulation condition (10 min) consisted of six blocks for each hand and the post-stimulation condition consisted of four blocks for each hand.

Go/No-Go Task (GNG Task)

The experimental timeline for the GNG task is depicted in **Figure 1B**. One GNG trial with a total duration of 2.5–3 s followed the following time course: first, a fixation cross was presented on a screen, which lasted 1 s and was followed by a 250 ms warning cue (yellow square) (Joundi et al., 2012). Subsequently, a 250 ms target cue was presented with a varied latency of 250–750 ms based on an underlying, linearly increasing hazard rate, in line with (Schoffelen et al., 2005). Subjects exerted a maximal horizontal force on the lever only when a "go" cue (green circle) appeared (91%), while 9% of target cues were "no-go" cues (red circle) (Schoffelen et al., 2005). The hazard rate and the low probability of "no-go" trials were utilized to ensure optimal inhibition-related activity (Schoffelen et al., 2005; Wessel, 2018). The response period was limited to 750 ms.

During the GNG task, when no response was required, subjects maintained a horizontal isometric force of 4% of maximum voluntary contraction, with the index fingerof the dominant hand on a lever, in line with (Kristevaet al., 2007). A low force output was used since it was shown to effectively enable corticospinal interaction and recruit most neurons in M1 (Evarts et al., 1983; Kristevaet al., 2007). The predetermined force was monitored throughout task execution and verbal feedback was givenin case of deviations.

Prior to the experiment, participants were instructed and practiced 10 GNG trials. One block consisted of 37 GNG trials

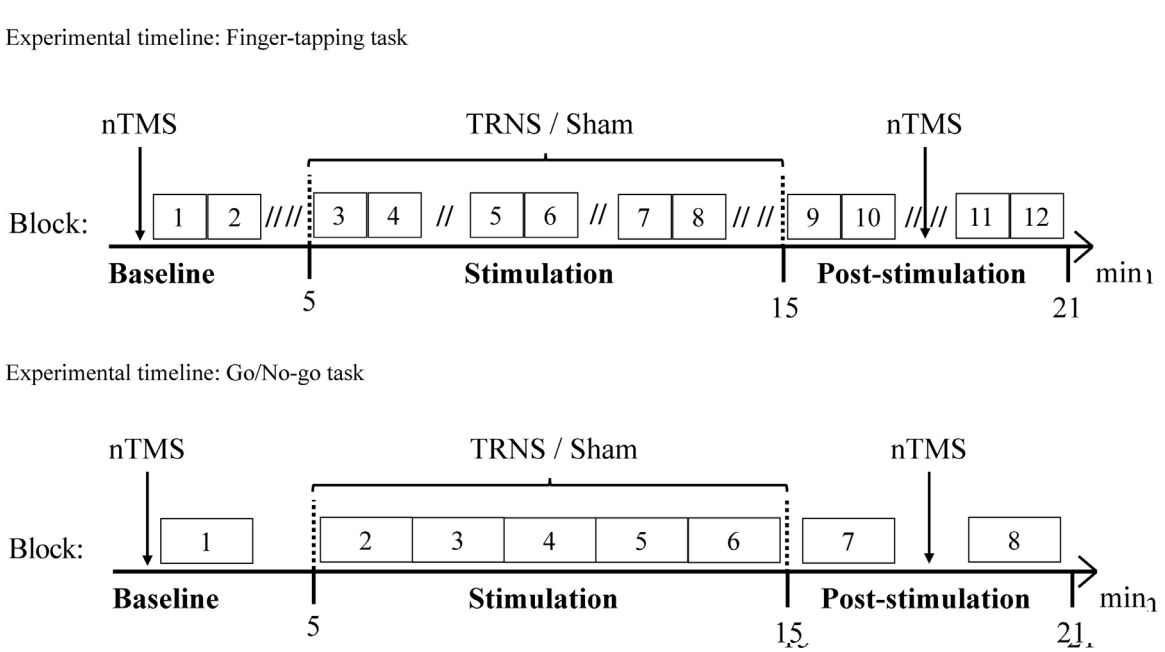

FIGURE 1 | Experimental timelines. Behavioral measurements of the FT task (A) and the GNG task (B) were conducted along with nTMS to evaluate CSE. Behavioral and electrophysiological measurements were acquired in a baseline condition and a post-stimulation condition. Offline measurements were complemented by online behavioral assessments during 10 min stimulation with tRNS or sham stimulation. (A) Experimental timeline of the FT task. 15 participants performed the FT task. During one block of 60 s, one hand was tapping for 30 s before switching to the other hand for 30 s. Double slashes ("//") denote a 60 s pause between blocks ("// //" = 120 s), to avoid excessive fatigue. (B) Experimental timeline of the GNG task. 15 participants performed the GNG task. One block consisted of 37 GNG trials and ended with a 15 s pause, resulting in 2 min per block.

and a 15 s pause, resulting in 2 min per block. The baseline condition consisted of one block, the stimulation condition(10 min) consisted of five consecutive blocks (i.e., a total of 5×37 trials = 185 trials) and the post-stimulation conditions consisted of two blocks.

Transcranial Random Noise Stimulation (tRNS)

Random noise stimulation was applied by a multi-channel low-voltage stimulation and EEG device certified for clinical use (NextWave, EBS Technologies GmbH, Kleinmachnow, Germany), which delivered weak random noise stimulation through conductive-rubber electrodes (NeuroConn GmbH, Ilmenau, Germany), placed in two saline-soaked sponges. One electrode (circular, 12.5 cm^2) was situated over the dominant motor cortex at the C3 EEG electrode position (since all subjects were right-handed), the other electrode (rectangular electrode, 30 cm^2) was placed over the contralateral frontopolar cortex (Moliadze et al., 2012). For tRNS, a peak-to-peak stimulation intensity of 1.51 mA (0.8 mAeffective current intensity) was applied for 10 min with noDC offset. The random signal was drawn from a uniform probability density with a sample rate of 1280 Hz anddigitally filtered to ensure a frequency distribution of 100–640 Hz, based on Terney et al. (2008). For sham stimulation,a 15 s ramp-up and 15 s ramp-down current was used inline with recommendations for tDCS (Nitsche et al., 2008; Schmidt et al., 2013a). Respective sessions of tRNS and sham stimulation were at least 7 days apart to avoid carry-over effects.

Navigated Transcranial Magnetic Stimulation (nTMS)

Single pulse nTMS (eXimia VR TMS, Nexstim, Helsinki, Finland) with optical tracking and subject-specific magnetic resonance images was used in combination with a biphasic figure- of-eight coil (70-mm wing diameter) to evaluate CSE with optimal control of physical parameters (Schmidt et al., 2015). Compared to conventional, non-navigated TMS, nTMS was shown to reduce MEP amplitude variance by 27% (Schmidt et al., 2009). Electromyography activity in response to nTMS was recorded from the dominant first dorsal interosseus muscle with Neuroline 700 surface electrodes (Ambu VR, Ballerup, Denmark) arranged in belly-tendon montage. MEP amplitude was defined by peak-to-peak measurement. The stimulation target was the "center of gravity" of the dominant first dorsal interosseus (Wassermann et al., 1992). Resting motor threshold was defined as the stimulation intensity required to elicit a 500 μV MEP appearing with 50% probability using the maximum-likelihood threshold detection method and a 95% confidence interval, ensuring an individually calibrated intensity prior to data acquisition in each session (Awiszus, 2003). CSE was then assessed with 20 MEPs

prior to and after electrical stimulation at the timepoints specified in **Figure 1**.

Analysis and Statistics

Two subjects withdrew consent to participate in the study before completion. The remaining 28 subjects (13 in the FT group, 15 in the GNG group) were included in the analysis and statistics.

CSE data was manually reviewed and outliers, definedas values above or below 2.2x the interquartile range, were identified in each session and removed (Hoaglin and Iglewicz, 1987). CSE was estimated by using an in-house algorithm that accounted for physiological and physical confounders, such that MEPs associated with confounding prestimulus muscle contraction (preinnervation) above 20 μV and 100 ms prior to stimulation were excluded and further physical and physiological covariance was partitioned out of CSE estimation with step-wise regression (Schmidt et al., 2015). Mean CSE data was then baseline normalized by subtracting baseline values from post-stimulation values. Normality of data was graphically confirmed with histograms and by using the Shapiro–Wilk test. Levene's test confirmed homogeneity of variances. Statistical analysis was conducted using a mixed model ANOVA to compare the main and interaction effects on CSE, with TASK (i.e., GNG, FT) as between-subjects factor and STIMULATION (i.e., tRNS, sham) as within-subjects factor.

Go/no-go task RTs, GNG task accuracy, FT ITI and FT taps were manually reviewed, which lead to exclusion of three subjects in the GNG group due to technical artifacts in the data. RTs and ITIs were outlier corrected, baseline normalized and z-transformed on a per subject basis over each session, in line with recommendations for within-subject designs and psychophysiological data (Bush et al., 1993). GNG accuracy data and FT taps were outlier corrected and baseline normalized for statistical analysis. Outlier correction involved trimming data by 5% of highest and lowest scores (Bush et al., 1993; Whelan, 2017). For GNG RTs specifically, trials without response and RTs below 100 ms after target cue presentation were rejected (Joundi et al., 2012). Baseline normalization required the mean of the baseline condition to be subtracted from the data. Z-transformation was used to increase power in comparison to raw means by accounting for intraindividual variability across subjects (Bush et al., 1993). A normal distribution could be confirmed both graphically as well as mathematically by the Shapiro–Wilk test. A linear mixed model for repeated measures was used to analyze the effect of tRNS on behavioral performance in the FT task and GNG task. It was used in favor of a repeated measures ANOVA due to its extended flexibility with regard to unbalanced data and precision in giving less biased estimates of fixed effects in repeated, correlated measurements (Cnaan et al., 1997; Krueger and Tian, 2004). As fixed effects, STIMULATION (i.e., tRNS/sham) and TIME (i.e., block) was entered into the model. SUBJECTS was entered as random effects. For a significant interaction of STIMULATION × TIME, *post hoc* tests for individual blocks were controlled for multiple comparisons using Bonferroni correction.

All digital signal processing was carried out with custom-made scripts within the MATLAB programming environment (MATLAB R2014a, The MathWorks, Inc., Natick, MA, United States). All statistical analysis was performed using SPSS Statistics with statistical significance level set at $\alpha = 0.05$ (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY, United States: IBM, Corp.). Results are presented as mean values and standard errors of the mean unless stated otherwise.

RESULTS

Corticospinal Excitability (CSE)

Effects of tRNS on CSE are depicted in **Figure 2**. Mean uncorrected baseline CSE for the FT group was similar for the tRNS (691 ± 89 μV) and the sham condition (FT, sham: 686 ± 125 μV) [$t(12) = 9.032$, $p = 0.975$]. Baseline CSE for the GNG group was also similar for the tRNS (530 ± 71 μV) and the sham condition (500 ± 105 μV) [$t(14) = 0.250$, $p = 0.806$]. In the mixed model ANOVA, there was no significant main effect of TASK [$F(1,26) = 1.961$, $p = 0.173$, $\eta_p = 0.07$] and STIMULATION [$F(1,26) = 1.814$, $p = 0.19$, $\eta_p = 0.05$] on CSE. However, there was a significance for the interaction STIMULATION × TASK [$F(1,26) = 5.474$, $p = 0.027$, $\eta_p^2 = 0.17$], indicating that excitability changes were dependent on the specific stimulation applied during task execution. Pairwise comparisons revealed that in the FT group, baseline corrected MEP responses were significantly facilitated following tRNS (381 ± 146 μV) compared to sham stimulation (14 ± 133 μV) ($p = 0.018$, $\eta_p^2 = 0.2$). In the GNG group, tRNS (−36 ± 97 μV) did not influence MEP responses compared to sham stimulation (−63 ± 93 μV) ($p = 0.473$, $\eta_p = 0.02$). This shows that tRNS specifically increased CSE

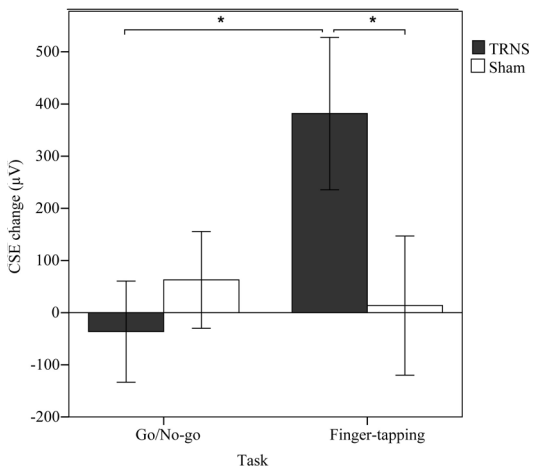

FIGURE 2 | Effects of tRNS on corticospinal excitability. Mean CSE change (μV) was calculated by subtracting baseline CSE measurements from post-stimulation measurements. CSE change is depicted for respective task type (GNG or FT) performed during 10 min of stimulation with either tRNS or sham stimulation. Error bars depict the standard error of the mean. In the FT group, MEP responses were significantly facilitated (*) after tRNS compared to sham stimulation and tRNS in the GNG group.

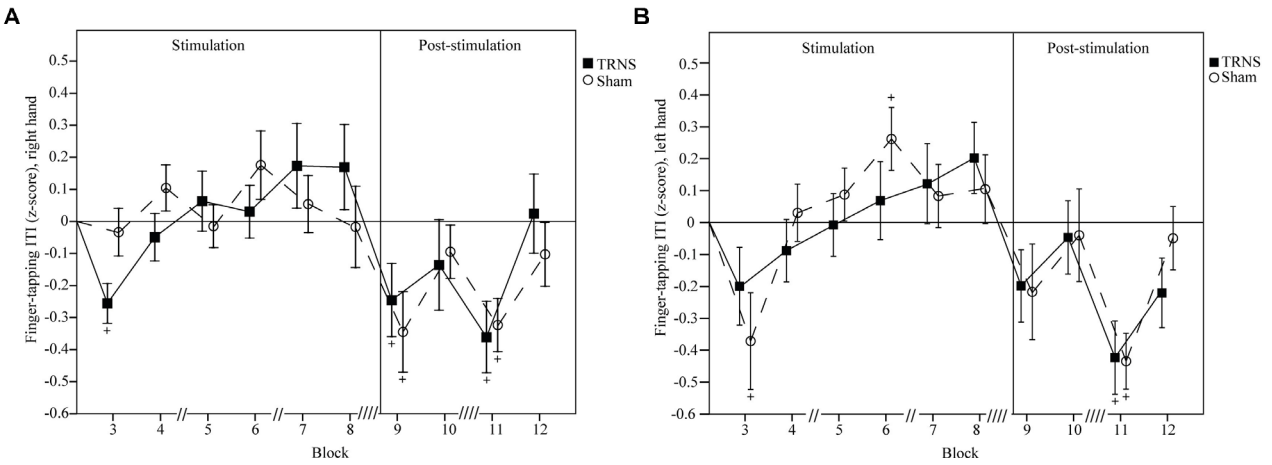

FIGURE 3 | Effects of tRNS on FT ITI. Mean FT ITIs are baseline corrected and z-transformed. Blocks 3–8 (30 s per block) depict ITIs during electrical stimulation, while blocks 9–12 present data post-stimulation. Double slashes ("//") denote a 60 s pause between blocks ("// //" = 120 s), to avoid excessive fatigue. Mean ITI is displayed with standard error of the mean. Significant changes from baseline are marked with "+." ITIs of the right hand (A) and the left hand (B) were not significantly different between the tRNS condition compared to the sham condition. For both hands, singular significant reductions in ITIs in block 3 of one condition likely represent a rebound effect after a prior pause. Reductions in ITIs post-stimulation for both the tRNS and sham conditions imply motor learning.

after the FT task but not after the GNG task (p = 0.022, η_p^2 = 0.19) (**Figure 2**).

FT: Intertap Interval (ITI)

Effects of tRNS on FT ITIs are depicted in **Figure 3A** (right hand) and **Figure 3B** (left hand). Uncorrected baseline ITIs were shorter for the right hand (tRNS, 148 ± 6 ms; sham, 149 ± 5 ms) compared to the left hand (tRNS, 170 ± 6 ms; sham, 170 ± 6 ms).

For the right hand, a linear mixed model did not show a significant main effect of STIMULATION on FT ITIs [$F(2) = 2.35$, $p = 0.6$]. However, a significant interaction of STIMULATION × TIME could be observed [$F(20) = 3.03$, $p < 0.001$]. *Post hoc* tests revealed significant reductions in ITIs after both tRNS (block 9, −0.246 ± 0.104, $p = 0.02$; block 11, −0.361 ± 0.101, $p < 0.001$) and sham stimulation (block 9, −0.345 ± 0.105, $p = 0.001$; block 11, −0.323 ± 0.101, $p = 0.001$). ITIs at the beginning of stimulation in block 3 were significantly faster only in the tRNS condition (−0.256 ± 0.101, $p = 0.012$). Bonferroni corrected pairwise comparisons between individual blocks and stimulation did not reach significant results (**Figure 3A**).

For the left hand, a linear mixed model did not show a significant main effect of STIMULATION on FT ITIs[$F(2) = 2.86$, $p = 0.58$]. However, a significant interaction of STIMULATION × TIME could be observed [$F(20) = 3.29$, $p < 0.001$]. *Post hoc* tests revealed significant reductions in ITIs after both tRNS (block 11, −0.423 ± 0.117, $p < 0.001$) and sham stimulation (block 3, −0.371 ± 0.112, $p = 0.001$; block 11, −0.434 ± 0.112, $p < 0.001$). There was a significant increase in ITIs the sham condition in block 6 (0.262 ± 0.112, $p = 0.02$) during stimulation. Bonferroni corrected pairwise comparisons between individual blocks and stimulation did not reach significant results (**Figure 3B**).

FT: Finger Taps

Effects of tRNS on FT taps are depicted in **Figure 4A** (right hand) and **Figure 4B** (left hand). Mean uncorrected baseline finger taps were higher for the right hand (tRNS, 171.62 ± 7.18; sham 173.65 ± 7.32) compared to the left hand (tRNS 150.81 ± 6.17; sham 154.23 ± 6.11).

For the right hand, a linear mixed model with baseline corrected data did not show a significant main effect of STIMULATION on FT taps [$F(2) = 1.98$, $p = 0.14$]. However, a significant interaction of STIMULATION × TIME could be observed [$F(20) = 3.39$, $p < 0.001$]. *Post hoc* tests revealed significant increases in the number of finger taps versus baseline for tRNS (block 3, 5.69 ± 2.34, $p = 0.016$; block 9, 5.54 ± 2.34, $p = 0.019$; block 11, 8.38 ± 2.34, $p < 0.001$) and sham stimulation (block 9, 7 ± 2.44, $p = 0.004$; block 11, 8.88 ± 2.33, $p < 0.001$). Additionally, toward the end of tRNS, the number of finger taps was significantly reduced versus baseline (block 7, −5.54 ± 2.34, $p = 0.019$; block 8, −4.77 ± 2.34, $p = 0.043$). Bonferroni corrected pairwise comparisons between individual blocks and stimulation did not reach significant results (**Figure 4A**).

For the left hand, a linear mixed model with baseline corrected data showed a significant main effect of STIMULATION on FT finger taps [$F(2) = 3.45$, $p = 0.03$] with a significant increase in FT finger tap estimates of fixed effects for tRNS (2.06 ± 0.79) [$t(255) = -2.62$, $p = 0.09$] but not for sham (0.16 ± 0.8) [$t(255) = 0.2$, $p = 0.84$]. However, *post hoc* tests between tRNS and sham did not reveal a significant difference between stimulation conditions [$t(255) = -1.7$, $p = 0.09$]. A significant interaction of STIMULATION × TIME could be observed [$F(20) = 2.63$, $p < 0.001$]. *Post hoc* tests revealed significant increases in the number of finger taps versus baseline after both tRNS (block 9, 4.73 ± 2.37, $p = 0.047$; block 11, 8.58 ± 2.37, $p < 0.001$; block 12, 5.69 ± 2.34, $p = 0.029$) and sham stimulation (block 3, 5.69 ± 2.37, $p = 0.017$;

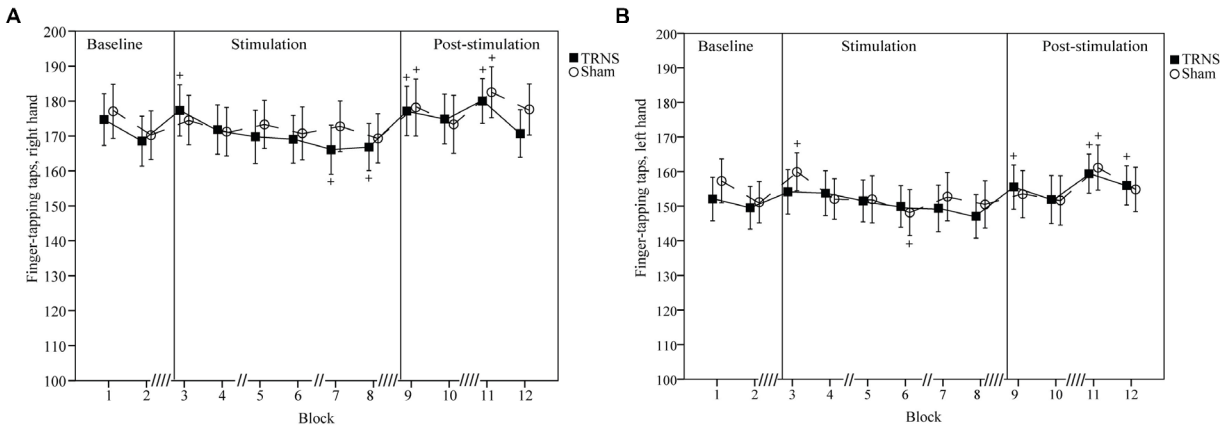

FIGURE 4 | Effects of tRNS on FT taps. Mean FT number of taps are shown which illustrate an overall higher tapping performance of the right hand (A) compared to the left hand (B) and complement changes in FT ITIs observed in Figure 3. Blocks 3–8 (30 s per block) depict finger taps during electrical stimulation, while blocks 9–12 present data post-stimulation. Double slashes ("//") denote a 60 s pause between blocks ("// //" = 120 s), to avoid excessive fatigue. Mean finger taps are displayed with standard error of the mean. Significant changes from baseline are marked with "+." (A,B) Number of finger taps for both hands were not significantly different between the tRNS condition compared to the sham condition. For both hands, singular significant increases in the number of finger taps in block 3 of one condition likely represent a rebound effect after a prior pause. Significant reductions during stimulation represent fatigue. Increased number of finger taps post-stimulation for both tRNS and sham conditions imply motor learning.

block 11, 6.92 ± 2.37, $p = 0.004$). Additionally, toward the end of sham stimulation, the number of finger taps was significantly reduced versus baseline (block 6, −6.08 ± 2.37, $p = 0.011$). Bonferroni corrected pairwise comparisons between individual blocks and stimulation did not reach significant results (**Figure 4B**).

GNG: Reaction Time (RT)

Effects of tRNS on GNG RT are depicted in **Figure 5A**. Mean uncorrected baseline RT for the tRNS condition was 303 ± 5 ms, and 313 ± 7 ms for the sham condition. A linear mixed model showed a significant main effect of STIMULATION on GNG RTs [$F(2) = 11.69$, $p < 0.001$] with a significant increase in estimates of fixed effects for tRNS (0.21 ± 0.045) [$t(160) = 4.65$, $p < 0.001$] but not for sham (0.06 ± 0.045) [$t(160) = 1.33$, $p = 0.19$]. Importantly, *post hoc* tests between tRNS and sham revealed a significant difference between stimulation conditions [$t(160) = -2.35$, $p = 0.019$]. Breaking down the main effect of STIMULATION into a stimulation period (blocks 2–6) and a post-stimulation period (blocks 7–8), the linear mixed model for RTs post-stimulation was significant [$F(2) = 5.48$, $p = 0.007$], with a significant difference in estimates of fixed effects: GNG RTs were attenuated after tRNS (0.24 ± 0.085, $p = 0.06$) compared to sham (−0.14 ± 0.085, $p = 0.107$) [$t(44) = -3.19$, $p = 0.002$]. There was no significant difference between tRNS and sham during the stimulation period [$t(114) = -0.79$, $p = 0.43$). A significant interaction of STIMULATION × TIME could also be observed [$F(14) = 2.57$, $p = 0.002$]. *Post hoc* tests showed attenuated RTs for tRNS in block 5 (0.284 ± 0.122, $p = 0.021$) and block 7 (0.32 ± 0.122, $p = 0.01$) and at the start of sham stimulation (block 2; 0.256, ± 0.117, $p = 0.031$). Bonferroni corrected pairwise comparisons between individual blocks and stimulation did not reach significant results. Together, these results show that tRNS specifically attenuated RTs in the GNG task in thepost-stimulation period (**Figure 5A**).

GNG: Task Accuracy

Effects of tRNS on GNG task accuracy are depicted in **Figure 5B**. Mean uncorrected baseline GNG task accuracy for the tRNS condition was 96.88 ± 0.91 and 98.34 ± 0.58% for the sham condition. A linear mixed model showed a significant main effect of STIMULATION on baseline corrected GNG accuracy [$F(2) = 18.01$, $p < 0.001$] with a significant increase in estimates of fixed effects for tRNS (1.89 ± 0.32) [$t(173) = 5.94$, $p < 0.001$] but not for sham (0.29 ± 0.33) [$t(173) = 0.86$, $p = 0.39$]. Importantly, *post hoc* tests between tRNS and sham revealed a significant difference between stimulation conditions [$t(173) = -3.49$, $p < 0.001$]. Breaking down the main effect of STIMULATION into a stimulation period (blocks 2–6) and a post-stimulation period (blocks 7–8), the linear mixed model for GNG accuracy during stimulation was significant [$F(2) = 12$, $p < 0.001$], with a significant difference in estimates of fixed effects: GNG accuracy was increased during tRNS (1.74 ± 0.36, $p < 0.001$) compared to sham (0.37 ± 0.38, $p = 0.973$) [$t(123) = -2.62$, $p = 0.009$]. GNG accuracy was also significantly increased in the post-stimulation period [$F(2) = 5.97$, $p = 0.005$] with significant differences in estimates of fixed effects after tRNS (2.27 ± 0.66, $p = 0.001$) compared to sham (0.083 ± 0.68, $p = 0.904$) [$t(48) = -2.30$, $p = 0.023$). A significant interaction of STIMULATION × TIME could also be observed [$F(14) = 2.78$, $p = 0.001$]. *Post hoc* tests showed increased task accuracy during tRNS in block 3 (1.85 ± 0.86, $p = 0.033$), block 5 (2.69 ± 0.86, $p = 0.002$) and after tRNS in block 7 (2.69 ± 0.86, $p = 0.002$) and block 8 (1.85 ± 0.86, $p = 0.033$). The sham condition did not reach significant results. Bonferroni corrected pairwise comparisons between individual blocks and stimulation did not reach significant results. Together,

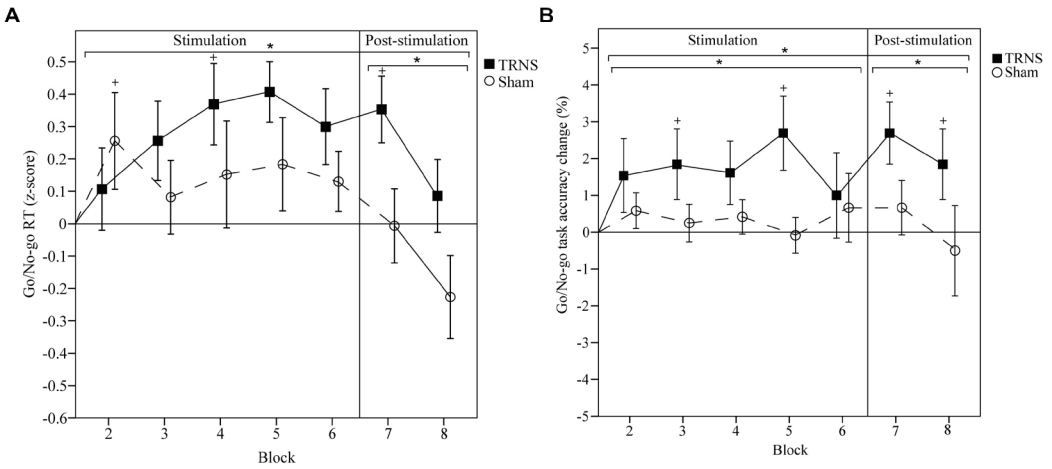

FIGURE 5 | Effects of tRNS on GNG RT and task accuracy. (A,B) Mean GNG RT and task accuracy are baseline corrected. RTs are z-transformed. Blocks 2–6 (2 min per block) depict RTs and task accuracy change during electrical stimulation, while blocks 7 and 8 present data post-stimulation. Means are displayed with standard error of the mean. Significant changes from baseline are marked with "+." Significant changes compared to sham are marked with "∗." (A) RTs were significantly longer in the tRNS condition compared to sham. (B) Task accuracy was significantly improved during and after tRNS compared to sham. Together, these results suggest that tRNS specifically strengthened motor inhibition and inhibitory control in the GNG task.

these results show that tRNS specifically increased task accuracy in the GNG task during stimulation and in the post-stimulation period (**Figure 5B**).

DISCUSSION

The purpose of this study was to investigate the task dependency of tRNS-induced neuromodulation in the motor system. The main results of this study show task-dependent dissociated effects on CSE and behavioral performance following tRNS during a FT task versus a GNG task. After motor training (FT task), characterized by repetitive motor activation, tRNS led to significant facilitation of CSE compared to sham stimulation, while behavioral performance was not significantly different to sham stimulation. Conversely, in the inhibitory control task (GNG task), tRNS-enhanced inhibition led to an attenuation of RTs without effects on CSE. Together, these findings support the notion that tRNS enhances the predominant task-dependent brain state. Our results highlight the interaction between tRNS and task-dependent brain activity and provide further evidence for tRNS' proposed mechanisms of action.

Motor Activation

In the simple motor training task (FT), online tRNS significantly facilitated CSE as compared to sham stimulation. To our knowledge, we are the first to show CSE enhancements after task execution during tRNS. CSE enhancements after tRNS have been previously shown only in idling subjects.In idling subjects, reliable CSE increases lasting 60 min are possible (Terney et al., 2008). Additionally, with regards to tRNS parameters, high frequency tRNS (100–640 Hz) (Terney et al., 2008) at high current intensities (1 mA) (Moliadze et al., 2012) with a duration of at least 5 min (Chaieb et al., 2011) was also shown to reliably increase CSE. In contrast, online tRNS was previously reported to impede CSE enhancements: CSE was found to be slightly attenuated for a cognitive task and strongly attenuated for a motor task (Terney et al., 2008). Attenuation after the motor taskwas suggested to be associated with task-induced fatigue (Terney et al., 2008).

Results from this study suggest that CSE facilitation after the FT task with online tRNS reflects an enhancement of task-dependent activation, i.e., additional motor activation in primed neural populations. Simple tapping tasks are well-established as prototype tasks to study motor training-induced neuroplasticity in the primary motor cortex (for a review see, Ljubisavljevic, 2006; Bezzola et al., 2012). Maximal sequential movements of the FDI ensure a maximum task-related activation of its cortical representation in M1, minimizing a confounding influence from other brain areas (Bezzola et al., 2012). Motor activation is independent of the physical tapping speed of subjects, since the amount of neural effort determines maximal neurophysiological activation (Lutz et al., 2005). Motor training leads to larger muscle representations, specific to the muscles involved in the task, and increased CSE (Pascual-Leone et al., 1994; Muellbacher et al., 2001; Koeneke et al., 2006).

The observation of further enhancement of task-dependent activation with tRNS fits well in line with the current understanding of tRNS' proposed mechanism of action: increase of CSE via transmission of subthreshold neural signals – a phenomenon known as stochastic resonance (Terney et al., 2008). Stochastic resonance, i.e., the mechanism by which an optimal noise condition improves signal detection in non-linear systems, has been known in the physics community since at least the early 1980s and has been universally observed in various neural systems including the human brain (Moss et al., 2004; Schmidt et al., 2013b).

Unchanged CSE levels after sham stimulation suggest that the tapping training duration was not sufficiently long to increase functional recruitment in the absence of tRNS. Motor training studies typically last 30–60 min (Classen et al., 1998; Muellbacher et al., 2001; Koenekeet al., 2006). These studies highlight the crucial addition of online tRNS in our study to dramatically reduce the required time for motor training-induced neuroplasticity in the primary motor cortex.

Motor Fatigue and Motor Learning

The FT task is a simple motor training task involving motor fatigue and motor learning indexed by a change in ITIs. It has been utilized as a clinical tool to characterize motor deficits in Parkinson's disease, cerebellar dysfunction, stroke and as a result of aging (Shimoyama et al., 1990; Arias et al., 2012).

In the present study, a linear increase in mean ITIs and finger taps *during* electrical- and sham stimulation represents task-induced motor fatigue (Rönnefarth et al., 2018). Fatigue inevitably occurs within seconds of task initiation (Shimoyama et al., 1990; Aoki et al., 2003; Rönnefarth et al., 2018). It involves not only peripheral, but also central mechanisms (central motor fatigue) as evidenced by reduced CSE after a fatiguing task (Kluger et al., 2012). Therefore, fatigue is a potential confounder in brain stimulation studies aiming to enhance CSE levels and likely explains CSE disruptions previously observed after online tRNS in the motor system (Antal et al., 2007; Terney et al., 2008). Several measures were taken in our study to tune the FT task to reduce the influence of fatigue (see section "Finger-Tapping Task (FT Task)). These measures were effective in preventing fatigue outlasting the stimulation condition, since post-stimulation ITIs and finger taps were equal to or lower than baseline levels and CSE inhibition, typically seen after excessive fatigue, was absent. Reduced ITIs and increased number of finger taps compared to baseline in block 3 (right hand), at the beginning of tRNS were not significant compared to sham stimulation and likely represent a rebound effect after a prior pause of 120 s. This might also explain the analogous phenomenon in block 3 of the left hand, at the beginning of sham stimulation.

The significant ITI enhancements and increased number of finger taps *after* tRNS and sham stimulation (between blocks 9–12) show that the utilized FT task was efficient in inducing motor learning. These unspecific effects on motor learning gain special significance when interpreted with corresponding CSE results: although the FT task improvements in the right hand were also observed in the sham condition, facilitation of CSE occurred only after tRNS. This implies that electrical stimulation might be associated with an enhanced potential for learning (Koeneke et al., 2006). Motor learning is known to occur as a result of motor training (for a review see, Ljubisavljevic, 2006), and to be closely associated with CSE facilitation and ITI improvements in simple tapping tasks (Koeneke et al., 2006). Further studiesalso emphasize the robust relation between motor learning and excitability enhancements, e.g., CSE levels return to baseline once subjects overlearn a task (Muellbacher et al., 2001) and improvement retention is disrupted when CSE is specifically suppressed over M1 (Muellbacher et al., 2002).

The robust behavioral improvements in the FT task after stimulation could not be differentiated (i.e., tRNS, sham), possibly due to a ceiling effect. In the young, healthy participants of this study, underlying motor learning processes are likely to be already optimized. Additionally, maximum task-related activation of M1 is thought to leave no roomfor further performance gains, especially in early stages of motor learning (Bezzola et al., 2012). Other measures ofFT task performance, e.g., force and tapping duration might expose tRNS-specific behavioral gains with higher sensitivity (Muellbacher et al., 2001; Rönnefarth et al., 2018). Providing evidence for neuromodulation of motor learning would be particularly relevant in the context of novel interventions following brain injury (Pascual-Leone et al., 2005).

Motor Inhibition

Unlike the simple motor training task, random noise stimulation in the inhibitory control task (GNG task) left CSE unchanged in both the tRNS and sham conditions, suggestive of an underlying inhibitory task-dependent brain state counteracting the facilitatory tRNS effects reported in idle subjects (Terney et al., 2008). We hypothesized a decrease in CSE after GNG and tRNS, reflecting enhanced motor inhibition. Methodological limitations and task complexity might have contributed to the absence of a clearer MEP decrease:

Firstly, CSE measurements after tRNS were not obtained on a trial-by-trial basis during GNG task execution and do not trace the time course of transient inhibitory state fluctuations per trial. The GNG task is a hallmark for motor inhibition encompassing periods of response preparation and response inhibition reflected by changes in CSE, for a review see Greenhouse et al. (2015) and Bestmann and Duque (2016). As subjects engage in the task and prepare to respond, motor inhibition, characterized by reduced MEPs, prevents a premature response (Greenhouse et al., 2015). The warning cue further enhances inhibitory processes (Boulinguez et al., 2009; Criaud et al., 2012) and the specificity of suppression to the muscles involved in the task (Greenhouse et al., 2012). If a "no-go" target cue appears, response inhibition acts as an active breaking process leading to global suppression of motor cortical activity with concurrent MEP suppression (Stinear et al., 2009; Greenhouse et al., 2012; MacDonald et al., 2014; Bestmann and Duque, 2016). Since CSE was investigated with single pulse nTMS *after* task execution, any potential transient enhancement of motor inhibition *during* the GNG task would not be detected in our paradigm.

Secondly, inhibition is interrupted by "go" cues requiring motor activation with concurrent brief facilitation of CSE (Stinear et al., 2009; MacDonald et al., 2014). These short but frequent motor responses might have contributed to the absence of a clear MEP suppression. Yet, rare "no-go" trials (<20%) are required to ensure sufficient inhibition-related activity anda 9% "no-go" probability has been shown to induce suchactivity (Schoffelen et al., 2005; Wessel, 2018). As becomes apparent, the inhibitory state associated with the GNG task is comparably more complex than the FT task. It includesthe subcomponents response preparation, response inhibition,

response activation and poses the methodological challenge of tracking these dynamically overlapping state changes with sufficient temporal resolution.

Inhibitory Control

Considering limitations arising from using single pulse nTMS to measure CSE after task completion, RT and task accuracy data acquired online, during the GNG task, serve as an easily assessable, more adequate parameter. RT and task accuracy are behaviorally relevant and trace dynamic state changes with a higher temporal resolution. RTs were significantly slowed in the tRNS condition, especially after electrical stimulation, while task accuracy was enhanced. Slowing of RTs in "go" trials is commonly used as a surrogate parameter for motor inhibition and is positively correlated to task accuracy (Bezdjian et al., 2009; Leotti and Wager, 2010). Response slowing is associated with suppression of MEPs, very similar to mechanisms involved in response inhibition (Jahfari et al., 2010).

The speed-accuracy trade-off is modulated by intraindividual inhibitory control: patients with impulse control disorders such as attention deficit and hyperactivity disorder (ADHD) andin patients who stutter, the speed-accuracy trade-off is shifted toward deficient inhibitory control with faster RTs and lower task accuracy (Bezdjian et al., 2009; Eggers et al., 2013). In turn, longer RTs and better task accuracy as signs of enhanced inhibitory control are achieved in patients with ADHD by pharmacological agents such as Modafinil (Turner et al., 2004). This phenomenon can likewise be observed in healthy subjects depending on gender (enhanced in female) and motivation (Bezdjian et al., 2009; Leotti and Wager, 2010). Consequently, we propose slowed RTs and enhanced task accuracy during and after tRNS to result from strengthened motor inhibition and inhibitory control outlasting stimulation. Our data suggests that tRNS impedes movement initiation by stabilizing the existing task-dependent brain state and delaying response initiation (Schmidt et al., 2013b). Future tRNS studies could try to modulate and optimize the speed-accuracy-tradeoff via task difficulty and in patients with deficient inhibitory control.

CONCLUSION

We provide evidence that tRNS-induced neuromodulation in the motor system is dependent on the task during stimulationsuch that CSE is enhanced in a FT task and inhibitory control is improved in a GNG task. Results confirm our hypothesis that transcranially applied random noise stimulation enhances the endogenous task-dependent brain state of healthy subjects. To our knowledge, we are the first to show CSE facilitation after online tRNS during a FT task. We argue in favor of online tRNS to avoid contradictory results and expose task specific regulatory processes to be modulated by transcranial stimulation techniques. Further confirmation of tRNS' mechanism of action is required to limit variability as a result of task dependency and to potentiate its neuroplastic effects in health and disease.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

SS, MS, and SB conceived the principle idea of the work. AJ, SS, MS, SB, LH, AK, MR, and RF designed the experiments. MS developed the software for experimental procedures and electrical stimulation. AJ, LH, and AK performed the measurements. AJ, SS, LH, LK, and RB-P conducted computational and statistical analyses of the data. All authors participated in the interpretation of the data. The manuscript was drafted by AJ and critically revised and approved by all authors.

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REFERENCES

Antal, A., Chaieb, L., Moliadze, V., Monte-Silva, K., Poreisz, C., Thirugnanasambandam, N., et al. (2010). Brain-derived neurotrophic factor(BDNF) gene polymorphisms shape cortical plasticity in humans. *Brain Stimul.* 3, 230–237. doi: 10.1016/j.brs.2009.12.003

Antal, A., Terney, D., Poreisz, C., and Paulus, W. (2007). Towards unravelling task-related modulations of neuroplastic changes induced in the human motor cortex. *Eur. J. Neurosci.* 26, 2687–2691. doi: 10.1111/j.1460-9568.2007.05896.x

Aoki, T., Francis, P. R., and Kinoshita, H. (2003). Differences in the abilities of individual fingers during the performance of fast, repetitive tapping movements. *Exp. Brain Res.* 152, 270–280. doi: 10.1007/s00221-003- 1552-z

Arias, P., Robles-Garcia, V., Espinosa, N., Corral, Y., and Cudeiro, J. (2012). Validity of the finger tapping test in Parkinson's disease, elderly and young healthy subjects: Is there a role for central fatigue? *Clin. Neurophysiol.* 123, 2034–2041. doi: 10.1016/j.clinph.2012.04.001

Awiszus, F. (2003). TMS and threshold hunting. *Suppl. Clin. Neurophysiol.* 56, 13–23. doi: 10.1016/S1567-424X(09)70205-3

Bestmann, S., and Duque, J. (2016). Transcranial magnetic stimulation:decomposing the processes underlying action preparation. *Neuroscientist* 22, 392–405. doi: 10.1177/1073858415592594

Bestmann, S., and Krakauer, J. W. (2015). The uses and interpretations of the motor-evoked potential for understanding behaviour. *Exp. Brain Res.* 233, 679–689. doi: 10.1007/s00221-014-4183-7

Bezdjian, S., Baker, L. A., Lozano, D. I., and Raine, A. (2009). Assessing inattention and impulsivity in children during the Go/NoGo task. *Br. J. Dev. Psychol.* 27,365–383. doi: 10.1348/026151008X314919

Bezzola, L., Mérillat, S., and Jäncke, L. (2012). Motor training-induced neuroplasticity. *GeroPsych* 25, 189–197. doi: 10.1024/1662-9647/a000070

Boulinguez, P., Ballanger, B., Granjon, L., and Benraiss, A. (2009). The paradoxical effect of warning on reaction time: demonstrating proactive response inhibitionwith event-related potentials. *Clin. Neurophysiol.* 120, 730–737. doi: 10.1016/j.clinph.2009.02.167

Brunoni, A. R., Amadera, J., Berbel, B., Volz, M. S., Rizzerio, B. G., and Fregni, F. (2011). A systematic review on reporting and assessment of adverse effects associated with transcranial direct current stimulation. *Int. J. Neuropsychopharmacol.* 14, 1133–1145. doi: 10.1017/S1461145710001690

Bush, L. K., Hess, U., and Wolford, G. (1993). Transformations for within-subject designs: a Monte Carlo investigation. *Psychol. Bull.* 113, 566–579. doi: 10.1037/ 0033-2909.113.3.566

Chaieb, L., Paulus, W., and Antal, A. (2011). Evaluating aftereffects of short- duration transcranial random noise stimulation on cortical excitability. *Neural Plast.* 2011:105927. doi: 10.1155/2011/105927

Classen, J., Liepert, J., Wise, S. P., Hallett, M., and Cohen, L. G. (1998). Rapid plasticity of human cortical movement representation induced by practice. *J. Neurophysiol.* 79, 1117–1123. doi: 10.1152/jn.1998.79.2.1117

Cnaan, A., Laird, N. M., and Slasor, P. (1997). Using the general linear mixed model to analyse unbalanced repeated measures and longitudinal data. *Statist. Med.* 16, 2349–2380. doi: 10.1002/(SICI)1097-0258(19971030)16:20<2349:: AID-SIM667>3.0.CO;2-E

Criaud, M., Wardak, C., Ben Hamed, S., Ballanger, B., and Boulinguez, P. (2012). Proactive inhibitory control of response as the default state of executive control.*Front. Psychol.* 3:59. doi: 10.3389/fpsyg.2012.00059

Eggers, K., De Nil, L. F., and Van Den Bergh, B. R. (2013). Inhibitory control in childhood stuttering. *J. Fluency Disord.* 38, 1–13. doi: 10.1016/j.jfludis.2012.10.001

Evarts, E. V., Fromm, C., Kroller, J., and Jennings, V. A. (1983). Motor Cortex control of finely graded forces. *J. Neurophysiol.* 49, 1199–1215. doi: 10.1152/jn.1983.49.5.1199

Fertonani, A., Pirulli, C., and Miniussi, C. (2011). Random noise stimulation improves neuroplasticity in perceptual learning. *J. Neurosci.* 31, 15416–15423. doi: 10.1523/JNEUROSCI.2002-11.2011

Gilbert, C. D., and Sigman, M. (2007). Brain states: top-down influences in sensory processing. *Neuron* 54, 677–696. doi: 10.1016/j.neuron.2007.05.019

Greenhouse, I., Oldenkamp, C. L., and Aron, A. R. (2012). Stopping a responsehas global or nonglobal effects on the motor system depending on preparation. *J. Neurophysiol.* 107, 384–392. doi: 10.1152/jn.00704.2011

Greenhouse, I., Sias, A., Labruna, L., and Ivry, R. B. (2015). Nonspecific inhibition of the motor system during response preparation. *J. Neurosci.* 35, 10675–10684. doi: 10.1523/JNEUROSCI.1436-15.2015

Hoaglin, D. C., and Iglewicz, B. (1987). Fine-tuning some resistant rules for outlier labeling. *J. Am. Statist. Assoc.* 82, 1147–1149. doi: 10.1080/01621459.1987.10478551

Hummel, F. C., Celnik, P., Pascual-Leone, A., Fregni, F., Byblow, W. D., Buetefisch, C. M., et al. (2008). Controversy: noninvasive and invasive cortical stimulation show efficacy in treating stroke patients. *Brain Stimul.* 1, 370–382. doi: 10.1016/j.brs.2008.09.003

Jahfari, S., Stinear, C. M., Claffey, M., Verbruggen, F., and Aron, A. R. (2010). Responding with restraint: What are the neurocognitive mechanisms? *J. Cogn. Neurosci.* 22, 1479–1492. doi: 10.1162/jocn.2009.21307

Joundi, R. A., Jenkinson, N., Brittain, J. S., Aziz, T. Z., and Brown, P. (2012). Driving oscillatory activity in the human cortex enhances motor performance. *Curr.Biol.* 22, 403–407. doi: 10.1016/j.cub.2012.01.024

Kluger, B. M., Palmer, C., Shattuck, J. T., and Triggs, W. J. (2012). Motor evoked potential depression following repetitive central motor initiation. *Exp. BrainRes.* 216, 585–590. doi: 10.1007/s00221-011-2962-y

Koeneke, S., Lutz, K., Herwig, U., Ziemann, U., and Jancke, L. (2006). Extensive training of elementary finger tapping movements changes the pattern of motor cortex excitability. *Exp. Brain Res*. 174, 199–209. doi: 10.1007/s00221-006- 0440-8

Kristeva, R., Patino, L., and Omlor, W. (2007). Beta-range cortical motor spectral power and corticomuscular coherence as a mechanism for effective corticospinal interaction during steady-state motor output. *Neuroimage* 36, 785–792. doi: 10.1016/j.neuroimage.2007.03.025

Krueger, C., and Tian, L. (2004). A comparison of the general linear mixed model and repeated measures ANOVA using a dataset with multiple missing data points. *Biol. Res. Nurs.* 6, 151–157. doi: 10.1177/1099800404267682

Lee, S. H., and Dan, Y. (2012). Neuromodulation of brain states. *Neuron* 76, 209–222. doi: 10.1016/j.neuron.2012.09.012

Leotti, L. A., and Wager, T. D. (2010). Motivational influences on response inhibition measures. *J. Exp. Psychol. Hum. Percept. Perform.* 36, 430–447. doi: 10.1037/a0016802

Li, L. M., Uehara, K., and Hanakawa, T. (2015). The contribution of interindividualfactors to variability of response in transcranial direct current stimulation studies. *Front. Cell Neurosci.* 9:181. doi: 10.3389/fncel.2015.00181

Ljubisavljevic, M. (2006). Transcranial magnetic stimulation and the motor learning-associated cortical plasticity. *Exp. Brain Res.* 173, 215–222. doi: 10. 1007/s00221-006-0538-z

Lutz, K., Koeneke, S., Wustenberg, T., and Jancke, L. (2005). Asymmetry of cortical activation during maximum and convenient tapping speed. *Neurosci. Lett.* 373, 61–66. doi: 10.1016/j.neulet.2004.09.058

MacDonald, H. J., Coxon, J. P., Stinear, C. M., and Byblow, W. D. (2014). The fall and rise of corticomotor excitability with cancellation and reinitiationof prepared action. *J. Neurophysiol.* 112, 2707–2717. doi: 10.1152/jn.00366. 2014

Moliadze, V., Atalay, D., Antal, A., and Paulus, W. (2012). Close to threshold transcranial electrical stimulation preferentially activates inhibitory networks before switching to excitation with higher intensities. *Brain Stimul.* 5, 505–511. doi: 10.1016/j.brs.2011.11.004

Moss, F., Ward, L. M., and Sannita, W. G. (2004). Stochastic resonance and sensory information processing: a tutorial and review of application. *Clin. Neurophysiol.* 115, 267–281. doi: 10.1016/j.clinph.2003.09.014

Muellbacher, W., Ziemann, U., Boroojerdi, B., Cohen, L., and Hallett, M. (2001).Role of the human motor cortex in rapid motor learning. *Exp. Brain Res.* 136,431–438. doi: 10.1007/s002210000614

Muellbacher, W., Ziemann, U., Wissel, J., Dang, N., Kofler, M., Facchini, S.,et al. (2002). Early consolidation in human primary motor cortex. *Nature* 415,640–644. doi: 10.1038/nature712

Nitsche, M. A., Cohen, L. G., Wassermann, E. M., Priori, A., Lang, N., Antal, A.,et al. (2008). Transcranial direct current stimulation: state of the art 2008. *Brain Stimul.* 1, 206–223. doi: 10.1016/j.brs.2008.06.004

Pascual-Leone, A., Amedi, A., Fregni, F., and Merabet, L. B. (2005). The plastichuman brain cortex. *Annu. Rev. Neurosci.* 28, 377–401. doi: 10.1146/annurev.neuro.27.070203.144216

Pascual-Leone, A., Grafman, J., and Hallett, M. (1994). Modulation of cortical motor output maps during development of implicit and explicit knowledge. *Science* 263, 1287–1289. doi: 10.1126/science.8122113

Pirulli, C., Fertonani, A., and Miniussi, C. (2013). The role of timing in the induction of neuromodulation in perceptual learning by transcranial electric stimulation. *Brain Stimul.* 6, 683–689. doi: 10.1016/j.brs.2012.12.005

Prichard, G., Weiller, C., Fritsch, B., and Reis, J. (2014). Effects of different electricalbrain stimulation protocols on subcomponents of motor skill learning. *BrainStimul.* 7, 532–540. doi: 10.1016/j.brs.2014.04.005

Rönnefarth, M., Bathe-Peters, R., Jooss, A., Haberbosch, L., Scholz, M., Schmidt, S.,et al. (2018). Force increase in a repetitive motor task inducing motor fatigue.*J. Mot. Behav.* doi: 10.1080/00222895.2018.1495172 [Epub ahead of print].

Saiote, C., Polania, R., Rosenberger, K., Paulus, W., and Antal, A. (2013). High-frequency TRNS reduces BOLD activity during visuomotor learning. *PLoS One* 8:e59669. doi: 10.1371/journal.pone.0059669

Schmidt, S., Bathe-Peters, R., Fleischmann, R., Ronnefarth, M., Scholz, M., and Brandt, S. A. (2015). Nonphysiological factors in navigated TMS studies; confounding covariates and valid intracortical estimates. *Hum. Brain Mapp.* 36, 40–49. doi: 10.1002/hbm.22611

Schmidt, S., Cichy, R. M., Kraft, A., Brocke, J., Irlbacher, K., and Brandt, S. A. (2009). An initial transient-state and reliable measures of corticospinal excitability in TMS studies. *Clin. Neurophysiol*. 120, 987–993. doi: 10.1016/j.clinph.2009.02.164

Schmidt, S., Fleischmann, R., Bathe-Peters, R., Irlbacher, K., and Brandt, S. A. (2013a). Evolution of premotor cortical excitability after cathodal inhibition of the primary motor cortex: a sham-controlled serial navigated TMS study. *PLoS One* 8:e57425. doi: 10.1371/journal.pone.0057425

Schmidt, S., Scholz, M., Obermayer, K., and Brandt, S. A. (2013b). Patterned brain stimulation, what a framework with rhythmic and noisy components might tellus about recovery maximization. *Front. Hum. Neurosci.* 7:325. doi: 10.3389/fnhum.2013.00325

Schoffelen, J. M., Oostenveld, R., and Fries, P. (2005). Neuronal coherence as a mechanism of effective corticospinal interaction. *Science* 308, 111–113. doi: 10.1126/science.1107027

Schulze, K., Luders, E., and Jancke, L. (2002). Intermanual transfer in a simple motor task. *Cortex* 38, 805–815. doi: 10.1016/S0010-9452(08)70047-9

Shimoyama, I., Ninchoji, T., and Uemura, K. (1990). The finger-tapping test.A quantitative analysis. *Arch. Neurol.* 47, 681–684. doi: 10.1001/archneur.1990.00530060095025

Silvanto, J., Muggleton, N., and Walsh, V. (2008). State-dependency in brain stimulation studies of perception and cognition. *Trends Cogn. Sci.* 12, 447–454. doi: 10.1016/j.tics.2008.09.004

Snowball, A., Tachtsidis, I., Popescu, T., Thompson, J., Delazer, M., Zamarian, L.,et al. (2013). Long-term enhancement of brain function and cognition using cognitive training and brain stimulation. *Curr. Biol.* 23, 987–992. doi: 10.1016/j.cub.2013.04.045

Stinear, C. M., Coxon, J. P., and Byblow, W. D. (2009). Primary motor cortex and movement prevention: where Stop meets Go. *Neurosci. Biobehav. Rev.* 33,662–673. doi: 10.1016/j.neubiorev.2008.08.013

Talelli, P., and Rothwell, J. (2006). Does brain stimulation after stroke have a future? *Curr. Opin. Neurol.* 19, 543–550. doi: 10.1097/WCO.0b013e32801080d1 Terney,

D., Chaieb, L., Moliadze, V., Antal, A., and Paulus, W. (2008). Increasing human brain excitability by transcranial high-frequency random noise stimulation. *J. Neurosci.* 28, 14147–14155. doi: 10.1523/JNEUROSCI. 4248-08.2008

Turner, D. C., Clark, L., Dowson, J., Robbins, T. W., and Sahakian, B. J. (2004). Modafinil improves cognition and response inhibition in adult attention-deficit/hyperactivity disorder. *Biol. Psychiatry* 55, 1031–1040. doi: 10.1016/j.biopsych.2004.02.008

Wassermann, E. M., Mcshane, L. M., Hallett, M., and Cohen, L. G. (1992). Noninvasive mapping of muscle representations in human motor cortex. *Electroencephalogr. Clin. Neurophysiol.* 85, 1–8.

Wessel, J. R. (2018). Prepotent motor activity and inhibitory control demands in different variants of the go/no-go paradigm. *Psychophysiology* 55:e12871. doi: 10.1111/psyp.12871

Whelan, R. (2017). Effective analysis of reaction time data. *Psychol. Rec.* 58, 475–482. doi: 10.1007/BF03395630

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Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

11. Komplette Publikationsliste

11.1 Originalarbeiten

Jooss A, Haberbosch L, Köhn A, Rönnefarth M, Bathe-Peters R, Kozarzewski L, Fleischmann R, Scholz M, Schmidt S, Brandt SA. Motor Task-Dependent Dissociated Effects of Transcranial Random Noise Stimulation in a Finger-Tapping Task Versus a Go/No-Go Task on Corticospinal Excitability and Task Performance. Front Neurosci. 2019;13.

Impact Factor: 3.707

Haberbosch L, Datta A, Thomas C, Jooss A, Kohn A, Ronnefarth M, Scholz M, Brandt SA, Schmidt S. Safety Aspects, Tolerability and Modeling of Retinofugal Alternating Current Stimulation. Front Neurosci. 2019;13:783.

Impact Factor: 3.707

Haberbosch L, Schmidt S, Jooss A, Kohn A, Kozarzewski L, Ronnefarth M, Scholz M, Brandt SA. Rebound or Entrainment? The Influence of Alternating Current Stimulation on Individual Alpha. Front Hum Neurosci. 2019;13:43.

Impact Factor: 3.209

Rönnefarth M, Bathe-Peters R, Jooss A, Haberbosch L, Scholz M, Schmidt S, Brandt SA. Force Increase in a Repetitive Motor Task Inducing Motor Fatigue. J Mot Behav. 2018:1-10.

Impact Factor: 1.348

Soll, D., Spira, D., Hollstein, T., Haberbosch, L., Demuth, I., Steinhagen-Thiessen, E., Bobber, T, Spranger, J., Kassner, U. (2019). Clinical outcome of a patient with lysosomal acid lipase deficiency and first results after initiation of treatment with Sebelipase alfa: A case report. Molecular Genetics and Metabolism Reports, 20, 100479.

Impact Factor: 0.507.

11.2 Abstracts

Roennefarth M, Jooss A, Haberbosch L, Koehn A, Fleischmann R, Brandt SA, Schmidt S. Frequency specific modulation of motor fatigue by beta- and gamma-tACS. Brain Stimulation: Basic, Translational, and Clinical Research in Neuromodulation. 2017;10(2):484.

Haberbosch L, Jooss A, Köhn A, Rönnefarth M, Fleischmann R, Schmidt S, Brandt SA. P 62 The effect of alternating current stimulation on individual alpha – A rebound phenomenon? Clin Neurophysiol. 2017;128(10):e358-e9.

Jooss A, Haberbosch L, Köhn A, Kozarzewski L, Rönnefarth M, Fleischmann R, Scholz M, Schmidt S, Brandt SA. P 63 Investigating the effects of tRNS variants and task dependency on cortical excitability. Clin Neurophysiol. 2017;128(10):e359.

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Rönnefarth M, Jooss A, Haberbosch L, Köhn A, Fleischmann R, Brandt S, Schmidt S. P 55 Frequency specific modulation of motor fatigue by beta- and gamma-tACS. Clin Neurophysiol. 2017;128(10):e356.

Rönnefarth M, Bathe-Peters R, Jooss A, Haberbosch L, Brandt S, Schmidt S. EP 92. Measuring force in fingertapping: A novel tool to detect motor fatigue. Clin Neurophysiol. 2016;127(9):e282.

Jooss A, Haberbosch L, Rönnefarth M, Fleischmann R, Scholz M, Brandt S, Schmidt S. EP 69. Brain state dependent inhibitory and facilitatory effects following transcranial random noise stimulation in two motor tasks. Clin Neurophysiol. 2016;127(9):e267-e8.

Köhn A, Konrad D, Haberbosch L, Jooss A, Bathe-Peters R, Fleischmann R, Schmidt S, Brandt SA. P147. Infrared-based kinematic read-out of TMS-elicited complex finger movements: A pilot study. Clin Neurophysiol. 2015;126(8):e135.

Haberbosch L, Jooss A, Fleischmann R, Rönnefarth M, Brandt S, Schmidt S. P78. Feasibility and safety aspects of retinofugal alternating current stimulation. Clin Neurophysiol. 2015;126(8):e132.

Köhn A, Schmidt S, Konrad D, Haberbosch L, Jooss A, Fleischmann R, Brandt SA. Motor imagery, navigated brain stimulation and the dynamics of hand-knob somatotopy. Brain Stimulation: Basic, Translational, and Clinical Research in Neuromodulation. 2015;8(2):399.

Haberbosch L, Schmidt S, Köhn A, Jooss A, Fleischmann R, Brandt SA. Influence of Alpha Suppression on Bandwidth Confined Electric Stimulation of the Visual System. Brain Stimulation: Basic, Translational, and Clinical Research in Neuromodulation. 2015;8(2):392.

Jooss A, Schmidt S, Haberbosch L, Köhn A, Scholz M, Brandt SA. Investigating the effects of noisy stimulation on the retinofugal pathway. Brain Stimulation: Basic, Translational, and Clinical Research in Neuromodulation. 2015;8(2):402.

Jooss A, Haberbosch L, Scholz M, Brandt S, Schmidt S. P146. Comparison of alpha-entrainment with oscillatory and noisy low-voltage stimulation. Clin Neurophysiol. 2015;126(8):e134-e5.

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