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FROM CORRELATION TOWARDS CAUSALITY – USING NON-INVASIVE NEUROMODULATION TO UNDERSTAND AND MODULATE PAIN

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ABSTRACT

Pain is a vital, protective phenomenon crucial to our survival. Pain can, however, also persist for extended periods of time without protecting the body. Such chronic pain conditions pose one of the greatest challenges to global healthcare systems, because pharmacological treatment of chronic pain remains difficult as illustrated by the recent opioid crisis. Thus, novel approaches for the treatment of chronic pain are urgently needed.

Recent insights into the brain mechanisms of pain open new perspectives for the development of such novel approaches. Accumulating evidence indicates that neural oscillations, i.e., rhythmic fluctuations in summed neural activity, play an important role in the brain processing of pain. Specifically, oscillations at alpha (8 to < 13 Hz) and gamma (> 30 to 100 Hz) frequencies in somatosensory and prefrontal regions play a significant role in the processing of nociceptive input and the emergence of acute and chronic pain. However, findings in humans mostly rely on correlative approaches, impeding mechanistic, causal inferences. To bridge this gap and progress from correlative towards causal evidence, techniques that allow researchers to manipulate neural oscillations in experimentally controlled settings are essential. Non-invasive neuromodulatory techniques such as transcranial alternating current stimulation (tACS) and neurofeedback are designed to modulate neural oscillations in a frequency specific manner and can be applied in humans in a safe and easy way. Hence, these techniques hold great potential for the investigation of the neural mechanisms underlying pain as well as the development of new treatment approaches for chronic pain.

Despite this appeal, studies applying non-invasive neuromodulatory techniques in the field of pain research are still limited in number or have produced inconclusive results. Thus, further systematic, high-quality research is warranted. To contribute to this effort, the current thesis presents the results of two projects investigating the effects of tACS and neurofeedback on experimental pain and discusses their implications. To enhance the replicability and interpretability of results, both projects adhere to most recent open science standards. Specifically, both studies were preregistered and include large sample sizes based on rigorous a priori-sample size calculations. In addition, both projects rely on Bayesian statistics to examine evidence not only for the presence but also for the absence of effects. To ensure full transparency, all data and study-related code are stored in a standardized format and made openly available to the research community.

The *first project* explored the potential of tACS to modulate pain by systematically applying alpha, gamma, or sham tACS over the somatosensory and prefrontal cortex during tonic experimental pain. Thereby, the study extends previous work by targeting a new location and frequency, both of which have been implicated in the processing of pain. Using conventional, standardized stimulation parameters, the results did not reveal tACS effects on tonic pain or brain activity recorded before and after the stimulation. However, several optimization approaches including the anatomy-informed and individualized tuning of stimulation parameters in the frequency, intensity, and spatial domains exist and merit further investigation.

The *second* project further explores the potential of EEG-based neurofeedback to modulate phasic experimental pain using a short-term neurofeedback training targeting somatosensory alpha oscillations. Motivated by the methodological challenges of neurofeedback studies and the inconsistency of previous results, the project was conceptualized as a registered report. This new publishing format aims at fostering high-quality research by minimizing analysis-, reporting-, and publication biases. Registered reports integrate an additional, stage 1, peer-review into the publication process. This stage 1 review takes place prior to data acquisition and can result in in-principal acceptance which guarantees the publication of the final study independent of its findings. The stage 1 review process of the second project demonstrated that registered reports represent a suitable tool to create a fully transparent, well-powered, and rigorous neurofeedback study in the field of pain research. However, registered reports also pose substantial methodological challenges for researchers, such as restrictions in flexibility and increased publication times due to stage 1 review delays. For the current project, the stage 1 review process amounted to 14 months impeding data acquisition and analysis within the time frame of this thesis. Hence, methodological, and procedural challenges of the format should be considered early on when planning a registered report.

In summary, tACS and neurofeedback do not yet deliver consistent findings in the field of pain research thereby challenging their scientific and clinical utility. Optimization approaches derived from integrative research efforts across disciplines, e.g., frequency, intensity, and spatial tuning, and a more thorough evaluation of the applied techniques through high-quality research, e.g., using new publication formats such as registered reports, are imperative to close this knowledge gap. Considering the urgent need for novel pain treatments as well as the conceptual plausibility and potentially broad clinical applicability of non-invasive neuromodulatory techniques to modulate pain, these efforts are worth pursuing and should be continued in the future.

ZUSAMMENFASSUNG

Schmerz ist ein effektives Warnsignal, das eine überlebenswichtige, protektive Funktion erfüllt. Schmerz kann jedoch auch über längere Zeiträume andauern, ohne den Körper zu schützen. Solche chronischen Schmerzzustände stellen in Ermanglung effektiver pharmakologischer Therapien eine der größten Herausforderungen für Gesundheitssysteme weltweit dar, wie die aktuelle Opioid-Krise verdeutlicht. Neue Ansätze zur Behandlung chronischer Schmerzen werden daher dringend benötigt.

Jüngste Einblicke in die Gehirnmechanismen von Schmerz eröffnen neue Perspektiven für die Entwicklung solcher Behandlungsansätze. Studien der letzten Jahre legen nahe, dass neuronalen Oszillationen, das heißt rhythmische Hirnaktivität in verschiedenen Frequenzbändern, eine besondere Rolle bei der Verarbeitung nozizeptiven Inputs und der Entstehung akuter und chronischer Schmerzen zukommt. Hierbei scheinen vor allem Alpha- (8 bis < 13 Hz) und Gamma-Oszillationen (> 30 bis 100 Hz) in somatosensorischen und präfrontalen Regionen eine besondere Rolle zu spielen. Bisherige humanexperimentelle Studien basieren jedoch weitestgehend auf korrelativen Untersuchungen von Schmerz und Hirnaktivität und lassen somit keine Aussagen bezüglich mechanistischer, kausaler Zusammenhänge zu. Der Übergang von korrelativen zu kausalen Schlussfolgerungen erfordert Verfahren, welche es Forscher*innen ermöglichen, neuronale Oszillationen experimentell zu manipulieren. Transkranielle Wechselstromstimulation (tACS vom Englischen *transcranial alternating current stimulation*) und Neurofeedback sind zwei Beispiele für solche nicht-invasiven neuromodulatorischen Verfahren. Sie wurden entwickelt, um neuronale Oszillationen frequenzspezifisch zu modulieren und können auf sichere und einfache Weise angewendet werden. Daher bergen diese Verfahren großes Potenzial für die Erforschung der neuronalen Mechanismen von Schmerz im menschlichen Gehirn sowie für die aus klinischer Sicht hochrelevante Entwicklung neuer Behandlungsansätze für chronischen Schmerz.

Trotz dieses Potentials gibt es aktuell nur wenige Studien, welche nicht-invasive neuromodulatorische Verfahren zur Erforschung von Schmerz anwenden, und die berichteten Ergebnisse sind inkonsistent. Daher ist der Bedarf nach weiteren, methodisch-hochwertigen Untersuchungen groß. Vor diesem Hintergrund aggregiert die vorliegende Dissertation die Ergebnisse zweier Projekte, welche die Auswirkungen von tACS und Neurofeedback auf experimentellen Schmerz untersuchen und diskutiert die resultierenden Implikationen. Um die Reproduzierbarkeit und Interpretierbarkeit der Ergebnisse zu verbessern, wurden beide Projekte entsprechend aktueller Open-Science

Standards konzipiert und durchgeführt. Beide Studien wurden präregistriert und umfassen große Stichprobengrößen basierend auf a priori Poweranalysen. Darüber hinaus stützen sich beide Projekte auf Bayesianische Statistik, welche es ermöglicht nicht nur das Vorliegen, sondern auch die Abwesenheit von Effekten zu untersuchen. Zusätzlich werden alle erhobenen Daten und Auswertungsskripte in einem standardisierten Format gespeichert und der Forschungsgemeinschaft offen zugänglich gemacht, um größtmögliche Transparenz zu schaffen und direkte Replikationen zu ermöglichen.

Das *erste Projekt* untersuchte das Potenzial von tACS zur Schmerzmodulation durch die systematische Anwendung von Alpha-, Gamma- oder Sham-tACS über dem somatosensorischen und präfrontalen Kortex. Dadurch erweiterte die Studie frühere Arbeiten um eine weitere Stimulationsregion sowie -frequenz. Die Ergebnisse zeigen unter Verwendung herkömmlicher, standardisierter Stimulationsparameter keine Effekte von tACS auf tonischen Schmerz oder Hirnaktivität. Die Anwendung optimierter und individualisierter Stimulationsparameter in den Bereichen Stimulationsfrequenz, -intensität und -montage erscheint jedoch vielversprechend und sollte in zukünftigen Studien systematisch untersucht werden.

Das *zweite Projekt* untersucht das Potential eines EEG-basierten Neurofeedback Trainings zur Schmerzmodulation, welches sich auf somatosensorisch Alpha-Oszillationen fokussiert. Motiviert durch die methodischen Schwächen und die inkonsistenten Ergebnisse früherer Neurofeedback-Studien nutzt das Projekt das neue Publikationsformat des *Registered Reports*. Dieses neue Publikationsformat zielt darauf ab, fragliche Forschungspraktiken während der Analyse, Berichterstattung und Veröffentlichung von Studien zu unterbinden und so qualitativ hochwertige Forschung zu fördern. Zu diesem Zweck beinhalten Registered Reports einen zusätzlichen Begutachtungsprozess, genannt Stage 1 Peer-Review, welcher vor der Datenakquise stattfindet. Methodisch hochwertige Studien können anschließend eine prinzipielle Annahme erhalten, was die Veröffentlichung der finalen Studie unabhängig von ihren Ergebnissen garantiert. Der Stage 1 Peer-Review Prozess des zweiten Projekts zeigte, dass Registered Reports ein geeignetes Instrument sind, um eine vollständig transparente und methodisch hochwertige Neurofeedbackstudie im Bereich der Schmerzforschung zu konzipieren. Registered Reports stellen Forschende jedoch auch vor erhebliche methodische und prozedurale Herausforderungen, darunter eingeschränkte Flexibilität und verlängerte Publikationszeiten. Der Stage 1 Peer-Review Prozess des zweiten Projekts betrug beispielsweise 14 Monate, was die Datenakquise und -analyse innerhalb des

Zeitrahmens dieser Arbeit verhinderte. Daher sollten die methodischen und prozeduralen Herausforderungen des Formats bei der Planung eines Registered Reports frühzeitig berücksichtigt werden.

Zusammenfassend liefern tACS und Neurofeedback im Bereich der Schmerzforschung bisher keine konsistenten Ergebnisse, was ihren wissenschaftlichen und klinischen Nutzen in Frage stellt. Die Exploration optimierter Stimulationsparameter, z.B. in den Bereichen Stimulationsfrequenz, -intensität, und -montage, sowie eine gründlichere Untersuchung der angewandten Verfahren in qualitativ hochwertigen Studien, z.B. unter Verwendung neuer Publikationsformate wie Registered Reports, sind zwingend erforderlich, um diese Wissenslücke zu schließen. In Anbetracht des dringenden Bedarfs an neuen Behandlungsansätzen für chronischen Schmerz sowie der konzeptionellen Plausibilität und potenziell breiten klinischen Anwendbarkeit nicht-invasiver neuromodulatorischer Techniken zur Schmerzmodulation erscheinen diese Bemühungen vielversprechend und sollten in Zukunft fortgesetzt werden.

ABBREVIATIONS

AAI	alpha asymmetry index
ACC	anterior cingulate cortex
ALT	attention left training
AMY	amygdala
ART	attention right training
BF	bayes factor
BFDA	bayes factor design analysis
BG	basal ganglia
CTC	communication-through-coherence hypothesis
EEG	electroencephalography
fMRI	functional magnetic resonance imaging
HD-tACS	high-definition transcranial alternating current stimulation
INS	insula
IPA	in-principal acceptance for publication
MEG	magnetencephalography
NPS	neurologic pain signature
NRS	numerical rating scale
PAG	periaqueductal grey
PCC	posterior cingulate cortex
PCI RR	peer community in registered reports
PET	positron emission tomography
PFC	prefrontal cortex
RM ANOVA	repeated measures analysis of variance
rTMS	repetitive transcranial magnetic stimulation

SBF+maxN	sequential Bayes factor design with maximal N
SIIPS-1	stimulus intensity independent pain signature-1
S1	primary somatosensory cortex
S2	secondary somatosensory cortex
T	thalamus
tACS	transcranial alternating current stimulation
tES	transcranial electrical stimulation
ToPS	tonic pain signature
tRNS	transcranial random noise stimulation
tTIS	transcranial temporal interference stimulation
VAS	visual analogue scale

1 INTRODUCTION

Pain is an unpleasant sensory and emotional experience that serves to protect the body. To achieve this vital function, pain does not simply mirror sensory information about potential threats, but it can also be substantially shaped by contextual factors including cognitive, emotional, and motivational processes[1]. Depending on current situational demands, sensory information and contextual factors are dynamically integrated, yielding a highly variable pain percept. Consequently, the same noxious (i.e., potentially tissue damaging) stimulus can lead to strong pain in some conditions (e.g., when fearing a medical procedure) whereas no or only modest pain is felt in others (e.g., when completing a long-distance run).

Accumulating evidence obtained using electroencephalography (EEG) and magnetoencephalography (MEG) suggests that rhythmic brain activity might be one of the mechanisms supporting this integration by flexibly routing information flow through the brain[2]. Rhythmic activity in the alpha (8 to <13 Hz) and gamma (>30 to 100 Hz) frequency bands, for instance, have been repeatedly associated with the processing of nociceptive stimuli and correlate with stimulus intensity and/or pain perception[3-8]. While informative and necessary, there is an increasing awareness that such correlative evidence fails to prove the necessity or sufficiency of a certain oscillation for pain and can, thus, not deliver mechanistic explanations for how pain emerges in the brain[9].

To overcome this limitation, techniques that allow researchers to manipulate neural oscillations in experimentally controlled settings and observe the impact on pain are required[10]. Non-invasive neuromodulatory techniques including transcranial alternating current stimulation (tACS) and neurofeedback open up such possibilities in healthy human participants[11,12] and could help identify brain mechanisms causally involved in pain. Beyond, such insights could aid the development of urgently needed neuromodulatory treatment approaches for chronic pain that directly target brain mechanisms causally involved in pain[13-15]. Despite this appeal, conclusive evidence from non-invasive neuromodulatory techniques in the field of experimental and chronic pain remains scarce warranting further investigation.

To contribute to this effort, the current thesis presents the results of two projects investigating the effects of two different neuromodulatory techniques, tACS and neurofeedback, on experimental pain (section 2 and 3, respectively) and discusses their implications (section 4). The preceding introduction (section 1) summarizes important concepts and previous research in three subsections. The first subsection introduces the neural mechanisms underlying pain with a focus on brain oscillations. The second subsection gives a general overview of non-invasive neuromodulatory techniques and their application in the field of pain research. Finally, the third subsection of the introduction outlines the aims of the current thesis.

1.1 Pain and the brain

1.1.1 Nociception

Pain is commonly preceded by a subconscious neurophysiological process referred to as nociception during which noxious stimuli are encoded in the peripheral and central nervous system[16]. Peripherally, potentially threatening mechanical, warm, or chemical stimuli are detected by the receptors (i.e., nociceptor) of specialized sensory neurons (i.e., nociceptive neurons) which then transmit the nociceptive signal to the dorsal horn of the spinal cord. In the spinal cord, nociceptive neurons form synaptic connections with secondary sensory neurons which predominantly ascend via the spinothalamic tract and project to the thalamus. Via third-order neurons, nociceptive information is then relayed to several subcortical regions and to the cerebral cortex where the transformation into a conscious pain percept occurs[17].

1.1.2 Brain regions of pain

While nociceptive processes in the periphery and spinal cord are well characterized[18], numerous open questions remain with respect to the cerebral integration of sensory information and contextual factors. Unlike other modalities, results from several imaging techniques including functional magnetic resonance imaging (fMRI), positron emission tomography (PET), MEG, and EEG indicate that this integration does not occur in a dedicated cerebral pain center exclusively devoted to pain[19,20]. Instead, pain is associated with complex spatial patterns of brain activity spread across the brainstem as well as subcortical and cortical regions which belong to different functional systems (Fig 1A)[19,20]. Brainstem and subcortical regions include the periaqueductal grey (PAG) as well as the amygdala (AMY), the basal ganglia (BG), and the Thalamus (T) which functions as relay center between brainstem and cerebrum. On a cortical level, pain-related regions include the primary and secondary somatosensory cortex (S1 and S2, respectively), which are linked to somatosensory processing, the anterior and posterior cingulate cortex (ACC and PCC, respectively), and the insula (INS). In addition, pain is associated with activity of the prefrontal cortex (PFC) which is, amongst others, linked to top-down control and emotional–motivational–evaluative processes[21-23] and plays an important role in descending pain modulation[19].

Based on this spatial extension of pain-related brain activity, pain is increasingly understood as a network phenomenon during which different functional regions dynamically interact depending on current situational demands[24,25]. Consequently, imaging efforts have shifted from the analysis of isolated brain regions to the analysis of activity and connectivity patterns across the entire brain[25,26]. Such whole-brain analyses have identified multivariate fMRI signatures including the neurologic pain signature (NPS)[27], the stimulus intensity independent pain signature-1 (SIIPS1)[28], and the tonic pain signature (ToPS)[29], which can predict experimental and, in the last case, even chronic pain by integrating information across brain regions. At the same time, the spatial extension of pain-related brain activity raises the question of how information can be exchanged and integrated between these regions at the short time scales observed in the context of pain (ms range). Anatomical connections provide the basis for communication within and between brain regions, yet changes in such connections cannot occur at the necessary, millisecond time scales[2]. Instead, increasing evidence supports the notion that rhythmic brain activity and its synchronization across brain regions could subserve the dynamic exchange and integration of information across the brain by enabling effective neural communication[30].

1.1.3 Brain rhythms of pain

Rhythmic brain activity, from here on referred to as neuronal oscillations, reflects periodic fluctuations of neural mass signals which can be recorded non-invasively using EEG or MEG[31]. Neural oscillations are mainly driven by periodic fluctuations in excitatory and inhibitory postsynaptic potentials which lead to periodic fluctuations in the excitability of neural populations[32]. Hence, when looking at the communication within and between brain regions, coherently oscillating populations could show properties for effective communication by aligning windows for spike output and sensitivity to synaptic input[30] (Fig 1B). According to the communication-through-coherence (CTC) hypothesis[30], for instance, anatomical connections in the brain are dynamically rendered effective or silenced through the presence or absence of oscillatory synchronization between pre- and post-synaptic neural populations. While presynaptic inputs arriving at the excitatory phase of the postsynaptic oscillatory cycle are thought to result in postsynaptic processing, presynaptic inputs arriving at random phases or during the inhibitory phase should not. Consequently, a postsynaptic neuronal population receiving several inputs should respond primarily to synchronized or coherent presynaptic groups resulting in selective communication. In addition, neuronal oscillations in different frequency bands are thought to contribute differentially to selective communication. While gamma oscillations (> 30 to 100 Hz) are thought to underlie local feedforward processing from

lower towards higher hierarchical brain regions, alpha (8 to < 13 Hz) and beta (13 to 30 Hz) oscillations are assumed to subserve feedback processing from higher towards lower brain regions[33]. Supporting the notion that neuronal oscillations are crucial for such essential brain functions as inter- and intraregional communication, neuronal oscillations are highly preserved across species[34] and are associated with various perceptual, cognitive, and behavioral functions, including pain[2].

Extending its spatial complexity to the frequency domain, pain is associated with complex spectral patterns covering a wide frequency range. In particular, pain-related oscillations have been observed at infraslow (< 0.1 Hz), theta (4 to < 8 Hz), alpha (8 to < 13 Hz), beta (13 to 30 Hz), and gamma (> 30 to 100 Hz) frequency ranges and vary depending on contextual factors and the type of pain under investigation[2] (Fig 1C). Consequently, none of these correlates can single-handedly predict pain. However, alpha and gamma oscillations seem to be particularly closely related to pain and might therefore be of particular importance for the processing of nociceptive input.

While findings with respect to chronic pain are rather inconsistent, studies on experimental pain have shown that both brief phasic (in the range of ms to s) and tonic pain (in the range of min), induce a strong suppression of *alpha oscillations*, in particular in somatosensory regions (Fig 1C)[2]. Importantly, the extent of this suppression and even spontaneous alpha power before a noxious event inversely relate to the perceived pain intensity[35-39]. Thus, higher pre- and post-stimulus alpha power is associated with less pain. These correlative findings are in line with the CTC hypothesis as well as current alpha theories “which propose that alpha oscillations gate sensory information processing in the human brain[40-42]. While lower alpha power in task-relevant, sensory regions is thought to facilitate processing of relevant sensory input, higher alpha power in task-irrelevant brain areas is thought to inhibit processing of irrelevant input. Attention represents one of the drivers that activate this alpha-based gating mechanism[43,44] and has also been shown to influence pain perception[45] as well as associated [pain-evoked and pain-induced] brain responses[46,47]. Correspondingly, attention-related alpha oscillations in somatosensory brain areas might serve the gating of nociceptive information in the processing of pain[46,48] and might therefore represent an ideal access point to the brain network underlying pain perception”[49].[a]

[a] *This thesis contains secondary publications, i.e., verbatim copies of text components published previously. Secondary publications are marked using quotation marks and references to the original publication. Deletions are marked using square brackets.*

Gamma oscillations, on the other hand, are positively related to pain intensity, with higher gamma power indicating more pain. Interestingly, a spatial shift of gamma oscillations seems to occur with increasing pain duration. Phasic experimental pain, for instance, is reliably tracked by gamma oscillations at central electrodes in both humans (Fig 1C) and rodents[50]. Furthermore, the optogenetic induction of gamma oscillations in S1 leads to enhanced nociceptive sensitivity and the display of pain behaviors in rodents indicating a causal role for pain[51]. However, when pain persists over longer time spans, e.g., during tonic experimental pain in healthy participants or during chronic pain in patients, higher pain intensity is associated with higher gamma oscillations in prefrontal brain regions[8,52,53]. Thus, with increasing duration, the representation of pain shifts from somatosensory regions commonly linked to sensory processing to prefrontal regions linked to functions like top-down control and emotional–motivational–evaluative processes[21-23]. Together, this points towards an important function of emotional-motivational-evaluative processes for longer lasting pain[9].

In summary, correlative evidence in humans and causal evidence in animals indicates that alpha and gamma oscillations in somatosensory and prefrontal regions play a crucial role for the processing of nociceptive input and the emergence of pain. Targeting these oscillations could, thus, enable access to the complex network underlying pain. Hence, their non-invasive modulation in humans is highly promising from both a basic science as well as a clinical perspective.

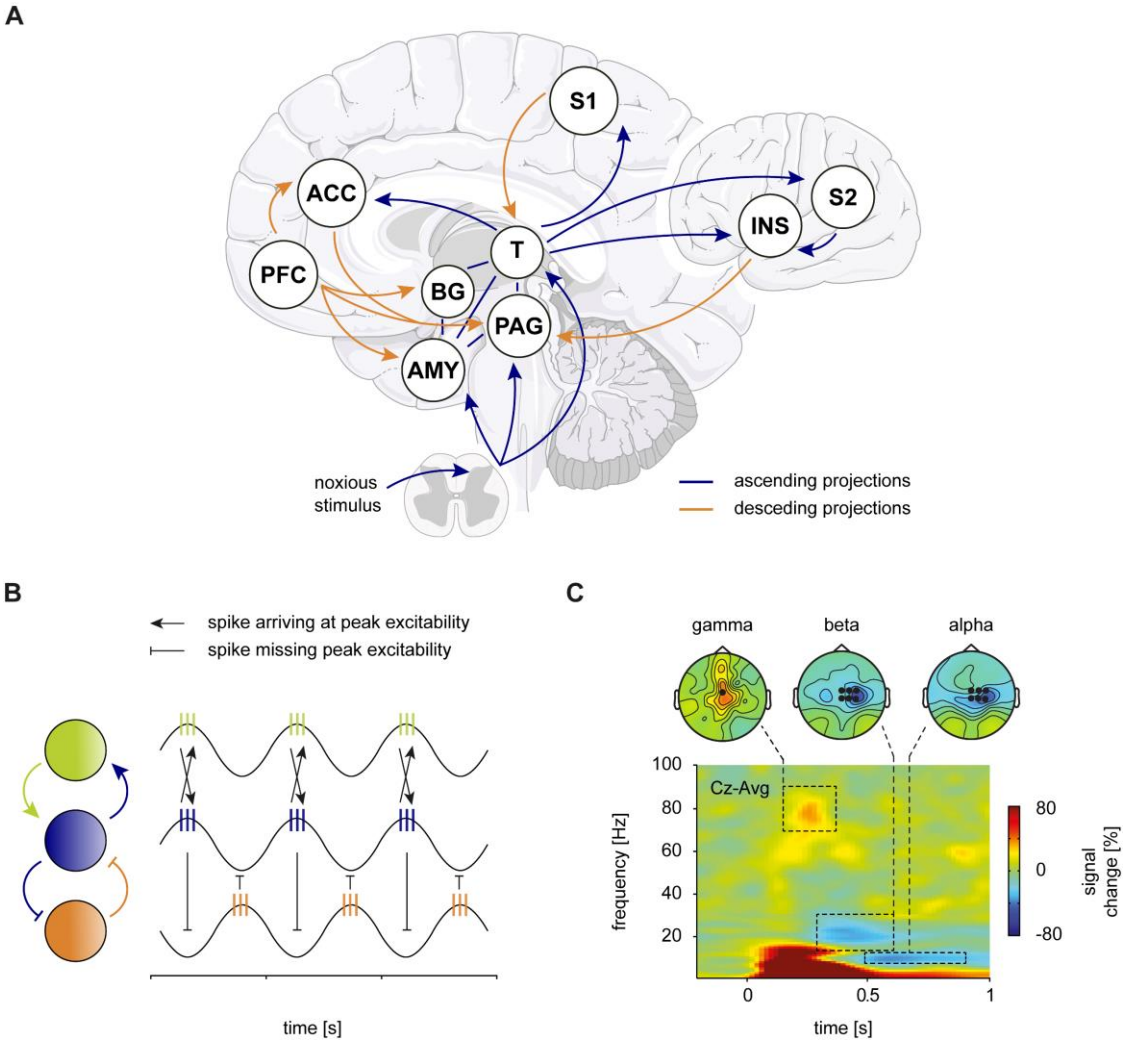

Figure 1. Brain regions and rhythms of pain. (A) Simplified overview of the brain regions included in ascending (blue) and descending (orange) pain pathways. (B) Schematic illustration of (in)effective communication between oscillating neuronal populations. Three populations of interconnected neurons (circles) are depicted together with their local field potential oscillations (waves) and action potentials (vertical lines). Action potentials either arrive at the postsynaptic neuron during a local field potential peak (arrows) or miss these peaks (blunt arrows). This leads to effective communication between the green and blue population, whereas communication is prevented between the blue and the orange population. (C) Oscillatory brain responses observed after brief, phasic experimental pain. The depicted time-frequency-representation (TFR) illustrates the central gamma enhancement (first dashed rectangle from the left) as well as the contralateral alpha and beta suppression (second and third dashed rectangle) typically observed after phasic pain (mean TFR averaged across trials and participants, n = 48, 20 trials, time point zero indicates application of a noxious stimulus). In addition, phasic pain leads to an enhancement in lower delta and theta frequencies (not marked). Alterations in these frequency ranges represent mainly phase-locked activity referred to as laser-evoked potential[54] and are commonly analyzed in the time-domain. Topographies depict the scalp distribution of neural activity for marked time-frequency windows. Black circles in the topographies indicate electrodes conventionally used to quantify the respective oscillatory brain response. For visualization purposes, the TFR is displayed as %-signal change relative to a prestimulus baseline (-3.3 to -2.8 s). Cz, Cz scalp electrode location according to the international 10-20 system; INS, insula; S2, secondary somatosensory cortex; S1, primary somatosensory cortex; T, Thalamus; PAG, periaqueductal grey; BG, basal ganglia; AMY, amygdala; ACC, anterior cingulate cortex; PFC, prefrontal cortex; Avg, average reference. Panel A was adapted by permission from Springer Nature: Neuroscience Bulletin, Ref. [25] (Modeling Pain Using fMRI: From Regions to Biomarkers, Reddan et al.), © (2021). Brain and spinal cord images were adapted by permission from smart.servier.com, Ref. [55-57], (CC BY 3.0). Panel B was adapted by permission from Elsevier, Trends in Cognitive Sciences, Ref. [58] (A mechanism for cognitive dynamics: neural communication through coherence, Fries), © (2005). Data in panel C are courtesy of PainLab-Munich, Department of Neurology, Technical University of Munich, Germany.

1.2 Non-invasive neuromodulation of pain

1.2.1 General overview of non-invasive neuromodulatory techniques

Non-invasive neuromodulatory techniques are an increasingly popular tool to study brain function in basic and translational neuroscience[11,12]. Using neuromodulatory techniques, neural measures of interest are experimentally manipulated and effects of this manipulation on perception and behavior are observed. Depending on the experimental design, perceptual and behavioral effects can either be observed during neuromodulation (online effects) or afterwards (offline effects). The same applies for the targeted brain activity, which should be assessed as a positive control to verify the success of the applied experimental manipulation. When neuronal oscillations are the focus of interest, applied neuromodulatory techniques typically include transcranial alternating current stimulation (tACS) and EEG-based neurofeedback[59]. Both techniques will be introduced in the following paragraphs and their application in the field of pain research will be reviewed.

1.2.2 Transcranial alternating current stimulation (tACS)

“Motivated by the rhythmic structure of endogenous brain activity, tACS uses weak alternating currents (< 4 mA peak-to-peak) of a certain frequency. These currents are applied to the scalp via 2 or more surface electrodes to modulate oscillatory brain activity, usually at a frequency thought to be involved in a certain condition or cognitive process[10,11]. In the brain, these currents are thought to induce periodic membrane potential fluctuations in affected brain areas, aligning the frequency and phase of endogenous oscillations[10,11]. This synchronization is commonly referred to as *entrainment*[60] and is supported by results from animal and computational modelling studies as well as behavioral effects during tACS in humans[10,11,61-63]. Besides online effects, also offline effects, which can persist for several hours after stimulation, are […] documented in humans on a behavioral[64,65] and neuronal level[66-70]. These are most probably induced by temporary alterations of synaptic plasticity[70,71] and are of particular relevance for potential clinical applications of tACS”[9].

Despite its appeal, only three studies have used tACS to investigate the neural mechanisms underlying pain and its modulation to date. “Applying short pressure pain stimuli of different intensities, a first study[72] could show that alpha tACS at 10 Hz over somatosensory cortices reduces pain ratings. This effect was confined to conditions in which the intensity of the upcoming stimulus was uncertain, indicating that expectations [might] influence pain-related tACS effects. Another study[73] points towards analgesic effects of somatosensory alpha tACS in chronic pain. Investigating both behavioral and

neurophysiological effects, the authors could show that 40 minutes of alpha tACS targeting the bilateral primary somatosensory cortex […] enhances alpha oscillations in the targeted regions. The extent of this increase was correlated with changes in pain severity and disability, indicating that stronger alpha increases lead to larger reductions in pain and the associated disability"[9]. Finally, a third pilot study focusing on fibromyalgia reported analgesic effects after beta or theta tACS over individualized locations, which were not present in the control condition entailing random noise stimulation (tRNS)[74]. However, methodological shortcomings of this study such as the lack of a second baseline assessment between conditions have been criticized[75] and warrant caution when interpreting the reported findings.

Summarizing, first tACS studies provide tentative evidence that neural oscillations can be altered using tACS and may be causally involved in the processing of phasic and chronic pain. However, due to their small number (n = 3), these studies require replication and elaboration by further systematic tACS studies. In addition, the conducted studies have focused mainly on somatosensory alpha oscillations and should be complemented by studies targeting other stimulation locations and frequencies such as prefrontal gamma oscillations which are conceptually plausible and feasible as well.

1.2.3 Neurofeedback

Neurofeedback is a form of brain-based biofeedback that relies on the assumption that oscillatory brain activity is responsive to operant conditioning[12,76]. Generally, the learning principle of operant conditioning states that the strength of a behavior is modified by its consequence: while positive consequences such as positive feedback and reward increase the probability of a behavior, negative consequences such as negative feedback and punishment decrease its probability[77]. Following these principles, neurofeedback uses real-time feedback signals to reinforce certain brain states. Specifically, brain activity is measured, analyzed, and fed back to users in real-time using sensory feedback signals (e.g., visual, auditory, tactile, or multimodal) which reflect the proximity of current brain activity to a desired brain state. Based on this feedback signal, users then try to self-regulate their brain activity towards the desired state, creating a closed-loop system. Thus, in contrast to other passive neuromodulatory techniques such as tACS, neurofeedback applications require active user engagement[76].

Although several neurofeedback studies targeting different brain regions and frequencies have been conducted in the context of pain research, its efficacy with respect to the modulation of pain remains unclear. While some studies report significant pain reductions after neurofeedback, others did not find analgesic effects[78-80]. “This inconsistency might be due to the particular methodological challenges of neurofeedback studies. One major challenge is to directly link modulations of brain activity to modulations of perception, e.g., by showing concomitant changes of brain activity and perception during neurofeedback instead of relying on pre-post neurofeedback changes only. Another crucial challenge is to define control conditions which effectively separate neurofeedback-specific effects from non-specific effects such as time and placebo effects[78,79]. So far, most pain-related studies have not met these challenges rendering the specificity and significance of previous findings unclear”[49]. Consequently, there is a general consensus that further, high-quality studies are needed[78-80].

1.3 Aims and outline

Building on the findings reviewed above, the goal of this thesis was two-fold. First, this thesis aimed at investigating the functional significance of alpha and gamma oscillations for phasic and tonic pain using non-invasive neuromodulatory techniques to progress from correlation towards causation. Second, this thesis aimed at exploring the ability of tACS and neurofeedback to modulate pain processing and perception which could support their utility in clinical settings such as chronic pain.

1.3.1 Project 1: Can we modulate pain using tACS?

The first project further explored the potential of tACS to modulate pain by systematically applying alpha, gamma, or sham tACS over the somatosensory and prefrontal cortex during tonic experimental pain. Thereby, the study extended previous work by targeting a new location and frequency, both of which have been implicated in the processing of pain. If tACS augments the targeted oscillatory brain activity, we expected to find alterations of pain ratings in active stimulation (verum) conditions compared to sham conditions without tACS.

1.3.2 Project 2: Can we modulate pain using neurofeedback?

The second project further explores the potential of EEG-based neurofeedback to modulate pain and underlying brain responses. Specifically, the proposed study investigates the impact of a short-term neurofeedback training targeting attention-related alpha oscillations in somatosensory brain regions on phasic experimental pain. In a sham-controlled, bi-directional regulation design, each participant will complete a total of 4 conditions in which attention-related alpha oscillations are either up- or downregulated

using true (verum) or sham neurofeedback. “If a direct link between attention-related alpha oscillations and pain exists, this bi-directional regulation should result in opposite behavioral effects[81] which should be less pronounced in the two sham conditions […]”[49].

Motivated by the methodological challenges associated with neurofeedback studies and the inconsistency of previous results, the project was conceptualized as a registered report and is currently still in the data acquisition phase. Thus, the corresponding sections will focus on the introduction of the registered reports format and its suitability for neurofeedback studies examining the modulation of pain rather than on the presentation of results.

1.3.3 Project-related publications

The results of project 1 have been published in a peer-reviewed journal[82] alongside a review-article motivating the usage of tACS to modulate pain[9]. Project 2 has been conceptualized as a registered report and has obtained an in-principal acceptance from a peer-reviewed journal. Original publications from both projects have been aggregated in this thesis to connect insights from two different non-invasive neuromodulatory techniques in the context of pain research and to illustrate how registered reports can be used to enhance the reliability and validity of neuromodulatory studies. Throughout this thesis, secondary publications are marked using quotation marks and references to the original publication. Deletions are marked using square brackets.

1.3.4 Data and code availability

Anonymized data sets and analysis code related to project 1 are publicly available at https://osf.io/pnd6g/. Likewise, all datasets and study materials related to project 2 will be made available at https://osf.io/qbkj2/ upon acceptance for publication (Stage 2 acceptance).To enhance re-usability, datasets were/will be uploaded in the standardized EEG-BIDS format[83].

2 PROJECT 1: CAN WE MODULATE PAIN USING TACS?

2.1 Methods

2.1.1 Participants

“The study protocol was approved by the local Ethics Committee of the Medical Faculty of the Technical University of Munich and pre-registered at ClinicalTrials.gov (NCT03805854). The study was conducted in accordance with the latest version of the Declaration of Helsinki and recent consensus guidelines for the application of tACS in humans[84]”[82]. Prior to any experimental procedures, all participants gave written informed consent. The study entailed six sessions and participants received a fixed financial compensation of 25 € per session plus a bonus of 50 € after completing the experiment. Thus, participants completing the experiment received 200 € in total.

“A priori sample size calculations using G*Power[85] determined a sample size of 28 participants for a repeated measures analysis of variance (RM ANOVA) design with 6 conditions (see below), a power of 0.95, an alpha of 0.05, and medium effect sizes of f = 0.25. This corresponds to an η^2 (proportion variance explained) of 0.06[86]. Based on these calculations, the final sample comprised 29 participants (all right-handed, 13 females, age: 25.7 ± 4.0 years [mean ± SD]). Overall, 39 healthy human participants were recruited. Ten participants were excluded during the course of experiment due to the absence of pain (n = 3) or intolerable pain (n = 3) during the first session, technical issues (n = 1: thermal stimulation was interrupted due to a broken cable, n = 1: technical defect of recording hardware), or meeting exclusion criteria during 1 of the sessions (n = 2). Inclusion criteria were age above 18 years and righthandedness. Exclusion criteria were pregnancy, neurological or psychiatric diseases, severe internal diseases including diabetes, skin diseases, current or recurrent pain, regular intake of medication (aside from contraception, thyroidal and, in 1 case, antiallergic medication), previous surgeries at the head or spine, previous syncopes or head traumas resulting in unconsciousness or concussion, metal or electronic implants, and any previous side effects associated with thermal, electrical, or magnetic stimulation. None of the included participants showed signs of clinical anxiety or depression according to the Hospital Anxiety and Depression Scale[87] with a cut-off of 8/21[88] (anxiety: 2.5 ± 2.0 [mean ± SD]; depression: 0.7 ± 0.9 [mean ± SD])”[82].

2.1.2 Paradigm

“In a within-subject design, each participant took part in 6 recording sessions. […] Each session comprised a fixed sequence of events (Fig 2A). In each session, tACS was applied over prefrontal cortex (PFC) or somatosensory cortex (S1) (Fig 2B) using alpha frequency (10 Hz) stimulation, gamma frequency (80 Hz) stimulation, or sham stimulation (Fig 2C). Concurrently, a tonic heat pain stimulus of varying intensity was applied to the left hand. During stimulation, participants continuously rated the currently perceived pain intensity. […]. Before and after the stimulation, 5 minutes of resting state EEG were recorded using the tACS electrodes”[82]. In addition to pain ratings, autonomic responses (skin conductance and electrocardiogram) were continuously measured during stimulation to examine whether tACS modulates pain-related autonomic responses. Due to its focus on pain perception, corresponding analyses and results will not be reported in this thesis but can be found in Ref. [82].

2.1.3 Noxious stimulation

“Tonic painful heat stimulation was applied to the participant’s left hand for 10 minutes using a thermode (TSA-II, Medoc, Ramat Yishai, Israel). Following an established paradigm[8,89,90], a predefined, fixed time course of stimulation (Fig 2A) consisting of 9 plateaus with 3 temperature levels (low, medium, and high) was applied. Temperature levels were individually adjusted for each participant by adding 0.5, 0.8, or 1.1°C to the individual pain threshold (see below), resulting in 3 intensity levels of thermal stimulation. The stimulation sequence consisted of 3 plateaus of 40, 50, and 60 s duration at each temperature level. The stimulation started from a baseline temperature of 40°C and changed with a rate of 0.1°C/s. All analyses were performed using an 8-minute-time window beginning at the start of the first plateau. Pain thresholds were determined for the left hand on the first recording day immediately before the pre-stimulation EEG resting state recording. In line with previous studies[8,89,90], over the course of 3 minutes, participants continuously adjusted the thermode temperature to their individual pain threshold using 2 buttons of a computer mouse with their right hand. Depending on the side of the button press, the thermode temperature either increased or decreased with a rate of 0.5° C/s. The individual pain threshold was defined as the average stimulus intensity during the last 10 s and was used to determine individual temperature levels for all 6 recording days. Thus, temperature levels were individually adapted but kept constant across all conditions for each single participant. We chose to keep the objective stimulus intensity constant across conditions to rule out different temperature levels as a confounding variable in our analyses. Mean pain threshold temperature was of 44.4 ± 1.7°C [mean ± SD]”[82].

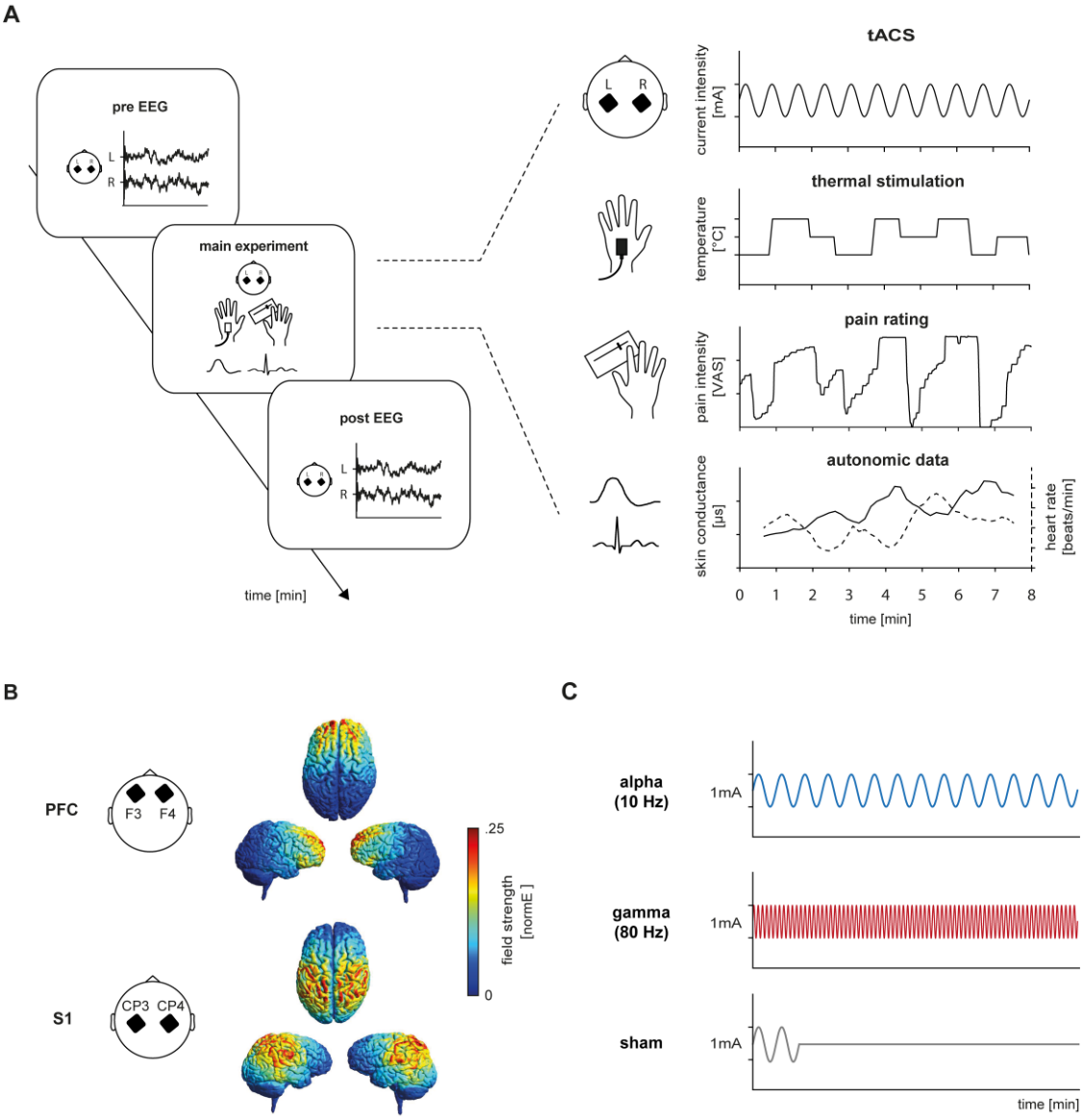

Figure 2. tACS paradigm. (A) Paradigm. "Each participant took part in 6 recording sessions which comprised a fixed sequence of events. During the main experiment, participants received tACS over prefrontal or somatosensory cortices using alpha, gamma, or sham stimulation while a tonic heat pain stimulus of varying intensity was applied to the left hand. Concurrently, participants continuously rated the currently perceived pain intensity and autonomic responses (skin conductance and electrocardiogram) were measured. Before and after the main experiment, 5 minutes of resting state EEG were recorded using the tACS electrodes"[82]. (B) tACS locations. "Using two 5*5 cm carbonized rubber electrodes placed according to the international 10-20 system, tACS of 1 mA peak-to-peak intensity was applied over PFC (electrode positions F3 and F4) or S1 (electrode positions CP3 and CP4). Electrode placement was validated through simulations performed with SimNIBS 2.150 using 1 mA intensity, standard conductivity parameters, and the SimNIBS template head model. Simulations of the induced electrical field strength are shown on the right"[82]. (C) tACS frequencies. "1 mA peak-to-peak tACS was applied at alpha or gamma frequencies or using sham stimulation. For alpha frequency stimulation, sinusoidal stimulation with a frequency of 10 Hz was applied. For gamma frequency stimulation, sinusoidal stimulation with 80 Hz frequency was applied. For sham stimulation, 30 s of 10 Hz sinusoidal stimulation were applied at the beginning of thermal stimulation only. All stimulations included 100 cycles fade-in and fade-out"[82]. EEG, electroencephalography; L, left; R, right; PFC, prefrontal cortex; S1, primary somatosensory cortex; tACS, transcranial alternating current stimulation; VAS, visual analogue scale. Figure 2 was adapted by permission from Elsevier: The Journal of Pain, Ref. [82] (Modulating Brain Rhythms of Pain Using Transcranial Alternating Current Stimulation (tACS) - A Sham-Controlled Study in Healthy Human Participants, May et al.), © (2021).

2.1.4 tACS

“Ten minutes of tACS were applied simultaneously to painful heat stimulation. The paradigm, thus, enables the exploration of immediate tACS effects (online effects) on pain rather than exclusively relying on aftereffects outlasting the stimulation (offline effects). tACS intensity was 1 mA peak-to-peak for all participants and conditions. We employed a Neuroconn DC-STIMULATOR MR (Neuroconn, Ilmenau, Germany) and 2 carbonized rubber electrodes with a size of 5×5 cm. To validate electrode placement, electrical fields induced by a 1 mA transcranial current stimulation were simulated beforehand using SimNIBS 2.1[91] with standard conductivity parameters and the SimNIBS template head model (Fig 2B and [82] Supplementary Fig S1). For stimulation of the PFC, electrodes were placed at positions F3 and F4 of the international 10-20 system. For stimulation of S1, electrodes were attached at positions CP3 and CP4. In line with recent recommendations[92], electrodes were firmly fixed to the scalp using an even layer of Ten20 conductive paste (D.O. Weaver, Aurora, CO, United States), rendering any additional fixation of electrodes unnecessary. Impedances were kept below 5 kΩ (1.7 ± 0.9 kΩ [mean ± SD across all subjects and conditions]) and were similar for all 3 stimulation conditions of both montages (PFC: $\chi^2(2) = 0.80$, $P = .672$; S1: $\chi^2 (2) = 3.07$, $P = .215$; Friedman tests). For alpha frequency stimulation, a 10 minute-sinusoidal stimulation with a frequency of 10 Hz was applied. For gamma frequency stimulation, a 10 minute-sinusoidal stimulation with 80 Hz frequency was applied. For sham stimulation, 30 s of 10 Hz sinusoidal stimulation were applied. All stimulations included 100 cycles fade-in and fade-out. Fade-in always started with the beginning of thermal stimulation. Thus, during the 8 minute-analysis window starting from the first plateau of thermal stimulation, participants received simultaneous, continuous tACS in the alpha and gamma frequency conditions, but no stimulation in the sham condition. For half of the participants, the 3 PFC sessions were performed first, followed by the 3 S1 sessions. For the other half, the order was reversed. Within the 3 sessions of each tACS location, the order of stimulation frequencies (alpha, gamma, sham) was counterbalanced to control for potential sequence effects of stimulation frequency”[82].

2.1.5 EEG recording

“The rationale of tACS is to modulate neural oscillations during tACS. Demonstrating such online effects directly requires the simultaneous measurement of neural oscillations during tACS. However, online EEG measurements are heavily contaminated by tACS artifacts and their significance is therefore uncertain[10]. To nevertheless check for a potential indicator of the neural efficacy of our stimulation, we investigated offline effects of our stimulation.”[82]. If present, these offline effects suggest the presence of online effects. Their absence, however, does not necessarily imply the absence of online effects as EEG after-effects are not consistently observed[68,69,82].

“To quantify potential tACS effects on oscillatory brain activity outlasting the stimulation, we recorded 5 minutes of resting state brain activity immediately before and after tACS (pre- and post-EEG). Participants were asked to stay in a relaxed, wakeful state, without any particular task, keeping their eyes open and their gaze rested on a centrally presented fixation cross. EEG data were recorded using the same 2 electrodes used for tACS, that is, placed at F3 and F4 for PFC sessions and at CP3 and CP4 for S1 sessions. Ag/AgCl electrodes attached to the nose and centrally on the forehead served as reference and ground, respectively. A bipolar Ag/AgCl electrode montage with electrodes below the outer canthus of the right eye and immediately below the hairline at the midline of the forehead was used to record eye movements. EEG data were sampled at 1000 Hz using the BrainAmp ExG MR amplifier (Brain Products, Munich, Germany) and bandpass-filtered between 0.016 and 250 Hz. Impedances were kept below 5 kΩ”[82].

2.1.6 Blinding

“Due to the attachment of electrodes, participants and experimenters were not blinded with respect to the location of tACS. However, we aimed at a double-blind design with respect to the tACS frequency (alpha, gamma, sham). To this end, each session was conducted by a main experimenter who was unaware of the stimulation frequency and interacted with the participant and a second experimenter who operated the tACS device. At the end of each session, blinding of the participant was assessed using a short questionnaire consisting of 3 questions: (1) “Did you have the impression that a continuous brain stimulation was applied today?”, (2) “Did you experience sensations at the scalp like tingling, prickling, or pulsing?”, and (3) “Did you experience light perceptions (phosphenes) like flickering?”. Question 1 was answered using a forced choice format (yes/no), whereas questions 2 and 3 were answered using a visual analogue scale (VAS) ranging from 0 (“no”) to 10 (“very strongly”)”[82].

2.1.7 Analysis of tACS effects on pain

“We first assessed whether tACS modulated pain perception (Fig 3A). For this purpose, the 8 minute-pain rating and -temperature time courses were smoothed using a sliding-window approach with a window length of 1 s and a step size of 0.1 s. Smoothed pain rating and temperature time courses represented the basis of further analyses. Subsequently, analyses of tACS effects on pain intensity were performed. To investigate whether tACS influenced the overall pain level, we computed a summary measure of pain intensity by averaging pain ratings across the 8 minute-interval and compared the resulting averages between conditions. To investigate whether tACS influenced pain intensity at any time during the 8 minutes of thermal stimulation, pain rating time courses were compared between alpha, gamma, and sham conditions in a time-resolved fashion. Lastly, we asked whether tACS might selectively alter pain intensity at certain temperature levels and compared the average pain intensity separately for low, medium, and high temperature levels. Since tACS might also influence the stability of pain ratings or the translation of noxious stimuli into pain rather than pain directly, we next examined tACS effects on pain variability and the relation of pain to thermal stimulation. To this end, we first obtained summary measures across the 8 minute-time course. For pain variability, the standard deviation of pain ratings was calculated across the entire time course. For the relation of pain and thermal stimulation, Pearson correlations between pain ratings and temperature were calculated. For additional time-resolved analyses, time courses of both measures were calculated by applying a sliding-window approach to the 8 minute-pain rating and -temperature time courses using a window size of 60 s and a step size of 10 s[89]. Subsequently, the standard deviation of pain ratings and Pearson correlations between pain ratings and temperature were calculated for each window”[82].

2.1.8 Analysis of tACS effects on brain activity

“We further investigated whether tACS induced neuronal changes outlasting the stimulation (offline effects, Fig 3B). To this end, EEG data obtained before and after tACS were downsampled to 250 Hz. A visual artifact correction was performed, manually rejecting data segments contaminated by muscle activity. All analyses focused on the electrode contralateral to the stimulated hand, that is, F4 for the PFC and CP4 for the S1 electrode montage. In addition, the 60 s data segments closest to tACS were selected, that is, the last minute of the 5 minute pre-stimulation EEG and the first minute of the 5 minutes post-stimulation EEG. Data were cut into 1 s epochs with 50 % overlap and frequency specific power between 1 and 100 Hz was calculated using a Fast Fourier Transformation with a Hanning window resulting in a frequency resolution of 1 Hz. Subsequently, power spectra were averaged across all epochs for pre- and post-stimulation EEGs separately. Pre-stimulation power spectra were then subtracted from post-stimulation power spectra for each of the 6 conditions and each participant individually. During statistical analyses, these difference power spectra were compared between the active tACS conditions (PFC/S1 alpha/gamma stimulation) and the respective sham conditions (see below).

In addition, we performed several control analyses. First, we repeated the same analysis calculating difference power spectra based on the complete 5 minutes rather the last and first 1 minute pre- and post-EEG data. A second control analysis used average power spectra across both prefrontal electrodes rather than the contralateral prefrontal electrode since prefrontal activations during pain do not show a clear lateralization[6,8,53]. Third, we log-transformed power spectra before statistical contrasts to account for the non-Gaussian distribution of EEG data. Finally, we also checked for potential tACS effects in the non-targeted frequency band by performing contrasts of gamma power spectra in the alpha tACS conditions and contrasts of alpha power spectra in the gamma tACS conditions. Alpha and gamma oscillations are thought to reflect complementary inhibitory and excitatory feedback processing, respectively[30]. Thus, it is conceivable that tACS targeting one frequency band might alter oscillatory activity in the other, non-targeted frequency band”[82].

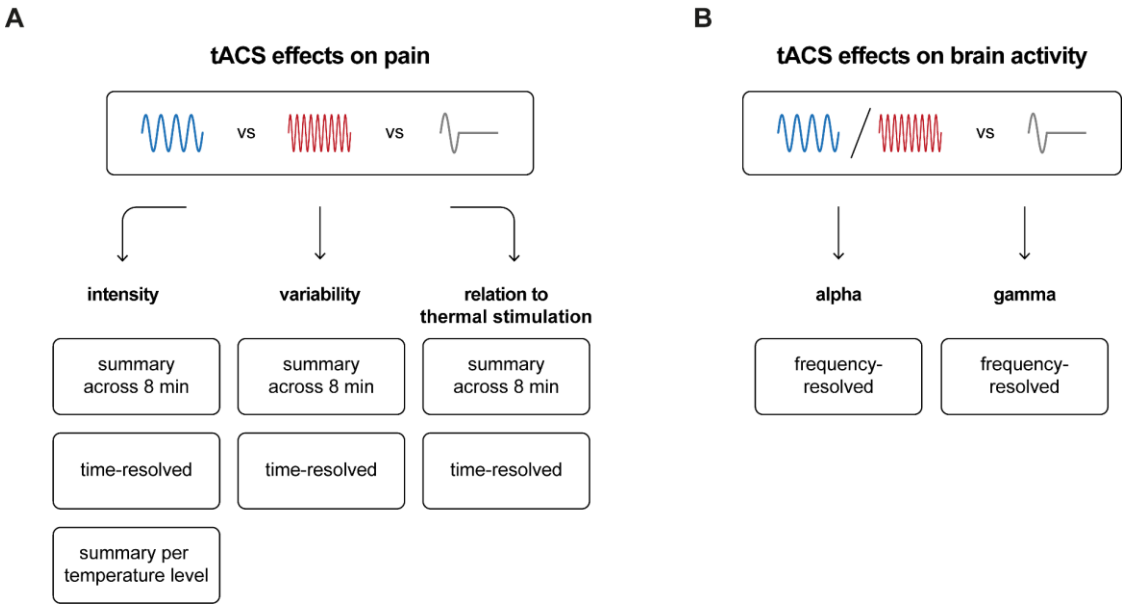

Figure 3. tACS analysis pipeline. (A) Investigation of tACS effects on pain. "tACS effects on pain were investigated with respect to the intensity of pain, the variability of pain, and the relationship of pain to thermal stimulation. All variables were analyzed across the entire 8-minute analysis interval (summary measures), in a time-resolved fashion, as well as per temperature level in the case of pain intensity. […] To detect tACS effects, all variables were compared between the 3 tACS conditions (alpha, gamma, sham) separately for both tACS locations (PFC, S1)"[82].(B) tACS effects on brain activity were investigated with respect to alpha and gamma oscillations in a frequency-resolved fashion. To detect tACS effects, power in the alpha/gamma band was compared between the respective active tACS condition and the sham conditions separately for both tACS locations (PFC, S1). tACS, transcranial alternating current stimulation. Panel A was adapted by permission from Elsevier: The Journal of Pain, Ref. [82] (Modulating Brain Rhythms of Pain Using Transcranial Alternating Current Stimulation (tACS) - A Sham-Controlled Study in Healthy Human Participants, May et al.), © (2021).

2.1.9 Statistics

“Statistical analyses were performed using Matlab (Mathworks, Natick, MA), the Matlab toolbox Fieldtrip[93], IBM SPSS Statistics for Windows (SPSS), version 26 (IBM Corp., Armonk, NY), and the statistical software package JASP, version 0.11.1 (JASP Team, 2019). Since Shapiro-Wilk tests indicated that some variables were not normally distributed, non-parametric tests were used for statistical analysis. These included Cochran’s Q-tests, Friedman tests, and non-parametric cluster-based permutation statistics[94,95]. Post hoc tests with Bonferroni correction were conducted when necessary and included McNemar tests for Cochran’s Q-tests and Wilcoxon matched-pairs signed rank tests for Friedman tests. Cluster-based permutation tests based on F-tests were followed up by pairwise post hoc cluster-based permutation tests based on t-tests. Additionally, Bayesian RM ANOVAs were performed to complement analyses relying on null-hypothesis significance testing[96]. They were followed up by post hoc Bayesian dependent samples t-tests for analyses yielding conclusive evidence for the alternative hypothesis or inconclusive evidence”[82].

“The blinding of participants was examined using a Cochran’s Q-test for question 1, which compared the frequency of yes responses across all 6 experimental conditions. VAS scores from question 2 and 3, which addressed the intensity of skin sensations and phosphenes, respectively, were investigated using Friedman tests. To investigate whether skin sensations and/or phosphenes differed between tACS locations, data from all frequency conditions were aggregated for each location and then compared using a Friedman test with the within-subjects factor location (PFC, S1). To investigate whether skin sensations and/or phosphenes differed between tACS frequencies, data from the 3 frequency conditions (alpha, gamma, sham) were compared using a Friedman test with the within-subjects factor frequency for both locations separately. All subsequent analyses were conducted separately for the PFC and S1 location as blinding questionnaires indicated that participants were successfully blinded for the S1 but not for the PFC location”[82].

“Friedman tests with the factor frequency (alpha, gamma, sham) were used to compare summary measures of pain intensity, pain variability, the relation of pain to thermal stimulation as well as […] summary measures of pain intensity for each temperature level. Cluster-based permutation statistics clustering across time were used to investigate tACS effects on time courses of pain intensity, pain variability, and the relation of pain to thermal stimulation[…]. Specifically, time courses were compared between alpha, gamma, and sham conditions using cluster-based permutation statistics based on dependent samples F-tests, clustering across time. Cluster-based permutation

statistics clustering across frequencies were used to investigate tACS offline effects on brain activity power spectra. To this end, pre- and post-stimulation difference power spectra from the electrode contralateral to thermal stimulation were compared between the active tACS conditions and the respective sham conditions using non-parametric cluster- based permutation statistics based on dependent samples t-tests, clustering effects across frequencies. Specifically, PFC alpha and gamma conditions were compared to the PFC sham condition and S1 alpha and gamma conditions were compared to the S1 sham condition resulting in 4 pairwise comparisons. When investigating the effect of alpha frequency stimulation, this analysis was applied to a frequency band from 8 to 12 Hz. When investigating gamma frequency stimulation, the frequency band was 70 to 90 Hz. To control for multiple comparisons, all p values were subjected to false discovery rate (FDR) control of Type I error[97]. Corrections were conducted separately for pain […] and brain activity considering the number of all statistical analyses performed for the respective measure (see Fig 3 for an overview […]). This resulted in an FDR control for 14 statistical tests (7 tests x 2 tACS locations) for pain ratings […] and an FDR control for 4 statistical tests (2 tests x 2 tACS locations) for brain activity. Throughout the manuscript, corrected p values are reported. [Uncorrected p values for all analyses are summarized in Supplementary Table S1.] If not stated otherwise, statistical tests were performed 2-sided with a significance level (α) of $p < .05$. Analyses relying on null-hypothesis significance testing were complemented by Bayesian RM ANOVAs. The Bayesian approach to hypothesis testing considers the likelihood of the observed data under the null and the alternative hypothesis. The comparison of the resulting probabilities is reflected by the Bayes Factor (BF_{01} = likelihood of the data given the H0/likelihood of the data given the H1)[96,98]. Thus, Bayes factors allow to specifically evaluate evidence in favor of the null hypothesis. Bayesian RM ANOVAs were performed for the pain intensity summary measure […] across 8 minutes. As before, the analyses included the factor frequency (alpha, gamma, sham) and were conducted separately for both tACS locations. For all effects, JASP default prior options were chosen"[82].

2.2 Results

2.2.1 Participants were blinded for tACS over S1, but not over PFC

“After each session, the blinding of participants was assessed using questionnaires (Supplementary Fig S2). When asked whether a continuous stimulation was applied or not, participants’ reports did not differ between the 6 experimental conditions ($\chi^2(5) = 7.46$, $p = .189$). Likewise, skin sensations did neither differ between tACS locations (χ^2 (1) = .75, $p = .385$) nor between tACS frequencies for either of the locations (PFC: $\chi^2(2) = 2.11$, $p = .348$; S1: $\chi^2(2) = 0.75$, $p = .688$). However, phosphenes were stronger for tACS over PFC than over S1 ($\chi^2(1) = 7.23$, $p = .007$). In addition, phosphenes differed between frequencies for tACS over PFC, but not over S1 (PFC: $\chi^2(2) = 8.90$, $p = .012$; S1: $\chi^2(2) = 0.19$, $p = .910$). Post hoc tests showed that phosphenes were significantly stronger in the PFC alpha condition than in the PFC gamma condition ($Z = -3.13$, $p = .006$). Hence, participants were successfully blinded for tACS over S1 but not for tACS over PFC. Thus, all further analyses investigated tACS effects separately for PFC and S1 locations“[82].

2.2.2 tACS did not modulate pain

“We first investigated whether tACS influenced pain intensity averaged across the entire 8 minutes of thermal stimulation. To this end, we compared average pain intensity during alpha, gamma, and sham tACS for both locations (Fig 4). The results did not show any statistically significant difference, neither during tACS over PFC nor during tACS over S1 ($p > .05$ for all tests; see Supplementary Table S1 for test statistics and uncorrected p values of all pain analyses). We further assessed whether tACS influenced pain intensity at any time during the 8 minutes of thermal stimulation. To this end, we compared pain intensity time courses during alpha, gamma, and sham stimulation for both tACS locations (Fig 4). For both PFC and S1, cluster-based permutation tests did not show significant differences in pain intensity at any time ($p > .05$ for all clusters). We further asked whether tACS might selectively alter pain intensity at certain temperature levels. For instance, tACS might particularly modulate pain at the lowest level at which pain ratings are closest to pain threshold and possibly most uncertain. We therefore compared the average pain intensity separately for low, medium, and high temperature levels (Supplementary Fig S3). However, no significant tACS effects on pain intensity were found for any temperature level ($p > .05$ for all tests). Lastly, we asked whether tACS might influence the stability of pain ratings or the translation of noxious stimuli into pain rather than pain intensity directly. To this end, we investigated whether tACS influenced the variability of pain or the relation of pain to thermal stimulation (Fig 5).

Comparisons of summary measures across 8 minutes did not yield significant tACS effects on pain variability or the relationship of pain to thermal stimulation ($p > .05$ for all tests). Likewise, time-resolved analyses of both measures did not show any significant tACS effects at any time ($p > .05$ for all clusters). Taken together, we did not find tACS effects on different measures of tonic pain."[82]

"Next, we used Bayesian statistics to evaluate direct evidence for a lack of tACS effects on pain intensity. We specifically performed Bayesian RM ANOVAs with the factor tACS frequency (alpha, gamma, sham) for both PFC and S1 locations. These analyses resulted in a Bayes factor (BF_{01}) which quantifies the relative likelihood of the data given the null hypothesis of no tACS effect over the alternative hypothesis postulating tACS effects. BF_{01} values below 0.33 are commonly classified as evidence for the alternative hypothesis, values from 0.33 to 3 are classified as inconclusive evidence, and values above 3 are classified as evidence for the null hypothesis[99]. The analysis of pain intensity resulted in a BF_{01} of 5.727 for the PFC and a BF_{01} of 2.082 for the S1 location, indicating that evidence for the null hypothesis was moderate for the PFC but inconclusive for the S1 location. To follow up the inconclusive result for tACS over S1, we performed pairwise comparisons between tACS frequencies (alpha, gamma, sham) using Bayesian dependent samples t-tests. These revealed moderate evidence for the null hypothesis when comparing the gamma and sham conditions ($BF_{01\ gamma \neq sham} = 3.893$) but inconclusive evidence for both comparisons entailing the alpha condition ($BF_{01\ alpha \neq sham} = 0.935$; $BF_{01\ alpha \neq gamma} = 2.491$)."[82]

"Taken together, frequentist statistical analyses did not provide evidence for a modulation of tonic pain by tACS at alpha or gamma frequencies over PFC or S1. Bayesian analyses provided moderate evidence for a lack of tACS effects on tonic pain except for alpha tACS over S1 where evidence was inconclusive"[82]. These group-level findings were further supported by exploratory subgroup analyses. Specifically, group-level analyses were repeated for (1) participants showing strongest evidence for frequency specific tACS offline effects as well as for (2) participants whose endogenous alpha peak frequency most closely resembled the tACS frequency of 10 Hz and did not yield tACS effects on pain (see Ref. [82] for details regarding the analysis pipeline and the corresponding results).

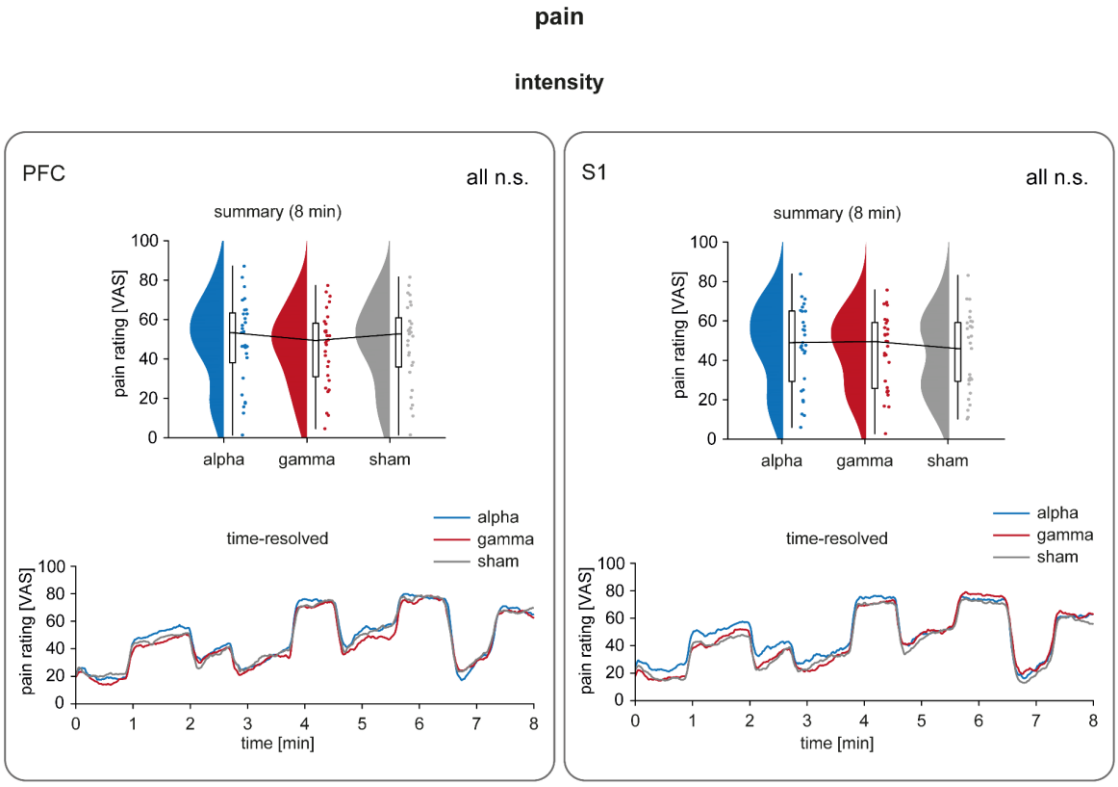

Figure 4. tACS effects on pain intensity. tACS effects on pain intensity are shown separately for PFC (left) and S1 (right) tACS locations. "For both locations, upper rows display summary measures obtained by averaging pain ratings (0−100; VAS) across the 8-minute analysis window. Raincloud plots[99] show unmirrored violin plots displaying the probability density function of the data, boxplots, and individual data points. Boxplots depict the sample median as well as first (Q1) and third quartiles (Q3). Whiskers extend from Q1 to the smallest value within Q1 - 1.5* interquartile range (IQR) and from Q3 to the largest values within Q3 + 1.5* IQR. Lower rows depict time-resolved analyses of pain rating time courses in the alpha, gamma, and sham tACS conditions. None of the analyses revealed significant differences between alpha, gamma, and sham stimulation indicating no tACS effects on the perceived pain intensity (N = 29; $PFC_{summary}$: p = .885, $PFC_{time\text{-}resolved}$: no cluster found, $S1_{summary}$: p = .864, $S1_{time\text{-}resolved}$: p = .857; Friedman tests and cluster-based permutation statistics; FDR-corrected p values)"[82]. n.s., not significant; PFC, prefrontal cortex; S1, somatosensory cortex; VAS, visual analogue scale. Figure 4 was adapted by permission from Elsevier: The Journal of Pain, Ref. [82] (Modulating Brain Rhythms of Pain Using Transcranial Alternating Current Stimulation (tACS) - A Sham-Controlled Study in Healthy Human Participants, May et al.), © (2021).

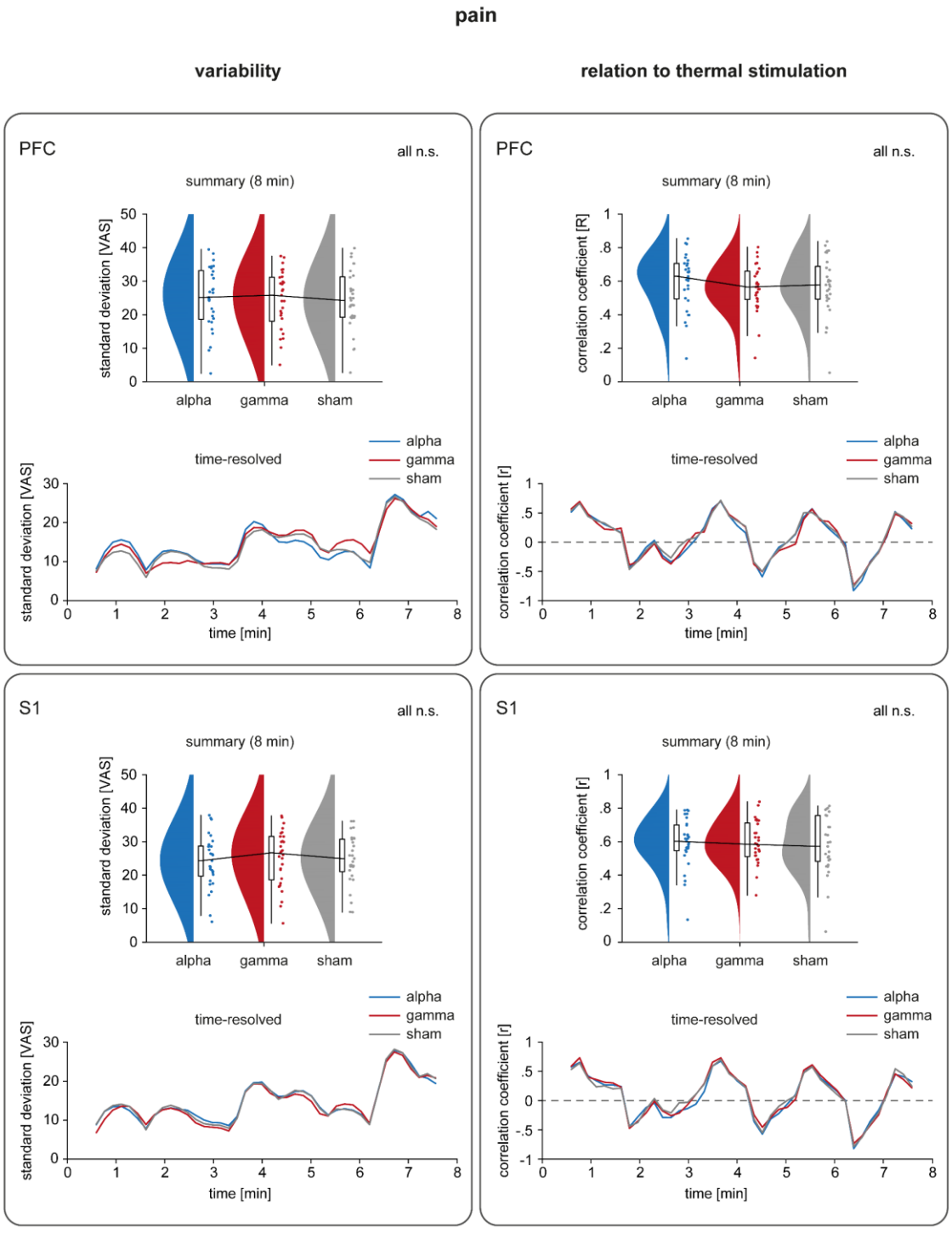

Figure 5. tACS effects on pain variability and relation to thermal stimulation. In line with Figure 4, tACS effects on pain variability and the relation of pain to thermal stimulation are shown separately for PFC and S1 tACS locations. "None of the analyses revealed significant differences between alpha, gamma, and sham stimulation, indicating no tACS effects on the stability of pain ratings or the translation of the noxious stimulus into pain (N = 29; pain variability: $PFC_{summary}$: p = .864, $PFC_{time\text{-}resolved}$: p = .857, $S1_{summary}$: p = .857, $S1_{time\text{-}resolved}$: p = .864; relation of pain to thermal stimulation: $PFC_{summary}$: p = .943, $PFC_{time\text{-}resolved}$: p = .857, $S1_{summary}$: p = .943, $S1_{time\text{-}resolved}$: p = .857; Friedman tests and cluster-based permutation statistics; FDR-corrected p values)"[82]. n.s., not significant; PFC, prefrontal cortex; S1, somatosensory cortex; VAS, visual analogue scale. Figure 5 was adapted by permission from Elsevier: The Journal of Pain, Ref. [82] (Modulating Brain Rhythms of Pain Using Transcranial Alternating Current Stimulation (tACS) - A Sham-Controlled Study in Healthy Human Participants, May et al.), © (2021).

2.2.3 tACS did not yield outlasting effects on brain activity

“We next investigated whether tACS induced neuronal changes outlasting the stimulation (offline effects) as a potential indicator of the neural efficacy of our tACS protocol. To this end, we calculated power spectra of EEG activity during the last minute before and the first minute after stimulation (Fig 6). We further calculated post – pre difference power spectra of the electrode contralateral to the thermal stimulation and compared them between the active tACS conditions (PFC/ S1 alpha/gamma stimulation) and the respective sham conditions. Cluster-based permutation statistics did not show any significant clusters for tACS over PFC or S1 in the targeted frequency bands ($p > .05$ for all clusters, 1- sided). Control analyses including the entire 5 minutes pre- and post-EEG data for power spectra calculation, using both prefrontal electrodes, and log-transforming power spectra before contrasts confirmed this finding ($P > .05$ for all clusters, 1-sided). Likewise, no tACS effects in the non-targeted frequency bands were observed ($p > .05$ for all clusters, 1-sided). Hence, tACS did not evoke effects on brain activity outlasting the stimulation”[82].

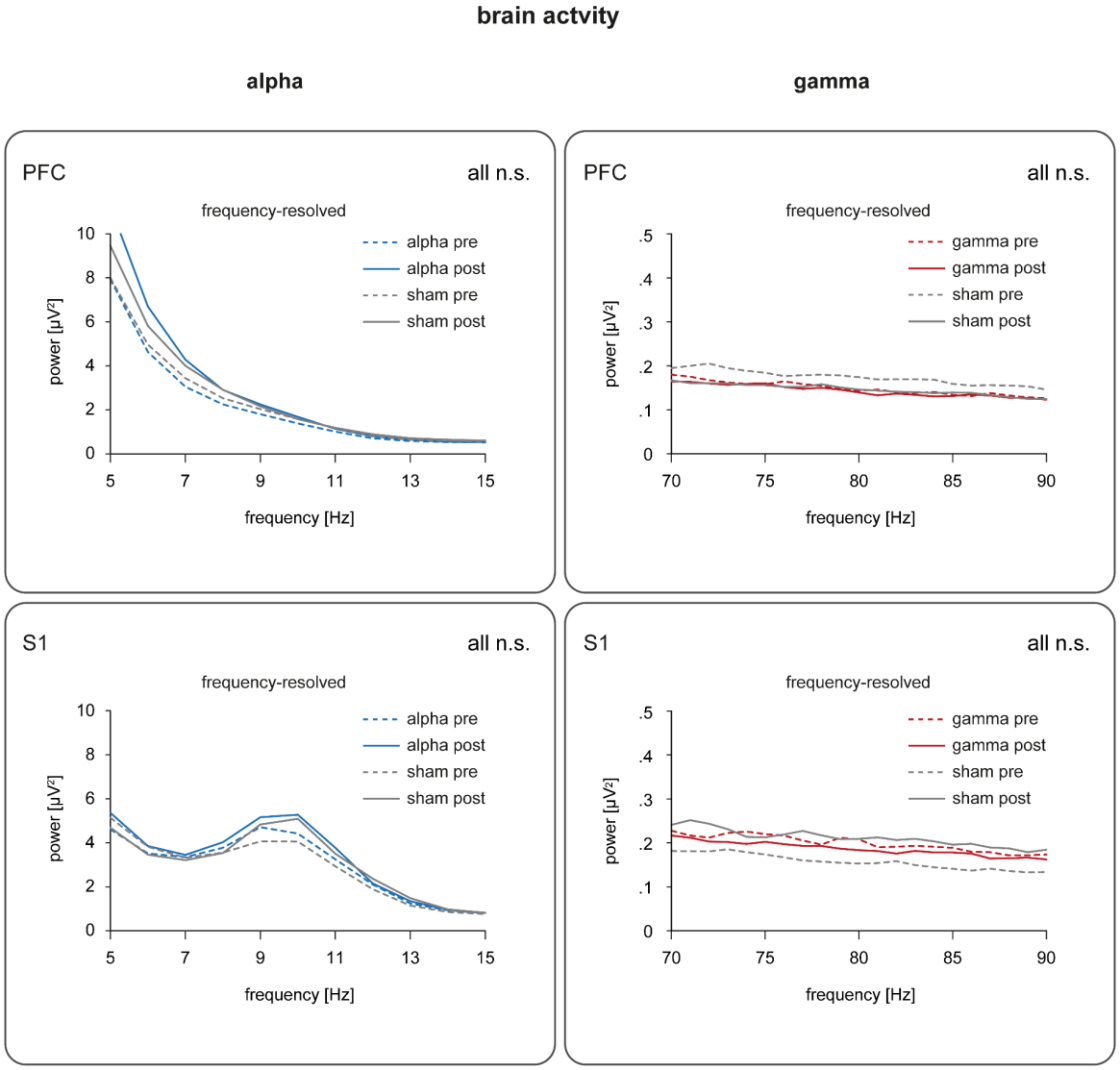

Figure 6. tACS effects on brain activity. "Power spectra of pre- (dashed lined; based on last minute of the recording) and post-stimulation EEGs (solid line; based on first minute of the recording) are shown separately for PFC (upper panel) and S1 (lower panel) locations. Left and right plots display power spectra for alpha and gamma frequency bands, respectively. For statistical analysis, prestimulation power spectra were subtracted from post-stimulation power spectra for each of the 6 conditions and each participant individually (not shown here). Subsequently, the resulting difference power spectra were compared between the active tACS conditions and the respective sham conditions (PFC_{alpha} vs PFC_{sham}, PFC_{gamma} vs PFC_{sham}, $S1_{alpha}$ vs $S1_{sham}$, $S1_{gamma}$ vs $S1_{sham}$). Analyses did not reveal significant power increases in the targeted frequency bands indicating that tACS did not evoke effects on brain activity outlasting the stimulation (N = 29; PFC_{alpha}: no cluster found, PFC_{gamma}: no cluster found, $S1_{alpha}$: p = .240, $S1_{gamma}$: no cluster found; Cluster-based permutation statistics; 1-sided FDR-corrected p values)"[82]. n.s., not significant; PFC, prefrontal cortex; S1, somatosensory cortex. Figure 6 was adapted by permission from Elsevier: The Journal of Pain, Ref. [82] (Modulating Brain Rhythms of Pain Using Transcranial Alternating Current Stimulation (tACS) - A Sham-Controlled Study in Healthy Human Participants, May et al.), © (2021).

3 PROJECT 2: CAN WE MODULATE PAIN USING NEUROFEEDBACK?

3.1 Methods

3.1.1 Registered reports

Registered reports represent a new publishing format aimed at fostering high-quality studies regardless of their outcome[101]. To this end, registered reports extend the standard publishing model through an additional peer-review process taking place prior to data collection. During this stage 1 review process, a detailed study protocol including the preregistration of all hypotheses and analyses as well as a priori sample size calculations is evaluated. Reviewed protocols are either rejected, revised, or receive *in-principal acceptance for publication* (IPA) in which case journals commit to publishing the study irrespective of its outcome. Hence, publication decisions are based primarily on the quality of the research question and the proposed methodology. Once an IPA has been obtained, authors conduct the proposed study and summarize their findings in a final manuscript which is evaluated in a second, stage 2 review process. Importantly, the stage 2 review process only verifies whether researchers followed the pre-specified study protocol; the motivation and methods are not relitigated, nor do the findings themselves influence the final editorial decision. Consequently, registered reports should mitigate publication bias (i.e., the tendency to publish only positive findings), prevent questionable research practices and underpowered studies, and improve study protocols by fostering peer discussion early in the scientific process.

Since their introduction in 2013, more than 300 journals have adopted registered reports, including several multidisciplinary and neuroscientific journals such as eNeuro, NeuroImage, PLOS Biology, and Nature Communications (see Ref. [102] for an updated list of participating journals). Over 500 registered reports have been published in these journals at the time of writing[103] creating a tentative basis for the evaluation of the publication format. In line with their objectives, registered reports seem to enhance sample sizes, data sharing, and reproducibility[104,105] and mitigate publication bias[106,107]. Initial evidence on the rate of null findings in the field of psychology, for instance, suggests that hypotheses are more often disconfirmed in registered reports than in traditional articles with estimated rates of 50-60 % versus 5 %, respectively[106,107]. At the same time, the elaborate preregistration required for registered reports does not seem to undermine novelty and creativity of examined research questions as feared by some sceptics[105]. Given the urgent need for innovative, high-quality studies with reproducible

and replicable results[78,79], the registered reports format therefore seems particularly promising when investigating whether pain can be modulated using neurofeedback.

3.1.2 Participants

The study protocol was approved by the local Ethics Committee of the Medical Faculty of the Technical University of Munich and preregistered at ClinicalTrials.gov (NCT05570695) and osf.io (https://osf.io/qbkj2/). The study will be conducted in accordance with the latest version of the Declaration of Helsinki and recent consensus guidelines for neurofeedback studies[108] (see https://osf.io/qbkj2/ for the corresponding checklist). “Prior to any experimental procedures, all participants will provide written informed consent. The study will entail two sessions and participants will receive a fixed financial compensation of 25 € plus a variable, performance-based compensation of up to 25 € per session. Thus, participants will receive between 50 and 100 € in total”[49].

“We will recruit healthy, right-handed participants aged between 18 and 45 years with a good command of German using convenience sampling. Exclusion criteria will comprise pregnancy, neurological or psychiatric diseases, severe internal diseases including diabetes, skin diseases, current or recurrent pain, regular intake of medication (aside from contraception, thyroidal, and antiallergic medication), previous surgeries at the head or spine, any previous side effects associated with thermal stimulation, contact to a person with a SARS-CoV-2 infection within the last 2 weeks, and current symptoms of a cold or flu. To be included in the analyses, participants will have to be right-handed according to the short version of the Edinburgh handedness inventory (cut-off score: laterality quotient > 60)[109], attend both sessions, and comply with instructions throughout the experiment. In addition, pain ratings will be screened for floor and ceiling effects during the first session. Participants with average pain ratings below 5 or above 95 prior to neurofeedback ($pain_{pre}$ assessment, see below) will be excluded from the analyses and their participation will be terminated prematurely. […]”[49]

“The sample size will be determined by a sequential Bayesian sampling plan that defines a maximal sample size[110]. Specifically, we will continuously analyze incoming data using Bayesian statistics until compelling evidence for an effect or its absence (BF_{01} or $BF_{10} \geq 10$) is found for specified primary analyses (see Table 1) or until a maximum of 95 participants has been included in our primary analyses[110,111]. The maximal sample size $N_{max} = 95$ was defined due to resource limitations and determined using a Bayes factor design analysis (BFDA) for Bayesian paired samples t-tests[110]. […] To account for potential non-responders[12,112], the BFDA was conducted with a weighted effect size estimate of Cohen’s d = 0.41 based on the following assumptions:

70 % responders with an effect size estimate of Cohen's d = 0.5 and 30 % non-responders with an effect size estimate of Cohen's d = 0.2"[49]. Further details can be found in the Supplementary Materials of Ref. [49] in the section *Bayes factor design analysis* and Fig S1.

3.1.3 Paradigm

"Methodology and terminology of this study are based on recent recommendations for EEG and neurofeedback studies[83,108,113]. Our EEG-based neurofeedback study will employ a within-subjects, bi-directional control design with two verum and two sham conditions which will be completed during two sessions (Fig 7). The design was adapted from recent neurofeedback studies which successfully modulated the asymmetry of alpha oscillations[114,115]. In a first verum neurofeedback condition, participants will be instructed to focus attention on their right hand and the up-regulation of alpha oscillations in the right hemisphere relative to alpha oscillations in the left hemisphere will be incentivized through neurofeedback (*attention right training*, ART_{NF}). In a second verum neurofeedback condition, participants will be instructed to focus attention on their left hand and the down-regulation of right relative to left alpha oscillations will be incentivized (*attention left training*, ALT_{NF}). To control for non-specific effects of the neurofeedback procedure such as expectation effects evoked by the explicit instruction, the two verum conditions will be accompanied by two matched sham neurofeedback conditions administered in a separate session. During the sham neurofeedback conditions, participants will obtain the same instructions, i.e., focus attention on your right hand (*sham attention right training*, ART_{sham}) or focus attention on your left hand (*sham attention left training*, ALT_{sham}). However, the feedback signal will not mirror their brain activity. Instead, the feedback signal and the corresponding reward of the last matching verum condition completed by a previous participant, i.e., ART_{NF} for ART_{sham} and ALT_{NF} for ALT_{sham}, will be replayed (yoked feedback). Thus, on a group level, the feedback signal and compensation will be identical between verum and sham conditions supporting blinding of participants and experimenters. To implement sham conditions for the first participant(s), data from a "participant zero" will be collected, who will only complete the verum trainings and will be excluded from the analyses. To avoid order effects, sessions will be separated by 7 days at least and the order of conditions between (verum vs sham) and within sessions (ART vs ALT) will be pseudo-randomized. "[49]

“Both sessions will comprise a fixed sequence of events aimed at capturing changes of alpha asymmetry, pain perception, and pain processing that occur during the neurofeedback training (Fig 7C). In addition, baseline measures of pain sensitivity and brain activity will be obtained to evaluate predictors of regulatory success during exploratory analyses”[49]. Details regarding these measurements and analyses will not be reported in this thesis but can be found in Ref. [49] and the corresponding Supplementary Materials. “Each experimental condition will entail 40 trials with 15 s of neurofeedback training, which will be followed by a brief noxious laser stimulus applied to the dorsum of the left hand. Three seconds after the noxious stimulus, participants will verbally rate the perceived pain intensity on a numerical rating scale (NRS) ranging from 0 (“no pain”) to 100 (“worst tolerable pain”). To enhance motivation, participants will receive feedback regarding their performance-based financial compensation at the end of each trial. Thus, the total neurofeedback training time per condition will be 10 min. Alpha asymmetry during these training runs will serve as read-out of regulation success. Pain ratings will serve as read-out for pain perception. Brain responses to noxious stimuli will serve as read-out for pain processing”[49].

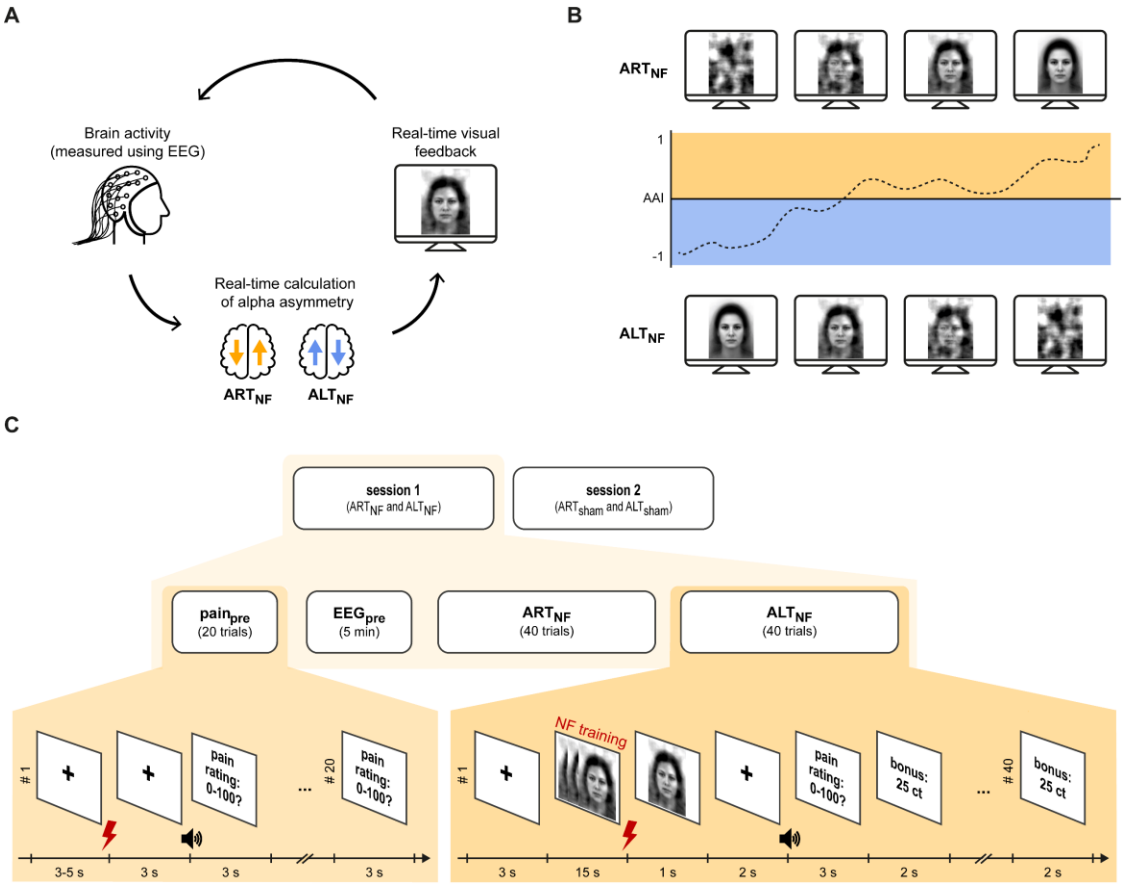

Figure 7. Neurofeedback paradigm. (A) Attention-based neurofeedback training. "During neurofeedback, the somatosensory alpha asymmetry index (AAI) will be calculated in real-time based on 1000 ms EEG data segments and will be fed back to participants using the visibility of neutral face images41. This feedback cycle will be updated every 100 ms. To control for non-specific effects, the experiment will entail two verum neurofeedback conditions during which alpha asymmetry will be regulated in opposite directions as well as two sham neurofeedback conditions with identical instructions but yoked feedback. During the attention right training (ART_{NF}), participants will be instructed to focus attention on their right hand and the up-regulation of right relative to left alpha oscillations will be incentivized. During the attention left training (ALTNF), participants will be instructed to focus attention on their left hand and the down-regulation of right relative to left alpha oscillations will be incentivized"[49]. (B) Real-time visual feedback. "AAI values and corresponding feedback signals for the verum conditions are displayed. For ART, AAI values above -0.6 will lead to an increase of image visibility until full visibility is reached at an AAI of 0.6. For ALT the relationship between AAI and image visibility is reversed. In addition, a small, central fixation cross will be superimposed on the images to support image fixation"[49]. (C) Paradigm. "Verum and sham neurofeedback conditions will be completed in a pseudo-randomized order during two separate sessions. Each session will entail verum or sham conditions (ALT_{NF} and ART_{NF} or ALT_{sham} and ART_{sham}, 40 trials each) as well as baseline assessments of pain sensitivity (painpre, 20 trials) and brain activity (EEG_{pre}, 5 min resting state with eyes open). The sequence of events of neurofeedback trials is shown on the right. After a fixation period of 3 s, the regulation period of 15 s will begin. Immediately afterwards, a noxious stimulus will be applied. To avoid that pain-related brain responses are confounded by visual offset responses, the last feedback signal of the regulation period will remain on the screen for another second before turning into a fixation cross. Two seconds later, an auditory and visual cue will prompt participants to rate the perceived pain intensity. Finally, the financial reward earned on a given trial will be displayed"[49]. AAI, alpha asymmetry index; ALT, attention left training; ART, attention right training; EEG, electroencephalography; NF, neurofeedback. Figure 7 was reprinted by permission from Ref. [49] (How Do Alpha Oscillations Shape the Perception of Pain? – An EEG-based Neurofeedback Study, Hohn et al.), (CC BY-NC-ND 4.0).

3.1.4 Hypotheses

“To test whether a mechanistic relationship between attention, alpha oscillations, and pain exists, we will compare the findings to four predicted result patterns capturing different combinations of attention, neurofeedback, and time effects (Fig 8) on alpha oscillations (hypothesis 1) and on pain perception and underlying brain responses (hypothesis 2). In addition, we will perform a multi-level mediation analysis based on single trial data to test whether changes in attention, alpha oscillations, and pain perception can be integrated into a single, mechanistic model (hypothesis 3). All analyses will rely on Bayesian hypothesis testing allowing us to quantify evidence in favor as well as against our hypotheses[111].

Hypothesis 1: Alpha oscillations are up- and down-regulated. Based on the literature and pilot data (Fig 9), we expect that lateralized attention leads to an up-regulation of alpha asymmetry in ART conditions and a downregulation in ALT conditions. Moreover, we expect that these attention effects are enhanced through verum compared to sham neurofeedback and increase over time (H1.1-3, Fig 1, pattern 1).

Hypothesis 2: The perception of pain and underlying brain responses are up- and downregulated. Assuming that attention-related alpha oscillations are causally involved in the emergence of pain, we also expect pain ratings to be up- and downregulated. During ART, the brain should be optimally tuned for the processing of stimuli from the right body side, thus leading to lower pain ratings in response to the applied left-sided pain stimuli. During ALT, the brain should be optimally tuned for the processing of stimuli from the left side of the body which should result in higher pain ratings. Hence, pain ratings should be lower during ART compared to ALT conditions. As for alpha asymmetry, we expect this attention effect to be enhanced through verum compared to sham neurofeedback (H2.1-2, Fig 1, pattern 1 or 3). Time effects will not be analyzed due to confounding effects of habituation or sensitization. To examine whether attention affects neural pain processing in a similar fashion, corresponding hypotheses will be tested for brain responses to noxious stimuli[116] (H2.3-4, Fig 1, pattern 1 or 3).

Hypothesis 3: Alpha oscillations partially mediate attention effects on pain perception. To test this hypothesis, we will employ multi-level mediation analysis. Mediation analysis not only assesses the effects of an independent variable (X) on a dependent variable (Y), but quantifies to which extent a third variable termed mediator (M) contributes to these effects[117]. Using this model, we expect that alpha oscillations (M) partially mediate the relationship between ART/ALT neurofeedback conditions (X) and pain ratings (Y), and that this mediation effect is more pronounced for the verum than for the sham neurofeedback (moderator) (H3)"[49].

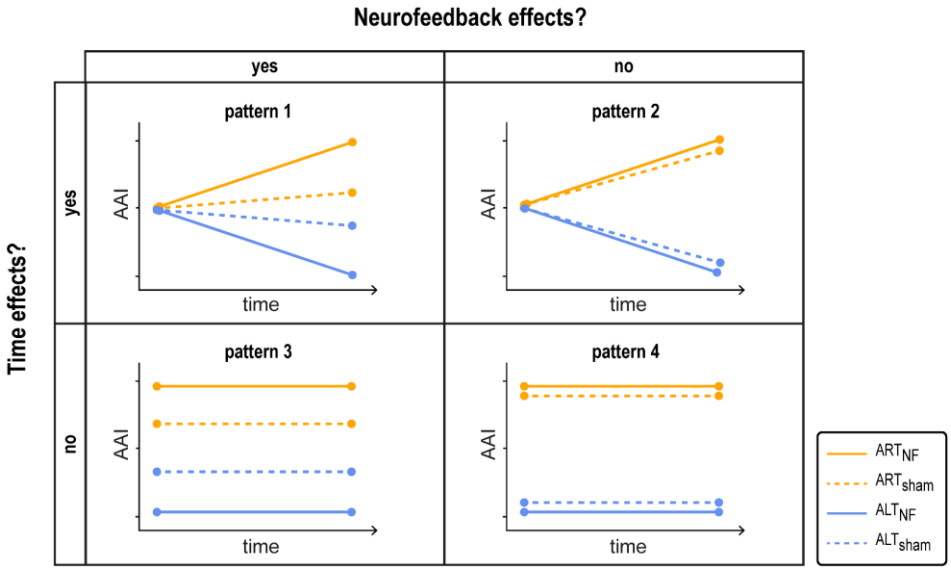

Figure 8. Predicted result patterns characterizing attention effects on alpha asymmetry. "Four different result patterns characterizing attention effects on alpha asymmetry indices (AAI, quantified as the normalized difference of alpha oscillations over right minus left somatosensory brain areas) will be investigated. Attention effects on AAIs manifest as difference between ART and ALT (orange and blue lines) and can be complemented by neurofeedback and time effects. Neurofeedback effects quantify differences between verum and sham neurofeedback conditions (solid and dashed lines). Time effects quantify differences between the first and second half of the data. For pain ratings and underlying brain responses, a reversal of attention effects, i.e., a down-regulation of pain during ART and an up-regulation during ALT, is expected due to the inverse relationship between alpha oscillations and pain. Note that the figure entails a schematic representation of the change of AAI values across time/trials and does not reflect expected absolute values"[49]. AAI, alpha asymmetry index; $ALT_{NF/sham}$, verum/sham attention left training; $ART_{NF/sham}$, verum/sham attention right training. Figure 8 was reprinted by permission from Ref. [49] (How Do Alpha Oscillations Shape the Perception of Pain? – An EEG-based Neurofeedback Study, Hohn et al.), (CC BY-NC-ND 4.0).

3.1.5 Noxious stimulation

“Noxious stimuli will be applied using cutaneous laser stimulation, which enables the well-controlled stimulation of nociceptive pathways without concomitant stimulation of tactile pathways[118]. All stimuli will be applied to the dorsum of the left hand using a neodymium:yttrium-aluminum-perovskite (Nd:YAP) laser (Stimul 1340, DEKA M.E.L.A. srl, Calenzano, Italy) and the following settings. Stimulus duration will be set to 4 ms and stimulus diameter to 7 mm. Laser intensity will be set to 3.5 J, which induces stable brain responses while being well tolerated[50]. To avoid tissue damage and minimize habituation/sensitization effects, stimulation sites will be changed slightly after each stimulus. For safety reasons, both study personal and participants will wear safety goggles throughout the experiment”[49].

3.1.6 EEG recording

“Brain activity will be recorded using BrainAmp MR plus amplifiers and 64 actiCAP snap sensors placed according to the 64-channel extended international 10-20 system (Brain Products GmbH, Gilching, Germany). All sensors will be referenced to FCz and grounded at FPz. In addition, electrooculographic activity will be recorded with a bipolar BrainAmp ExG MR amplifier (Brain Products GmbH, Gilching, Germany) and Ag/AgCl sensors placed below the outer canthus of each eye. An Ag/AgCl sensor attached at the nasion will serve as ground. All recordings will be performed at a sampling rate of 500 Hz (0.2 µV resolution) and band-pass filtered between 0.016 and 250 Hz. Impedances will be measured directly before the $pain_{pre}$ run and sensors will be prepared until impedances below 20 kΩ are achieved for all active and passive sensors. Pain ratings will be manually added to the EEG data as markers during the experiment”[49].

3.1.7 Neurofeedback setup

“Motivated by recent studies[114,115,119], we have designed a short-term neurofeedback protocol with real-time data analysis and feedback visualization using MATLAB (version: R2020b, Mathworks, Natick, MA) and the MATLAB-based toolboxes FieldTrip (version: 20210212, [93]) and Psychtoolbox-3 (version: 3.0.17 beta, https://psychtoolbox.org). To this end, EEG data is streamed from an acquisition computer (operating system: Windows 10) to a second computer (operating system: Ubuntu 20.04.2 LTS) responsible for data processing and stimulus presentation in real-time and is stored in a buffer. Every 100 ms, 1000 ms segments are accessed from this buffer and analyzed as follows. Data are demeaned, and power estimates from 8 to <13 Hz are obtained with a 1 Hz resolution using a Hanning-tapered Fast Fourier Transformation. Subsequently, alpha power over right and left somatosensory regions is calculated by

averaging across 8-12 Hz and the channels C4, CP4, CP6 and C3, CP3, CP5, which overlie the somatosensory cortex and yield a higher signal-to-noise ratio than single channels[76]. Resulting power estimates are then used to calculate the alpha asymmetry index (AAI), defined as:

$$\mathrm{AAI}(t) = \frac{\alpha_{rS1}(t) - \alpha_{lS1}(t)}{\alpha_{rS1}(t) + \alpha_{lS1}(t)}$$

where $\alpha_{\mathrm{rS1}}(t)$ and $\alpha_{\mathrm{lS1}}(t)$ represent alpha power in time window t in right and left somatosensory regions, respectively. AAI values range from -1 to 1 when power is purely left- or right-lateralized, respectively, and will be communicated to participants by altering the visibility of neutral face images (see paragraph below and section *Image Visibility* in the Supplementary Materials for details)"[49].

"Participants will be instructed to use spatial attention towards the left or right hand to enhance the visibility of face images as much and as long as possible until the pain stimulus is applied[115]"[49]. Specifically, one of 8 neutral face images from the Averaged Karolinska Directed Emotional Faces (AKDEF) data set[120] (image IDs: F/MNEFL, F/MNEFR, F/MNEFHL, F/MNEHR) will be chosen randomly during each trial. "Depending on the training condition, image visibility will be altered in opposite directions. During ART_{NF}, AAI values above -0.6 will lead to an increase of image visibility until full visibility is reached at an AAI of 0.6. During ALT_{NF}, AAI values below 0.6 will lead to an increase of image visibility until full visibility is reached at an AAI of -0.6 (see section *Image Visibility* in the Supplementary Materials and Fig S2 for details). During sham conditions, the feedback signal of the last verum condition completed by another participant will be replayed (yoked feedback) irrespective of the current alpha asymmetry"[49]. To enhance motivation, participants will receive feedback regarding their performance-based financial compensation at the end of each trial. "This compensation will reflect the average AAI on a given trial and will amount to up to 0.25 € per trial (ART: linear increase from 0-0.25 € for positive AAI values with a bonus of 0.25 € if the AAI is larger than 0.6; ALT: linear increase from 0-0.25 € for negative AAI values with a bonus of 0.25 € if the AAI is smaller than -0.6)"[49].

3.1.8 Pilot data

To test our neurofeedback setup, we conducted a pilot study with n = 5 participants (Fig 9). The applied neurofeedback training protocol was identical to the one described above but entailed longer trial durations (36 trials with 15, 20, or 25 s duration) and no laser stimuli. Visual inspection of the data indicated no systematic differences between trial durations and attention effects on the group level, thus supporting the suitability of the proposed neurofeedback setup.

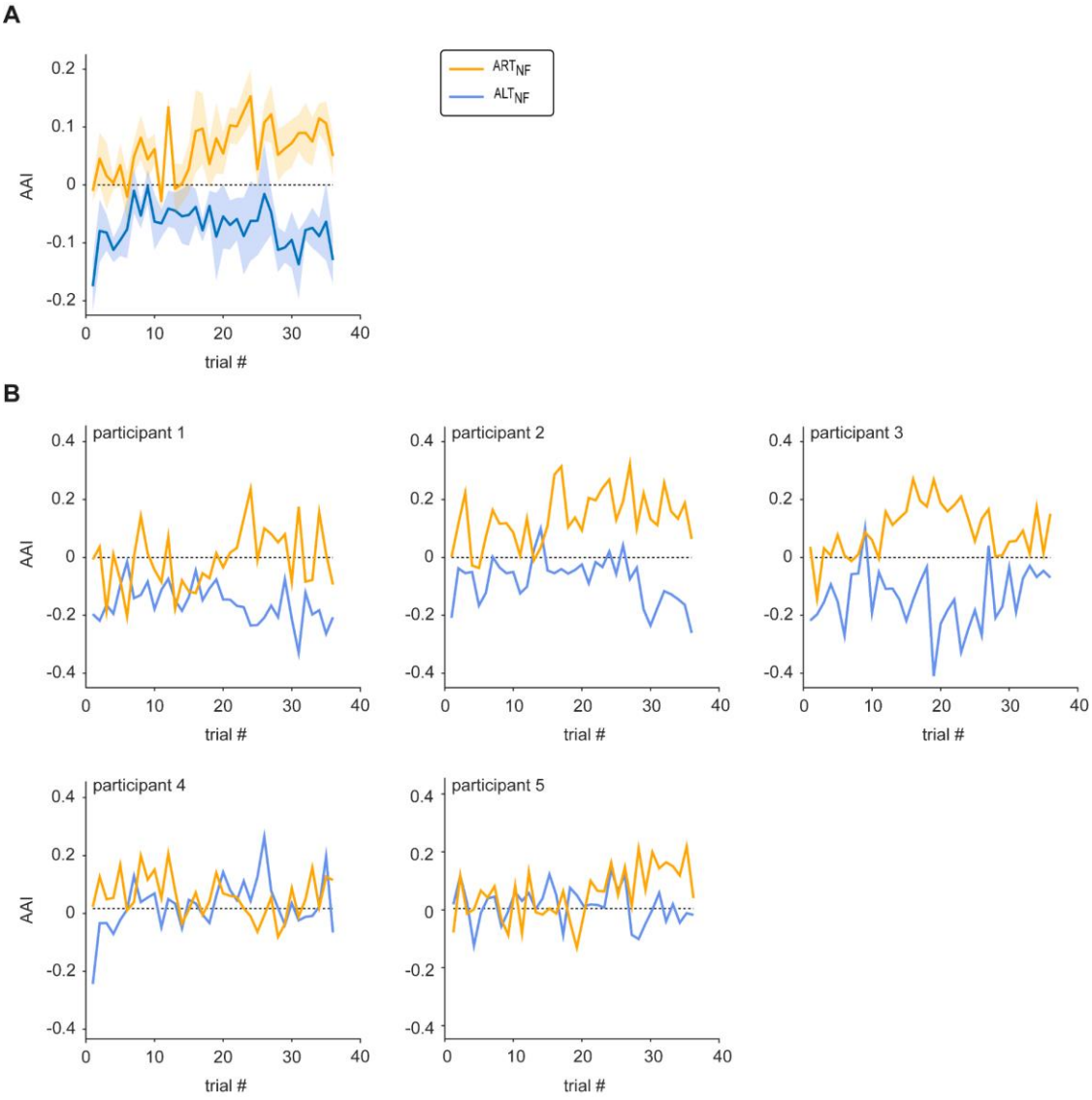

Figure 9. AAI modulation through neurofeedback training in pilot study (n = 5). "Results of a pilot study with n = 5 participants completing 36 trials of ART_{NF} and ALT_{NF} training. Plots show (A) mean group effects with shadings indicating the SEM as well as (B) individual data"[49]. AAI, alpha asymmetry index; ALT_{NF}, verum attention left training; ART_{NF}, verum attention right training; SEM, standard error of the mean. Figure 9 was reprinted by permission from Ref. [49] (How Do Alpha Oscillations Shape the Perception of Pain? – An EEG-based Neurofeedback Study, Hohn et al.), (CC BY-NC-ND 4.0).

3.1.9 Blinding

“Double-blinding will be enabled through the usage of sham conditions and participant-specific numeric codes encoding the order of conditions. During each session, these codes will automatically (1) determine the predefined training conditions and (2) generate file names. Thus, the *experimenter* will be blinded during data acquisition as well as during subsequent preprocessing and analysis steps. To ensure blinding of the *participants*, we will additionally provide a cover story. Specifically, we will inform participants that the experiment will investigate how two different neurofeedback trainings affect pain perception and whether training effects fluctuate over time. We will provide no information regarding the underlying feedback feature or our study hypotheses until the debriefing at the end of the last session. Since both verum and sham conditions comprise an identical sequence of events and resemble each other with respect to the feedback signal and reward, expectation effects which match our experimental hypotheses are highly unlikely. To evaluate blinding, participants will be asked to rate how well they could regulate the visibility of faces every 20 trials, i.e., twice per condition, on a visual analogue scale (VAS) ranging from 0 (“not at all”) to 100 (“very well”). In addition, participants will be asked to indicate whether they completed verum or sham neurofeedback during session 2 after debriefing”[49].

3.1.10 Preprocessing

“Preprocessing of EEG data will be conducted using the BrainVision Analyzer software (version: v2.1.1.327; Brain Products, Munich, Germany), MATLAB (version: R2020b, Mathworks, Natick, MA) and the MATLAB-based toolbox FieldTrip (version: 20210212, [93]). For each session, the preprocessing pipeline will be applied separately to $pain_{pre}$, EEG_{pre} and concatenated neurofeedback data sets (ART_{NF} and ALT_{NF} or ART_{sham} and ALT_{sham}).Thus, 6 data sets will be preprocessed for every participant. First, the electrode layout file will be added to the data set, bad channels will be rejected based on visual inspection, and the data will be filtered (fourth-order Butterworth 1 Hz high-pass filter, 49 to 51 Hz band-stop filter to dampen line noise). Subsequently, an independent component analysis (ICA)[121] based on the extended infomax algorithm will be performed on the filtered EEG data. To exclude speech artefacts from the ICA, data segments entailing speech will be omitted prior to the ICA. Specifically, a time interval ranging from -1000 to 5000 ms with respect to the auditory cue will be omitted from neurofeedback data sets. For $pain_{pre}$ data sets, a time interval ranging from -1000 to 3000 ms with respect to the auditory cue will be omitted. In addition, bad or missing channels will be excluded. The resulting components will be examined and those representing eye movements and muscle artifacts will be identified based on their topographies and time

courses. Subsequently, independent components representing artifacts will be subtracted from the raw, unfiltered EEG data. Next, missing channels will be interpolated using a weighted average of all neighbors. In addition, time intervals of 400 ms around signals exceeding an amplitude threshold of ±100 μV or displaying a gradient steeper than 30 μV/s will be automatically marked for rejection. This will be complemented by a final visual inspection during which remaining artefacts will be marked. Finally, EEG data will be re-referenced to the average reference and the online reference FCz will be added to the channel array. Resulting data will be exported to Matlab and further analyzed using FieldTrip along with custom written code. In Fieldtrip, preprocessed data will be segmented and transformed to BIDS format[83] before further analyses will be performed. Neurofeedback data sets will be segmented into 19 s epochs ranging from -17 to 2 s with respect to the laser stimulus"[49].

3.1.11 Analysis of hypothesis 1: Alpha oscillations are up- and down-regulated

"To examine changes in alpha oscillations, AAIs will be extracted from the preprocessed neurofeedback data sets following the same procedure as during neurofeedback. For each trial, data from the last 12 s of the training period will be extracted to limit the impact of the smoothing function used for AAI visualization (see section *Image Visibility* in the Supplementary Materials for details). Then, data will be segmented into 1000 ms epochs with 900 ms overlap and epochs with artefacts will be rejected (see section *Preprocessing* for details on applied artifact criteria). Remaining epochs will be demeaned and power estimates from 8 to <13 Hz will be obtained with a 1 Hz resolution using a Hanning-tapered Fast Fourier Transformation. For every epoch, alpha power will be quantified for right and left somatosensory regions by averaging across frequencies (8-12 Hz) and channels (C4, CP4, CP6 and C3, CP3, CP5, respectively) and alpha asymmetry indices will be calculated. Finally, single trial alpha asymmetry indices will be obtained by averaging asymmetry indices across all epochs per trial.

Subsequently, asymmetry indices will be analyzed using an adaptive analysis pipeline which will allow us to differentiate between 4 response patterns describing attention, neurofeedback, and time effects (see Table 1 and Fig S3 for a summary and visualization of the pipeline). As a basis for these analyses, single trial asymmetry indices of the first and second half of each condition (≙ 20 trials) will be averaged separately for every participant resulting in 8 averages per participant (2 time periods x 4 conditions). To examine changes in alpha asymmetry over time (*time effect*; H1.1), single-subject first and second half averages will be compared separately for ART_{NF} and ALT_{NF} conditions. If at least one comparison yields a time effect (increase for ART_{NF} and a

decrease for ALT_{NF}, respectively), second half averages will be used as basis for all subsequent analyses. If no time effect is found, first and second half averages will be averaged, and the resulting values will be used for subsequent analyses. To examine whether verum neurofeedback leads to larger increases/decreases in alpha asymmetry than sham neurofeedback (*neurofeedback effect*; H1.2), single-subject asymmetry averages from the verum conditions will be compared to the corresponding sham conditions. If at least one comparison yields a neurofeedback effect, verum-sham differences scores will be calculated for every participant, and the resulting ART and ALT difference scores will be compared to evaluate whether ART and ALT conditions yield opposite patterns of brain activity (*attention effect*; H1.3). Otherwise, data from corresponding verum and sham conditions will be averaged, and the resulting single-subject ART and ALT average scores will be compared"[49].

3.1.12 Analysis of hypothesis 2: The perception of pain and underlying brain responses are up-and downregulated

"To examine changes in pain perception, pain ratings will be extracted from the preprocessed neurofeedback data sets and will be analyzed by repeating the analysis steps used for alpha asymmetry indices (including decisions on data selection, see Table 1 and Fig S4 for a summary and visualization). Since alpha oscillations and pain ratings are inversely related, the direction of pairwise comparisons will be reversed, however. Subsequently, *neurofeedback effects* (H2.1), and *attention effects* (H2.2) on pain ratings will be evaluated. Time effects will not be analyzed due to confounding effects of habituation or sensitization.

Correspondingly, *neurofeedback* (H2.3) and *attention effects* (H2.4) will be evaluated for brain responses which are typically observed after a noxious stimulus (see Table 1 and Fig S4 for a summary and visualization). These include a characteristic sequence of evoked potentials referred to as N1, N2, and P2 responses[122,123]. In addition, noxious stimuli suppress neuronal oscillations in the alpha (8 to < 13 Hz) and beta (13 to 30 Hz) frequency bands and induce oscillations in the gamma (30 to100 Hz) frequency band[2,124]. Single-trial evoked and oscillatory brain responses to noxious stimuli will be quantified using established procedures[50,125] which have been validated on an unpublished dataset (Fig S5). To examine *evoked brain responses*, preprocessed data from the neurofeedback runs will be bandpass filtered between 1 and 30 Hz (fourth-order Butterworth). Then, a baseline correction will be applied using the fixation period preceding the neurofeedback training. Specifically, amplitudes from 2000 to 2500 ms with respect to the beginning of the fixation period will be subtracted from the post stimulus data. Subsequently, evoked responses will be quantified in a two-step

procedure. First, individual peak latencies of evoked responses will be determined based on averages across all trials of all neurofeedback conditions. To this end, local minima/maxima of the averaged waveform will be determined at predefined channels (N1: C4, N2: Cz; P2: Cz)[50,125] and in pre-defined time-windows (N1: 120-200 ms; N2: 180-300 ms; P2: 250-500 ms)[50]. Second, single-trial amplitudes will be obtained by averaging across a 30 ms window[125] centered at the previously defined peak latency. To quantify the N1 response, data will additionally be re-referenced to Fz[126] before calculating the average waveform across all trials. To examine *oscillatory brain responses*, preprocessed data from the neurofeedback runs will be filtered (fourth-order Butterworth 1 Hz high-pass filter, 41 to 51 Hz band-stop filter to dampen line noise). Next, single trial time-frequency estimates will be obtained using a Hanning-tapered Fast Fourier Transformation and a sliding window approach. To obtain alpha and beta responses, a sliding window with a length of 500 ms and a step size of 20 ms will be used. To obtain gamma responses, the window length will be shortened to 250 ms, while the step size will remain 20 ms. Finally, responses at alpha and beta frequencies will be assessed by calculating the mean power across 8-12 Hz and 14-30 Hz, respectively, across a time window of 500-900 ms[50] and across the channels Cz, CPz, C2, C4, CP2, CP4[50]. Responses at gamma frequencies will be assessed by calculating the mean power across 70-90 Hz[125], a time window of 150-350 ms[125] at Cz[50]. Brain activity in the theta frequency band will not be analyzed as it mainly represents time-locked activity, which is captured by the laser evoked potential analyses[2,127"49].

3.1.13 Analysis of hypothesis 3: Changes in alpha oscillations mediate attention effects on pain perception

"To link changes of alpha oscillations to changes in pain perception on a single-trial level, we will employ a moderated multi-level mediation analysis based on data from all neurofeedback conditions. Mediation models examine whether the covariance between two variables (X and Y) can be explained by an intermediate variable termed mediator (M). Additional moderators make it possible to assess whether the strength of mediation effects varies across different conditions or groups[128]. Applied to the current study, such analyses allow us to assess whether the relationship between ART and ALT neurofeedback conditions (X) and single-trial pain ratings (Y) can be explained by the AAI (M) on a given trial and whether this effect is more pronounced for verum than for sham neurofeedback (moderator) (H3). To this end, the bi-variate relationships between X, M, and Y variables are quantified and the mediation effect is determined based on the resulting path coefficients. Finally, path coefficients and the mediation effect can be compared between different levels of the moderator. Together, this procedure yields

two advantages. First, mediation analyses quantify the relationship between single-trial brain activity (AAIs) and behavior (pain ratings) which is increasingly analyzed by neurofeedback studies[108]. Second, mediation analyses go beyond bi-variate brain-behavior analyses by integrating all variables of interest (i.e., attention, AAIs, and pain ratings) into a single model and thereby fosters mechanistic insights"[49] (see Table 1).

3.1.14 Data exclusion criteria and control analyses

"To ensure data quality, the following exclusion criteria will be applied for primary analyses. First, single trials will be omitted from all analyses if (a) more than 50 % of data segments from the neurofeedback training period are rejected due to artefacts or (b) no/an invalid pain rating (outside to the range of 0-100) occurred. Second, participants (c) with less than 10 remaining trials for a neurofeedback condition or (d) focusing on the wrong hand will be omitted from all primary analyses. In addition, trials will be removed from the analysis of evoked and oscillatory responses, if artefacts occur during time periods used for baseline correction or the 1500 ms time interval following the laser stimulus"[49].

"Control analyses will assess the impact of artefacts and unblinding on the results of primary analyses[108] by examining whether artefacts and blinding vary systematically between ART and ALT conditions. To this end, we will calculate single-subject artefact (AI) and blinding indices (BI) for ART and ALT conditions and compare the resulting values between conditions. The A/BIs will be defined as follows:

$$\mathrm{AI}_{\mathrm{AR/LT}} = \frac{\mathrm{trials}_{\mathrm{AR/LT\_NF}} - \mathrm{trials}_{\mathrm{AR/LT\_sham}}}{\mathrm{trials}_{\mathrm{AR/LT\_NF}} + \mathrm{trials}_{\mathrm{AR/LT\_sham}}}$$

and

$$\mathrm{BI}_{\mathrm{AR/LT}} = \frac{\mathrm{blinding}_{\mathrm{AR/LT\_NF}} - \mathrm{blinding}_{\mathrm{AR/AL\_sham}}}{\mathrm{blinding}_{\mathrm{AR/AL\_NF}} + \mathrm{blinding}_{\mathrm{AR/AL\_sham}}}$$

with $\mathrm{trials}_{\mathrm{AR/LT\_NF/sham}}$ representing the number of trials rejected in the respective condition and $\mathrm{blinding}_{\mathrm{AR/LT\_NF/sham}}$ representing the average blinding score in the respective condition. Indices that are not defined (denominator equals 0) will be set to 0"[49].

3.1.15 Statistics

“All statistical analyses will be conducted in the R programming environment (version: 4.1.1, [129]). Pair-wise comparisons will be conducted using one-sided, Bayesian parametric paired samples t-tests or non-parametric signed rank tests depending on the data distribution. For parametric t-tests, the “BayesFactor” package will be used (version: 0.9.12-4.2., [130], parameters: Cauchy prior distribution with a scale parameter r = √(2)/2 and truncation at zero). For non-parametric rank tests, the functions “signRankGibbsSampler” and “computeBayesFactorOneZero” will be used ([131], parameters: Cauchy prior distribution with a scale parameter r = √(2)/2 and truncation at zero 1000 iterations). Resulting Bayes factors (BF_{10}) will be reported along with corresponding effect size estimates quantifying the median of the posterior Cohen’s δ distribution and its 95 % credibility interval. Thereby, tests quantify evidence for both the presence and absence of effects[111].

Multi-level mediation analysis will be conducted using the “bmlm” package (version: 1.3.4., [132,133]). Using Bayesian multilevel modeling and Markov chain Monte Carlo procedures, five path coefficients and corresponding confidence intervals will be calculated for each participant (first-level coefficients) as well as on a group level (second-level coefficients). These coefficients represent the relationship between X and M (path a), the relationship between X and Y (path c), the relationship between M and Y controlled for X (path b), the relationship between X and Y controlled for M (path c’), and the mediation effect (path ab)[134]. To quantify moderation effects, two multi-level mediation analyses will be conducted separately for verum and sham neurofeedback conditions and the resulting first-level coefficients will be compared between models separately for each path. This will be done using the parametric or non-parametric tests described above. Preceding the mediation analyses, X will be recoded (ART, -1 and ALT, 1) and M (single-trial AAIs) will be centered within-person[132”49].

3.1.16 Design table

Creating a seamless link between study hypotheses, sampling and analysis plans, and the contingent interpretation given different outcomes represents one of the key features and essential building blocks of registered reports. To elucidate this “preparatory chain of inference”[103] as precisely as possible, most registered reports include design tables which explicate every link in the chain.

Table 1. Design Table

Hypotheses	Sampling Plan	Analysis Plan	Interpretation
Q1: Are alpha oscillations up- and downregulated?			
H1: Alpha oscillations are up- and downregulated. **H1.1: Time up/downregulates alpha oscillations.** H1.1.a: $AAI_{ART\_verum\_2ndhalf} > AAI_{ART\_verum\_1sthalf}$ H1.1.b: $AAI_{ALT\_verum\_2ndhalf} < AAI_{ALT\_verum\_1sthalf}$	SBF+maxN design with the following parameters: Starting at $N_{min} = 20$, the sample size will be increased with $n_{step\_size} = 5$ until: - $BF_{10} \geq 10$ / $\leq 1/10$ for H1.3.a or - $N_{max} = 95$ is reached. Note: The precise comparison for the evaluation of H1.3.a depends on the previous evaluation of H1.1 (time effects) and H1.2 (neurofeedback effects).	One-sided Bayesian paired samples t-tests (Cauchy prior distribution with scale parameter r = $\sqrt{(2)}/2$ and truncation at zero) or one-sided Bayesian Wilcoxon signed rank tests (Cauchy prior distribution with scale parameter r = $\sqrt{(2)}/2$ and truncation at zero, 1000 iterations) if normality assumptions are violated according to Q-Q-plot.	**H1: Alpha oscillations are up- and downregulated.** If time AND neurofeedback AND attention effect: evidence for up- and downregulation according to pattern 1 If time AND attention effect: evidence for up- and downregulation according to pattern 2 If neurofeedback AND attention effect: evidence for up- and downregulation according to pattern 3 If attention effect: evidence for up- and downregulation according to pattern 4 If evidence for absence of attention effect: evidence for absence of up- and downregulation Otherwise: inconclusive evidence **H1.1: Time up/downregulates alpha oscillations.** If $BF_{10} \geq 3$ for H1.1.a OR H1.1.b: evidence for time effect If $BF_{10} \leq 1/3$ for H1.1.a AND H1.1.b: evidence for absence of time effect Otherwise: inconclusive evidence

Table continued.

Hypotheses	Sampling Plan	Analysis Plan	Interpretation
H1.2: NF up/downregulates alpha oscillations. If time effect: use averages across second half of trials (data_2ndhalf) Otherwise: use averages across all trials (data_total) H1.2.a: $AAI_{ART\_verum} > AAI_{ART\_sham}$ H1.2.b: $AAI_{ALT\_verum} < AAI_{ALT\_sham}$ **H1.3: Attention up/downregulates alpha oscillations.** If neurofeedback effect: H1.3.a: $AAI_{ART\_diff(verum,sham)} > AAI_{ALT\_diff(verum,sham)}$ Otherwise: H1.3.a: $AAI_{ART\_avg(verum,sham)} > AAI_{ART\_avg(verum,sham)}$			**H1.2: NF up/downregulates alpha oscillations.** If $BF_{10} \geq 3$ for H1.2.a OR H1.2.b: evidence for neurofeedback effect If $BF_{10} \leq 1/3$ for H1.2.a AND H1.2.b: evidence for absence of neurofeedback effect Otherwise: inconclusive evidence **H1.3: Attention up/downregulates alpha oscillations.** If $BF_{10} \geq 3$ for H1.3.a: evidence for attention effect If $BF_{10} \leq 1/3$ for H1.3.a: evidence for absence of attention effect Otherwise: inconclusive evidence

Table continued.

Hypotheses	Sampling Plan	Analysis Plan	Interpretation
Q2: Are the perception of pain and underlying brain responses up- and downregulated?			
H2: The perception of pain and underlying brain responses are up-and downregulated. **H2.1: NF up/downregulates pain ratings.** If AAI time effect: use averages across second half of trials (data_2ndhalf) to evaluate neurofeedback effect Otherwise: use averages across all trials (data_total) H2.1.a: $rating_{ART\_verum} < rating_{ART\_sham}$ H2.1.b: $rating_{ALT\_verum} > rating_{ALT\_sham}$	The sample size for this analysis will be determined by the analysis of H1.3.a.	One-sided Bayesian paired samples t-tests (Cauchy prior distribution with scale parameter r = √(2)/2 and truncation at zero) or one-sided Bayesian Wilcoxon signed rank tests (Cauchy prior distribution with scale parameter r = √(2)/2 and truncation at zero, 1000 iterations) if normality assumptions are violated according to Q-Q-plot.	**H2: The perception of pain and underlying brain responses are up-and downregulated.** Evaluated for pain ratings and each brain response separately. If time AND neurofeedback AND attention effect: evidence for up- and downregulation according to pattern 1 If time AND attention effect: evidence for up- and downregulation according to pattern 2 If NF AND attention effect: evidence for up- and downregulation according to pattern 3 If attention effect: evidence for up- and downregulation according to pattern 4 If evidence for absence of attention effect: evidence for absence of up- and downregulation Otherwise: inconclusive evidence **H2.1: NF up/downregulates pain ratings.** If $BF_{10} \geq 3$ for H2.1.a OR H2.1.b: evidence for neurofeedback effect If $BF_{10} \leq 1/3$ for H2.1.a AND H2.1.b: evidence for absence of neurofeedback effect Otherwise: inconclusive evidence

Table continued.

Hypotheses	Sampling Plan	Analysis Plan	Interpretation
H2.2: Attention up/downregulates pain ratings. If AAI neurofeedback effect: H2.2.a: $rating_{ART\_diff(verum,sham)} < rating_{ALT\_diff(verum,sham)}$ Otherwise: H2.2.a: $rating_{ART\_avg(verum,sham)} < rating_{ART\_avg(verum,sham)}$			**H2.2: Attention up/downregulates pain ratings.** If $BF_{10} \geq 3$ for H2.2.a: evidence for attention effect If $BF_{10} \leq 1/3$ for H2.2a: evidence for absence of attention effect Otherwise: inconclusive results
H2.3: NF up/downregulates brain responses. Evaluated for each brain response (BR) separately. If AAI time effect: use averages across second half of trials (data_2ndhalf) to evaluate neurofeedback effect Otherwise: use averages across all trials (data_total) H2.3.a: $BR_{ART\_verum} < BR_{ART\_sham}$ H2.3.b: $BR_{ALT\_verum} > BR_{ALT\_sham}$			**H2.3: NF up/downregulates brain responses.** Evaluated for each brain response separately. If $BF_{10} \geq 3$ for H2.3.a OR H2.3.b: evidence for neurofeedback effect If $BF_{10} \leq 1/3$ for H2.3.a AND H2.3.b: evidence for absence of neurofeedback effect Otherwise: inconclusive evidence
H2.4: Attention up/downregulates brain responses. Evaluated for each brain response (BR) separately. If AAI neurofeedback effect: H2.4.a: $BR_{ART\_diff(verum,sham)} < BR_{ALT\_diff(verum,sham)}$ Otherwise: H2.4.a: $BR_{ART\_avg(verum,sham)} < BR_{ART\_avg(verum,sham)}$			**H2.4: Attention up/downregulates brain responses.** Evaluated for each brain response separately. If $BF_{10} \geq 3$ for H2.4.a: evidence for attention effect If $BF_{10} \leq 1/3$ for H2.4.a: evidence for absence of attention effect Otherwise: inconclusive evidence

Table continued.

Hypotheses	Sampling Plan	Analysis Plan	Interpretation
Q3: Do alpha oscillations mediate attention effects on pain perception?			
H3.1: Alpha oscillations partially mediate attention effects on pain perception (mediation effect). H3.1.a: 0 ∉ 95% CI ab_{verum} AND 0 ∉ 95% CI c'_{verum} (partial mediation effect in verum conditions) H3.1.b: 0 ∉ 95% CI ab_{sham} AND 0 ∉ 95% CI c'_{sham} (partial mediation effect in sham conditions)	The sample size for this analysis will be determined by the analysis of H1.3.a.	Mediation effect: Bayesian multi-level mediation analyses conducted separately for verum und sham conditions (X: ART_{NF} and ALT_{NF} (verum model) or ART_{sham} and ALT_{sham} (sham model); M: single-trial AAIs; Y: single-trial pain ratings).	**H3.1: Alpha oscillations partially mediate attention effects on pain perception (mediation effect).** If 0 ∉ 95% CI $ab_{verum/sham}$ AND 0 ∉ 95% CI $c'_{verum/sham}$ for H3.1.a/H3.1.b: evidence for partial mediation effect in verum/sham conditions If 0 ∉ 95% CI $ab_{verum/sham}$ AND 0 ∈ 95% CI $c'_{verum/sham}$ for H3.1.a/H3.1.b: evidence for full mediation effect in verum/sham conditions If 0 ∈ 95% CI $ab_{verum/sham}$ for H3.1.a/H3.1.b: no evidence for mediation effect Note: Interpretability of mediation effect in verum condition is limited if no mediation effect is found in the sham conditions, because the causal sequence between X and M cannot be validated.
H3.2: Mediation effects are stronger for verum compared to sham NF (moderation effect). H.3.2.a: $ab_{verum} > ab_{sham}$		Moderation effect: One-sided Bayesian paired samples t-tests (Cauchy prior distribution with scale parameter r = $\sqrt{(2)}/2$ and	**H3.2: Mediation effects are stronger for verum compared to sham NF (moderation effect).** If $BF_{10} \geq 3$ for H3.2.a: evidence for stronger mediation effects for verum compared to sham neurofeedback If $BF_{10} \leq 1/3$ for H3.2.a: evidence for equally strong mediation effects for verum compared to sham neurofeedback

Table continued.

Hypotheses	Sampling Plan	Analysis Plan	Interpretation
		truncation at zero) or one-sided Bayesian Wilcoxon signed rank tests (Cauchy prior distribution with scale parameter r = √(2)/2 and truncation at zero, 1000 iterations) if normality assumptions are violated according to Q-Q-plot.	Otherwise: inconclusive evidence

Note. AAI, alpha asymmetry index; ALT, attention left training; ART, attention right training; BF, Bayes factor; BR, brain response; NF, neurofeedback; SBF+maxN, sequential Bayes factor design with maximal N. Table 1 was reprinted by permission from Ref. [49] (How Do Alpha Oscillations Shape the Perception of Pain? – An EEG-based Neurofeedback Study, Hohn et al.), (CC BY-NC-ND 4.0).

3.2 Results

3.2.1 Registered reports represent a suitable tool to assess whether pain can be modulated using neurofeedback

Following guidelines provided by Nature Communications[135], we conceptualized a fully transparent, well-powered, and rigorous registered report addressing the question of whether pain can be modulated using neurofeedback. This conceptualization was facilitated by two main factors: First, neurofeedback represents an established neuromodulatory technique which made it possible to design a well-controlled short-term neurofeedback training protocol adhering to recent recommendations[81,108]. This protocol was then tested using a pilot study (n=5) which visually confirmed the expected neurophysiological effects. Second, theoretical frameworks on the functional significance of neural oscillations[30,42] and their association with attention and pain enabled us to delineate clear hypotheses and to develop a comprehensive analysis pipeline. Importantly, this analysis pipeline was built mainly upon simple statistical tests[136] (t-tests), which facilitated the establishment of a clear mapping between hypotheses, statistical tests, and the interpretation of all potential outcomes. In this context, Bayesian hypothesis testing, which also quantifies evidence for the absence of an effect, proved to be especially suited. Resulting Bayes Factors distinguish between evidence for a(n) (un)successful neuromodulation and inconclusive evidence and, thus, enhance the interpretability of results[111]. In addition, the editorial and reviewer comments provided during the stage 1 review improved the proposed methodology, for instance, by encouraging the implementation of a fully Bayesian analysis pipeline and by inspiring several exploratory analyses. Overall, the registered reports format can thus be regarded as a suitable tool to assess whether pain can be modulated using neurofeedback.

3.2.2 Registered reports are associated with restrictions on flexibility and additional time cost

While there are many advantages to the registered reports format, we also identified two practical challenges associated with it. First, and most importantly, the conceptualization of a registered report was considerably more time consuming than the conceptualization of a study using the standard publishing model. Contributing factors were the preparation of the stage 1 manuscript including the elaboration of the design table (~ 10 months) and the subsequent stage 1 review process. In-principal acceptance for project 2 was obtained after 14 months of stage 1 review at two journals. A first review process at Nature Communications required > 13 months and resulted in rejection after five rounds of review with one negative and three positive evaluations. A second review

process at PLOS Biology then led to IPA within 4 weeks based on a re-evaluation of the review history from Nature Communications. Thus, the upfront time commitment for the conceptualization of our registered report amounted to almost two years. This upfront time commitment is unlikely to be offset by the subsequent, streamlined publication process because typical publication steps including data collection and analysis in addition to drafting and revising the final manuscript remain[106]. Overall, we therefore roughly expect a tripling of the publication time in comparison to the standard publishing model. The second practical challenge to consider is reduced flexibility. Registered reports impose substantial restrictions on methodological and analytical flexibility as any deviation from the proposed methodology and analysis pipeline can lead to a rejection during the stage 2 review process. To address this issue, we collected pilot data which helped us to streamline our experimental setup and analysis pipeline and to reduce the risk of post-hoc alterations[106].

4 DISCUSSION

Non-invasive neuromodulatory techniques such as tACS and neurofeedback have been designed to modulate oscillatory brain activity in a frequency-specific fashion and therefore represent promising tools to enhance our understanding of pain and its modulation. Despite this appeal, studies applying non-invasive neuromodulatory techniques in the field of pain research are still limited in number or have produced inconclusive results. Thus, further systematic, high-quality research is warranted. To contribute to this effort, the current thesis aggregates the results of two projects investigating the effects of tACS and neurofeedback on experimental pain. To enhance the replicability and interpretability of results, both projects adhere to most recent open science standards. Specifically, both studies were preregistered and include large sample sizes based on rigorous a priori-sample size calculations. In addition, both projects rely on Bayesian statistics to examine evidence not only for the presence of, but also for the absence of effects. Lastly, all data and study-related code are stored in a standardized format and are made openly available to the research community enabling full transparency and computational reproducibility. The following two sections discuss the findings of both projects highlighting methodological challenges and promising future directions. Finally, the third section summarizes the implications across projects.

4.1 Project 1: Optimizing tACS to modulate pain

Project 1 “systematically explored whether tACS can modulate pain […] in healthy human participants using a tonic heat pain paradigm. In 6 recording sessions, participants received tACS over PFC or S1 using alpha, gamma, or sham stimulation while pain ratings […] were collected. Analyses showed that, using the current setup, tACS did not modulate the perceived pain intensity, the stability of pain ratings or the translation of the noxious stimulus into pain”[82]. Likewise, tACS did not modulate brain activity recorded after stimulation. Bayesian analyses conducted for pain measures further indicated that this result did not reflect a lack of power. Instead, it provided evidence for the absence of an effect. “The only exception was alpha tACS over S1 where evidence for tACS effects on tonic pain intensity was inconclusive”[82].

The reported lack of behavioral and neurophysiological findings diverged from our hypotheses but is in accordance with the high variability of findings reported in tACS studies. Overall, the tACS literature is diverse and replications are rare, which has led to serious concerns regarding the effectiveness of tACS (see Ref. [137] for a comprehensive discussion). At the same time, this variability and the resulting criticism has fueled multi-disciplinary optimization attempts combining computational modelling[91] alongside

in vitro[138,139], in vivo[61,140], and ex vivo[141] studies in various populations to elucidate the mechanisms underlying tACS and improve its application[137]. With clinical utility in mind, the present study used established, yet fixed, stimulation parameters. Specifically, we applied fixed stimulation frequencies of 10 and 80 Hz, a fixed intensity of 1 mA peak-to-peak to enable blinding, and fixed bi-hemispheric montages with two electrodes placed according to the 10-20 system. While practical, these stimulation parameters might not have been optimal for modulating oscillatory brain activity, thus warranting further pain studies with optimized stimulation parameters. Given the vast parameter space of tACS, a comprehensive overview of optimization approaches is beyond the scope of this thesis. Instead, selected examples which address the main concerns with respect to tACS and, thus, hold promise for future pain studies will be discussed.

One of the main concerns raised in the context of tACS is its low intensity. To enable blinding and avoid adverse effects such as skin damage and transient headaches, most studies use stimulation intensities between 1 and 4 mA peak-to-peak which is the maximal intensity recommended by current safety guidelines[84]. While some argue that stimulation intensities within this range suffice[10] others argue that these might be too low to pass through the skull and modulate brain activity[141]. *Frequency tuning*[142], i.e., adjusting the stimulation frequency to the frequency of the endogenous target oscillation, represents one of the earliest optimization approaches focusing on this issue and is rooted in the idea that entrainment is a non-linear effect determined by the interplay of stimulation frequency and intensity[143]. According to the so-called Arnold tongue principle, the stimulation intensity required to achieve entrainment changes as a function of frequency mismatch, with higher intensities required the larger the deviation between external and endogenous frequencies[143-145] (Fig 10A). Thus, tuning the applied stimulation frequency to the frequency of the individual, endogenous target oscillation could reduce the required current intensity and enhance the likelihood of neurophysiological effects within the recommended intensity range[143,146]. Future studies investigating the modulation of pain could therefore use individual resting-state alpha peak frequencies and pain induced gamma peak frequencies. These could be quantified before the stimulation or repeatedly when using intermittent stimulation protocols. In addition, methods taking the aperiodic component or 1/f noise present in EEG data[147,148] into account might enhance the accuracy of peak frequency estimation by differentiating between the aperiodic component and the genuine periodic oscillation[146].

Other concerns raised in the context of tACS are the impact of interindividual differences in neuroanatomy[149] and the limited focality of the stimulation[150]. Indeed, recent studies using electrical field modelling indicate that the distribution and intensity of the induced electrical fields are strongly influenced by individual neuroanatomy leading to target intensities varying by more than 100 % when fixed stimulation intensities are used[149]. In addition, resulting field characteristics seem to predict neurophysiological offline effects of the stimulation[151] emphasizing the importance of current flow modelling as a tool to optimize tACS parameter selection. Building on modelling output, stimulation intensities can be, for instance, adjusted individually with the goal of eliminating interindividual differences in the electric field intensity at the cortical target site[149]. This *intensity tuning* could align neurophysiological effects across individuals leading to more stable and foreseeable results on the group level[149].

Beyond, optimization approaches based on electrical field modelling might also mitigate concerns regarding the limited focality of tACS. Derived models allow researchers to assess the extent to which peripheral (e.g., via the retina[152] or peripheral nerves[153]) and cortical co-stimulation beyond the target site can be expected and thus form the basis for *spatial tuning* via optimization of the applied electrode montage. Current flow modelling indicates that the bi-hemispheric montage chosen in the current study might have induced the highest field intensities close to the midline and not under the stimulation electrodes[150]. In addition, it might have induced anti-phase synchrony in the left and right hemisphere thereby potentially affecting not only the processing within each region but also the communication between them[82]. High-definition tACS (HD-tACS) and transcranial temporal interference stimulation (tTIS, Fig 10B)[154] might help to address these issues. HD-tACS typically involves several small electrodes (e.g., <5 cm^2 total electrode surface) arranged in a ring-fashion around a center electrode of opposite polarity[155] and might help to focus the induced electric field to more focal regions within one hemisphere[156]. Going even further, tTIS might allow researchers to selectively target deeper brain regions[154]. In its basic form, tTIS involves the simultaneous application of two high-frequency alternating currents which are too high to drive neural firing in isolation (e.g., in the kHz range) but differ by a value within the physiological range (e.g., 1 kHz and 1.01 kHz yielding a frequency difference (Δf) of 10 Hz). At their intersection, the interference of both fields creates an amplitude-modulated electrical field oscillating at the frequency difference Δf which is thought to selectively modulate affected neurons without driving neighboring or overlying regions only exposed to one of the fields. While tTIS is still in its infancy, first results in rodents[154] and based on human head models[157-159] support its enhanced stimulation focality making tTIS a highly

promising method for spatial tuning and the modulation of pain, which is associated not only with activity in several cortical but also subcortical brain regions[19,20].

Summarizing, the high prevalence of null findings including the present study has raised doubts regarding effectivity of tACS and fueled multi-disciplinary research efforts to elucidate its mechanisms of action and improve its application. Due to this development, several optimization approaches exist, ranging from anatomy-informed, individualized parameter selection to the interference of simultaneously applied electrical fields. Given the conceptual plausibility of tACS and its potential clinical utility, these approaches are worth pursuing. However, optimization often comes at the cost of increased complexity (e.g., additional EEG recordings for frequency tuning or brain scans for intensity and spatial tuning). Thus, more systematic research is needed to allow users to evaluate parameter choices based on the ratio between output gain and complexity.

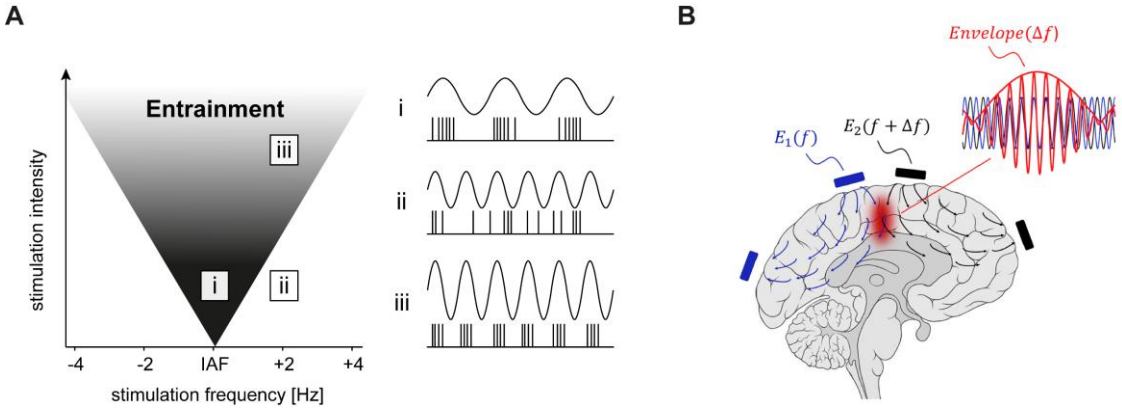

Figure 10. tACS optimization through frequency and spatial tuning. (A) Arnold tongue principle. According to the Arnold tongue principle, the stimulation intensity required to achieve entrainment increases with increasing deviation between external stimulation frequency and endogenous frequency represented by the individual alpha frequency (IF). When both frequencies are aligned, entrainment can occur at rather low intensities (i), whereas stimulation off the endogenous frequency is ineffective (ii) or requires increased stimulation intensities (iii). Consequently, the entrainment region (shaded in black) is characterized by a triangular shape. (B) Temporal interference stimulation. During tTIS two electric field vectors E1 (blue arrows) and E2 (black arrows) are simultaneously applied to the brain at kHz frequencies f and f + Δf. Importantly, these frequencies are too high to drive neural firing in isolation but differ by a value within the physiological range (e.g., 1 kHz and 1.01 kHz yielding a frequency difference Δf of 10 Hz). The interference field (red trace) drives neural activity at the frequency difference Δf in a focal manner affecting only regions where the envelope amplitude is sufficiently large (red shading). IAF, individual alpha frequency. Panel A was adapted by permission from Elsevier: Brain Research, Ref. [146] (Targeting Neural Oscillations with Transcranial Alternating Current Stimulation, Riddle & Frohlich), © (2021). Panel B was reprinted by permission from Elsevier: Cell, Ref. [154] (Noninvasive Deep Brain Stimulation via Temporally Interfering Electrical Fields, Grossman et al.), (CC BY 4.0).

4.2 Project 2: Optimizing neurofeedback to modulate pain – registered reports as suitable tool

Project 2 further explores the potential of EEG-based neurofeedback to modulate phasic experimental pain using a short-term neurofeedback training targeting somatosensory alpha oscillations. Motivated by the methodological challenges associated with neurofeedback studies and the inconsistency of previous results, the project was conceptualized as a registered report. Results demonstrated that registered reports represent a suitable tool to create a fully transparent, well-powered, and rigorous neurofeedback study on pain modulation due to the confirmatory nature of the study[160]: First, neurofeedback is an established neuromodulatory technique and guidelines on its conduct exist[108] which facilitated the development of a training protocol and study design. Second, previous evidence made it possible to delineate clear hypotheses embedded in existing theoretical frameworks and to develop a comprehensive analysis pipeline. To facilitate interpretation, this analysis pipeline was built mainly on simple statistical tests[136] and will be applied to a sufficiently large sample. Combined, these aspects enhance the credibility of obtained results and therefore address the urgent need for more high-quality research in the field of neurofeedback-based pain modulation[78,79]. In addition, registered reports ensure publication independent of results and, therefore, enhance the visibility of negative findings which are likely underrepresented in the neurofeedback literature. Factors such as small sample sizes, analytical flexibility, and ideological and financial interests, e.g., of private practitioners and equipment manufacturers, make the literature on EEG-based neurofeedback susceptible to a high false positive rate[161,162]. Thus, studies producing reliable and unbiased findings including null findings are crucial to create a reliable knowledge base that can inform clinical applications.

However, project 2 also identified downsides of the registered reports format, namely restrictions on flexibility and additional time costs. While restrictions on flexibility can, for instance, be addressed by conducting pilot studies to streamline the proposed methodology and examine its feasibility, additional time costs might be harder to address. With a stage 1 review duration of 14 months, the present registered report required a very high upfront time commitment which is unlikely to be offset during subsequent stages such as data acquisition or the stage 2 review process. Such stage 1 review delays pose a serious challenge when time resources are limited, e.g., for early career researchers in graduate programs or post-doctoral positions with fixed-term contracts and have been identified as one of the key challenges of registered reports[103,106]. To address this issue, several initiatives which attempt to accelerate stage 1 review processes while maintaining research quality have recently been launched[103].

The Royal Society Open Science, for instance, initiated a network of reviewers committed to *rapid reviews* (1-2 days of review leading to 7 days for the initial stage 1 review round) for COVID-related registered reports[103]. To date, over 1400 researchers[163] and 12 journals have joined the network and first publications indicate that rapid review processes are indeed possible with an average total review time of 18 days[103]. Another promising initiative focuses on the reduction of editorial handling times by offering *journal-independent registered reports*[103]. Specifically, the recently created non-profit platform *Peer Community in Registered Reports* (PCI RR) offers peer-reviews without involving journals. Instead, manuscripts are handled by so-called *recommenders*, i.e., researchers trained with respect to registered reports who act analogous to action editors. Recommenders issue a positive recommendation for manuscripts which have been favorably peer reviewed. After recommendation, authors can then choose to publish their final manuscript in one of 25 “PCI-friendly” journals without further peer review (see Ref. [164] for a list of participating journals). These initiatives present promising paths towards more efficient registered reports. However, they require expansion, e.g., with respect to the topics and journals included and the education of editors and reviewers, to enhance the efficiency of registered reports across a variety of research fields. Hence, it is advisable to consider additional time costs early on during the conception of a registered report as well as when planning questions of employment[106].

In summary, the registered reports format is a suitable tool to promote the development of rigorous neurofeedback studies examining the modulation of pain. However, when adopting the format, practical obstacles such as additional time costs and restrictions on flexibility should be considered.

4.3 Implications across projects and outlook

Accumulating correlative evidence in humans and causal evidence in animals indicates that neural oscillations play a significant role for the processing of nociceptive input and the emergence of pain. Non-invasive neuromodulatory techniques such as tACS and neurofeedback aim at modulating these features of brain activity non-invasively, thus fostering the transition from predominantly correlative towards causal evidence in humans. To date, however, tACS and neurofeedback do not deliver consistent findings leaving many open questions with respect to their scientific and clinical utility. Optimization approaches derived from integrative research efforts across disciplines, e.g., frequency, intensity, and spatial tuning, and a more thorough evaluation of the applied techniques through high-quality research, e.g., using new publication formats such as registered reports, are imperative to close this knowledge gap. Only if neuromodulatory techniques provide stable and predictable results based on a known mechanism of action will they advance our understanding of pain and novel approaches for its modulation. Such insights would not only be highly relevant from a basic science perspective, but also from a clinical perspective. Chronic pain poses one of the greatest challenges to global healthcare systems because it is highly prevalent affecting 20 - 30% of the adult population[165,166] and remains difficult to treat[167,168]. Current therapies often fail to alleviate symptoms or can cause serious side effects as tragically illustrated by the recent opioid crisis[169]. At the same time, the development of new pain therapeutics is stagnating[15]. Thus, novel, non-pharmacological treatment approaches for chronic pain are indispensible[13,14]. Considering their conceptual plausibility to modulate pain, and their potentially broad clinical applicability, non-invasive neuromodulatory techniques represent promising candidates for such novel treatment approaches. Thus, research efforts focusing on the optimization and clinical translation of neuromodulatory techniques are worth pursuing and should be continued in the future.

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