Nociception and pain in the electroencephalogram

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“There is no substitute for good data”

[Unknown / i.e., somebody said this to me at the beginning of my thesis and now I persistently emphasize it because it is indeed important]

“EEG recordings are almost always contaminated by artifacts” [1]

Publishing statement

The present cumulative thesis is a compilation of three peer-reviewed journal articles, together covering the results obtained within this dissertation, and an additional individual section that summarizes and reflects the results of all three publications in a broader context. All publications have been published open access under the CC BY 4.0 license, meaning that I am still the copyright holder. All co-authors agreed on including these three manuscripts into this thesis, and the respective co-author approval documents have been submitted. I will further add a declaration on the collaborative work in this thesis at the end of the manuscript to point out the individual contributions of each co-author to each paper that is included here. The publications that are included in this thesis are as follows:

1) [2] **Anders M***, Anders B, Kreuzer M, Zinn S, Walter C (2020) Application of Referencing Techniques in EEG-Based Recordings of Contact Heat Evoked Potentials (CHEPS). Frontiers in Human Neuroscience 14 (527). DOI: 10.3389/fnhum.2020.559969
   The published article is reproduced in the Chapter 8: Publication 1 (methodology establishment study) of the dissertation.

2) [3] **Anders M**, Anders B, Dreismickenbecker E, Hight D, Kreuzer M, Walter C, Zinn S (2023) EEG responses to standardised noxious stimulation during clinical anaesthesia: a pilot study. BJA Open Volume 5 (March 01). doi:10.1016/j.bjao.2022.100118
   The accepted manuscript is reproduced in the Chapter 9: Publication 2 (project IMPACE) of the dissertation. During copy editing, some orthographic changes were introduced and the title was changed from “EEG trajectories of standardized noxious stimulation during general anaesthesia in real patients – a pilot study” to the one in the above citation. In this thesis, the paper is included with the old title and without the orthographic changes that are present in the published version.

3) [4] **Anders M**, Dreismickenbecker E, Fleckenstein J, Walter C, Enax-Krumova EK, Fischer MJM, Kreuzer M†, Zinn S† (2022) EEG-based sensory testing reveals altered nociceptive processing in elite endurance athletes. Experimental brain research (Online ahead of print). doi:10.1007/s00221-022-06522-4 *† indicates a shared senior/last authorship*
   The accepted manuscript is reproduced in the Chapter 10: Publication 3 (project SPINE) of the dissertation. During copy editing, some orthographic changes were introduced. In this thesis, the paper is included without the orthographic changes that are present in the published version.

1 Abstract

The scope of this thesis is to elaborate on the use cases of the EEG in pain research. It has been submitted as a cumulative dissertation, meaning that the main part of this thesis has been previously published in international peer-reviewed journals [2-4]. The first part of this thesis begins with an introduction which describes the general methodoligcal considerations and theoretical background information that is needed to perform pain research using the EEG. Then, I will give a summary of the results of all three studies and the subsequently published manuscripts. The discussion will give an outlook on two ongoing projects and elaborate how the methodology that has been compiled throughout my time as a PhD student can be further applied to scientific problems in pain research. I will conclude with the possibilities and the limitations of the EEG in pain research.

The second part of this thesis consists of three publications that cover three individual studies, of which I am the lead/first author. These publications describe different use cases for the EEG in pain research. The first publication lays out the methodological backbone of this thesis, analyzing the exact EEG parameters that are needed to achieve the results in the following projects. Then, I present two additional studies. The first study describes the usefulness of pain-related evoked signatures after standardized noxious stimulation in the EEG in patients undergoing general anesthesia. The second study outlines differences in the pain processing of elite endurance athletes versus a normally active control group. Furthermore, it outlines how the function of the endogenous pain modulatory system can be measured in the EEG using CPM. All studys are discussed individually as per the journal guidelines.

2 Introduction

2.1 Introduction to pain and its testing methods

Pain is a ubiquitous problem that imposes great burdens from a clinical, psychological, economical, and sociological point of view [5,6]. The prevalence of chronic pain correlates with higher age, with numbers of 19.6 % for chronic low back pain in a population of individuals aged between 20 and 59 years [7]. The diagnosis and the treatment of pain results in huge costs for the healthcare system. An early prevention and a stratified treatment of pain and pain-related illnesses will not only decrease direct costs for society but can also significantly improve the quality of life of the affected individual. Hence, for the prevention of pain and chronic pain, methods to detect populations with higher risk factors for the development of pain and the quantification of those risk factors are needed to minimize or avoid the occurrence of chronic pain and pain-related illnesses. As pain is a subjective experience, methods to quantify pain in a clinical setting are helpful to evaluate the success of treatments such as medication. Pain is mostly characterized as "[…] whatever the experiencing person says it is, existing whenever and wherever the person says it does" – a definition that has become famous since it was used by McCaffery in 1968 [8]. A well-established pain testing panel is quantitative sensory testing (QST), which relies on the subjective pain ratings communicated by the subject to the investigator [9]. Subjective sensory testing can be usefully extended with dynamic pain models such as conditioned pain modulation (CPM) [10], which assesses the capacity of the endogenous pain modulation system, or the subjective McGill pain questionnaire (MPQ) [11]. Objective pain testing methods include the EEG [2] and fMRI [12].

2.2 Pain, nociception, and the EEG

Pain by its definition is a personal experience depending on biological, psychological, and social factors and, thus, is influenced by subjectivity [13]. The assessment of pain is tricky; it aims to objectively quantify pathophysiological changes besides assessing psychosocial variates [14,8]. Until today, subjective pain testing is the gold standard in research. However, advances in computerized analytics of the electroencephalogram (EEG) have enabled the integration of computational techniques into pain studies. The EEG provides an objective assessment of the processing of a (noxious) somatosensory stimulus, and its readout does not rely on the individuals' subjective feedback. High-density, multi-channel EEG recordings combined with standardized noxious stimulation may help to establish a better understanding of pain-related dynamics in the cerebral cortex and may unmask alterations in the perception of nociceptive events.

However, there is limited evidence that the EEG is a suitable tool for the evaluation of clinical pain [15]. For this use case and up to this day, methods to detect subjective pain are the most common, accurate, reliable, and reproducible way to describe an individual's experience of pain [16]. A variety of quantifying measures for subjective pain are used, such as visual or verbal rating scales. Hence, for the detection and treatment of pain, simply asking the patient to rate their pain with whatever method is suitable for the case is usually sufficient in standard patient care.

Objective methods such as the EEG can be used in nonverbal populations such as patients during general anesthesia, newborn infants, or animals [17-20]. Those objective methods may also serve uses in e.g., patients with dementia, where the subjective rating may not always be accurate [16]. On top of that, the EEG is an excellent tool to objectively compare the response of the brain to standardized noxious stimulation e.g., between an interventional group and a control group, while testing the efficiency of analgesic compounds [21]. Another use case would be to determine the differences in the characteristics of the objective EEG response to noxious stimulation between two groups that differ in a certain sociological factor, and where abnormalities in the nociceptive system are suggested by previous research. Finally, objective pain characteristics in the EEG can be compared between a control group and a group with a characterizing disease that results in pain as a symptom [22].

The characteristics in the EEG after standardized noxious stimulation do not fully represent the pain perception of the subject but are rather a surrogate parameter for the processing of noxious information in the somatosensory system, i.e., an indirect readout of the function of the nociceptive pathways [23]. The EEG is thus not an individual biomarker for pain, although the extracted signatures sometimes correlate with the subjective pain ratings [24,23,25]. However, similar to the QST testing panel, normative data for certain setups of standardized noxious stimulation and the research on the evoked response in the EEG has recently been published, aiming at simplifying the use of the EEG to detect anomalies in the nociceptive pathways [26-28].

2.3 Human EEG recordings

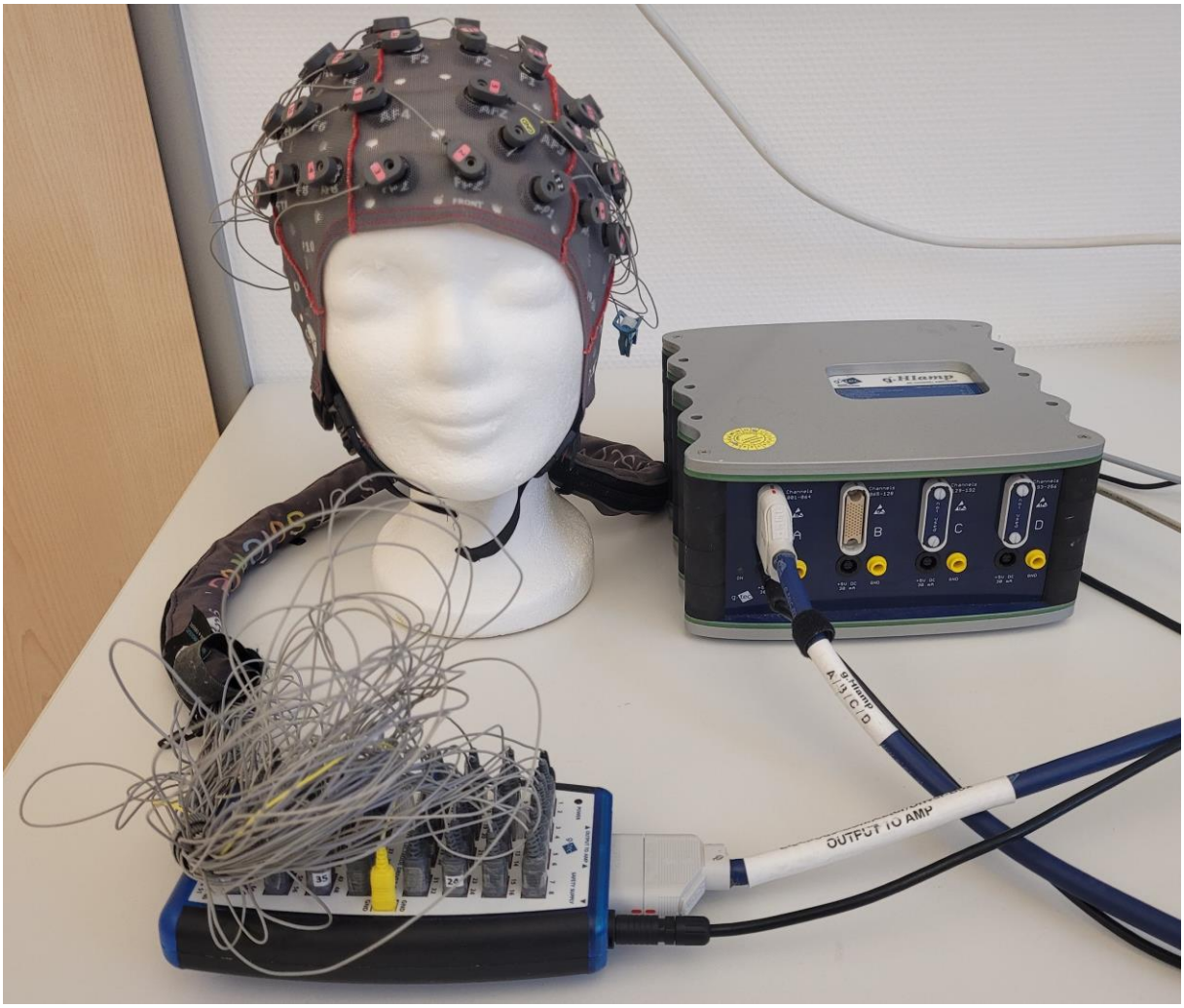

Figure 1: The EEG recording device g.HIamp (Guger Technologies, Schiedlberg, Austria) that we used for all recordings included in this thesis, including the 64 channel active electrode (g.Tec g.SCARABEO) EEG headcap. The headbox in the bottom only serves as a splitter without any additional function.

Since the 19th century, the EEG is used as an easy and cost-effective method to measure physiological processes in the brain. The concept of an EEG recording is the voltage difference between two points on the surface of the head [29,30]. Activity in the different regions of the brain (e.g., in the cortex) usually leads to the creation of action potentials (AP) that are transmitted as a current through the neurons, for which a certain voltage is needed. This voltage extends from the neurons to the surface of the skull and can then be measured with an adequate EEG device. It is, however, no surprise that the voltage is very small. Thus, the first crucial part of an EEG device is an amplifier that amplifies the voltage up to a certain extent. This can be either done centrally in the EEG recording device (a so-called passive EEG recording electrode montage), where the signal is recorded with plain wires and flows from the recording site (the head) directly into the amplifier where it is amplified, or directly at the

surface of the head within the electrode itself (a so-called active EEG recording electrode montage). In an active montage, the signal is amplified a second time in the EEG recorder. Passive EEG recordings are the most common, as they are cheap and reliable. However, they are highly influenced by artifacts caused by e.g., cable movement: if the unamplified small current flows through lengthy cables, small movements can already lead to the induction of a current on top of the physiological signal, which will be seen as noise. An active electrode system circumvents this in the way that it transmits an already pre-amplified current from the head to the second amplification stage. This way, the artifactual influence of small non-physiological currents and the interference with other electronic fields is minimized and higher recording quality is achieved [31]. For this thesis, active EEG electrodes were used, and the whole EEG setup is shown in Figure 1.

There is a variety of modern commercial EEG systems available on the market, but recent advantages in the manufacturing of integrated circuits allow for low-cost open-source EEG devices such as OpenBCI [32] or others [33]. Some of the systems also offer a wireless connection, e.g., via Bluetooth. This heavily limits the bandwith and subsequently the number of electrodes, and most of the available wireless EEG systems only support 32 channels up to a sample rate of 256 Hz. In addition, most of the low-cost devices are equipped with passive electrodes. Thus, for this thesis and the included studies, we relied on a commercially available system that uses a wired connection, proprietary active electrodes, connectors, recording software, and even ships with additional software for data analysis. However, there is also a variety of open source software for data analysis available, such as Fieldtrip [34] and EEGLAB [35], which are both plugins for the widely used commercial software MatLab (Mathworks, Natick, Massachusetts, United States of America). This is not only way cheaper than relying on proprietary software, but most often offers more frequent updates and subsequently the latest state-of-the-art analysis tools.

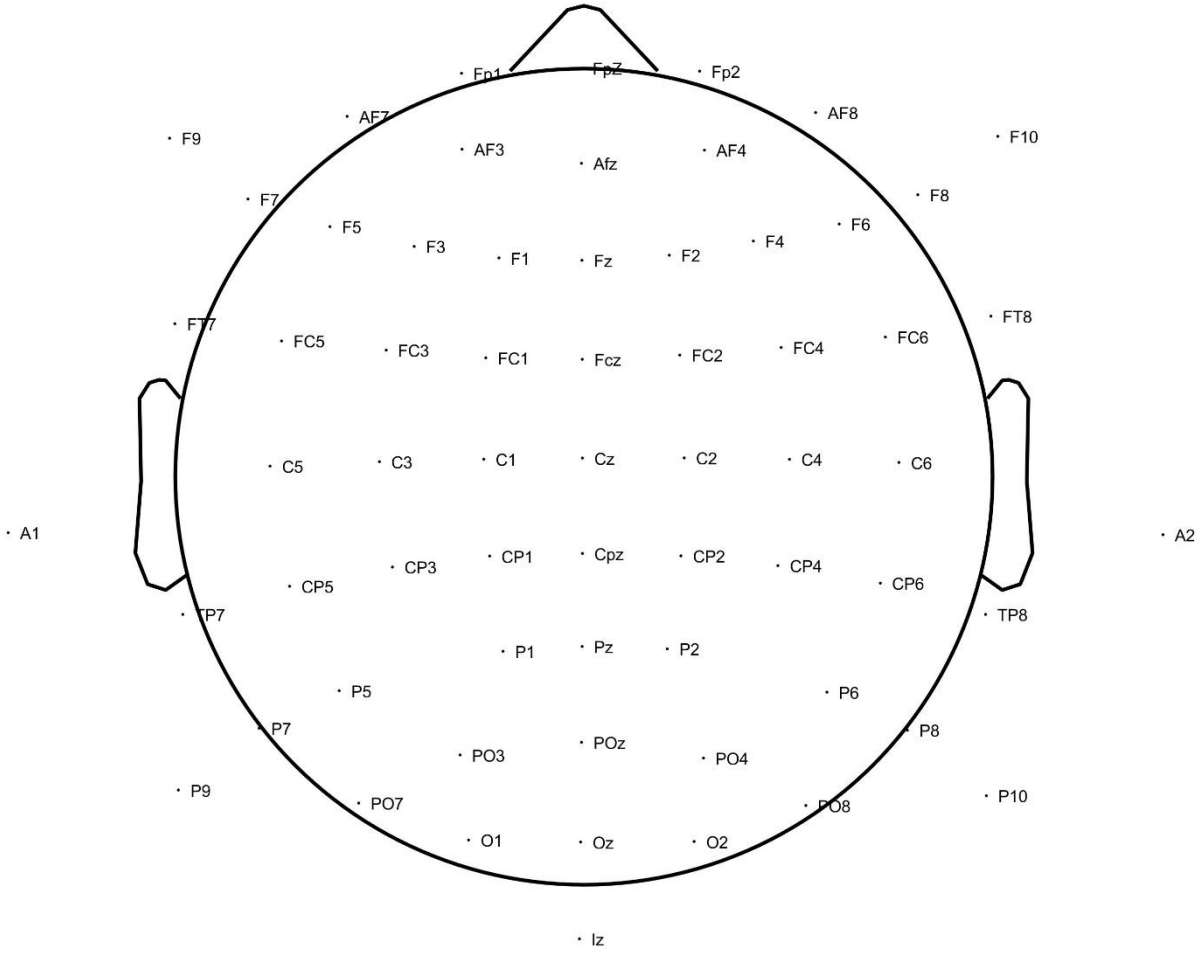

Figure 2: sample electrode layout in the 10-20 system for 64 electrodes which equally cover the whole head.

The recording quality of the EEG is dependent on the contact of the electrode to the surface of the head. The electrodes are arranged in a certain way on the head, with the most prominent arrangement system being the 10-20 system, as outlined in Figure 2 [36]. A higher density or electrode, i.e., a higher number of electrodes, increase the coverage of the scalp and foster the spatial resolution of the results. Standard EEG setups range from a couple of electrodes up to 256 for very high-density recordings, with 32, 64, or 128 electrodes being commonly used. The electrical impedance of the electrode connection to the surface of the scalp is usually decreased by using abrasive gel to smoothen the outer layer of the epidermis, and then adding a water-based conductive gel between the skin and the conducting material of the electrode [37]. In this way, impedances as low as 5 kOhm can be achieved, although higher impedances are usually fine for modern EEG systems as well [38]. The impedance between every single electrode and the skin can easily be measured by modern EEG systems by injecting a known current into the electrode and analyzing the voltage difference according to Ohm's law [39].

Depending on the shape of the head and the density of the hair, especially when using high-density electrode layouts, not every electrode will have a sufficiently low impedance to generate a usable EEG waveform. This is not only outlined by a high impedance in the online-impedance-check of the EEG recording software, where unusable electrodes are often highlighted black, yellow, or red. A skilled EEG technician (like the author of this thesis, of course) will usually spot an abnormal waveform in the raw EEG recording with features like unusually high amplitudes (> 100 µV), little correlation with adjacent channels, or high-frequency noise due to e.g., failing common mode rejection. During the course of this thesis, I have also observed recordings where the impedances in the EEG recording software g.Recorder (Guger Technologies, Schiedlberg, Austria) for a small number of electrodes was marked yellow, i.e., an impedance above 5 kOhm and below 50 kOhm. After visual inspection of the raw EEG of those single electrodes, I still deemed the EEG waveforms usable and did not encounter any problems during subsequent data analysis. This aligns with current literature that points out that most often, impedances slightly above 5 kOhms, i.e., less than 10 kOhms, still result in a usable EEG [38]. This is important to remember when recording high-density multi-channel EEGs with 64 or more electrodes, or when recording EEGs in a time-constricted environment. Both problems (a high number of electrodes in a time-constricted environment) were relevant to this thesis: if the examiner already spends an hour preparing the EEG electrodes, followed by three hours of constant painful stimulation, even the most vigilant participant will be subjected to tiredness and absent-mindedness and the data quality will inadvertently suffer. However, certain electrodes of interest for this thesis, especially electrode position Cz (central midline) and the earlobes, were always optimized for very low impedance, as we derive our pain-related signal from electrode Cz, and the earlobes were in some cases used as the common reference point.

The (pre-)amplified signal will then undergo the last stage during the recording process, which is analog to digital conversion via an analog-digital converter (ADC). The voltage curve measured from the brain is analog, i.e., an unlimited number of data points can be obtained for a given timeframe. This would result in an infinite file size when the signal is stored, which is something that exceeds the limits of today's computers. To obtain a discrete signal, a sample rate is set. For this thesis, I deemed a sample rate of $fs = 512\ Hz$ appropriate. As per the Nyquist–Shannon sampling theorem, this sample rate allows evaluating frequencies of up to 256 Hz, which far exceeds the human frequencies of 100 Hz or less that are researched within our projects. A higher sample rate has no further benefits besides a higher Nyquist frequency $f/2$ and by doubling the sample rate, the file size of the offline-stored EEGs would also double; subsequently also doubling the calculation time of every linear processing operation during data pre-processing as well. As most of the processing steps in this thesis were linear, the

computation time was also directly dependent on the hardware capabilities that were used, especially on processor speed and available random access memory (RAM). For processing, the EEG is stored on the volatile RAM instead of on a hard disk or solid state drive, as the RAM's access speed is vastly superior. For this thesis, modern state-of-the-art hardware with a high processor core count and a sufficient amount of RAM was utilized. Also, as a multi-core processor was used, most processing steps were carried out in parallel, utilizing MATLAB's parallel computing toolbox.

The discrete digital EEG then marks the end of the recording process. Modern EEG recording software offers a huge variety of other processing options which were all not used within the projects outlined here. Pre-processing operations can also be applied to the offline-stored EEG after the recording has taken place, and there is no need to decide on processing parameters a priori. If any, specific visualization-only filters can be used to inspect the EEG during the recording process, but the choice to store unprocessed data leaves more room for analysis later on in the process.

2.4 A word on artifacts

The low voltage that is derived from the neurons in the human head must be heavily amplified to be even recognizable and is also subjected to a variety of influential factors from the surrounding environment. Miniscule changes in the electromagnetic field surrounding the EEG equipment may or may not have dire consequences on the readout in the recording, rendering it unusable from time to time. Unfortunately, everything electric around us oscillates and transmits current, with those oscillations inducing another current into nearby conducting materials.

The most common artifactual influence on the EEG is line noise, i.e., the oscillations of the current that we draw from power outlets (Europe: 50Hz, USA: 60Hz). The line noise artifact is hugely visible in the raw un-processed EEG. It is not only injected into the EEG via induction processes of the surroundings. As an EEG machine is usually powered through the common 230 V power outlet (or 110 V or 120 V in other countries, e.g., the United States), the artifactual 50 Hz oscillations are injected into the EEG recorder via the grounding of the system. The artifact is limited to a very specific frequency (50 Hz) and is usually more or less stationary. It does not change its sinusoidal characteristics over time, only merely fluctuates in intensity and phase, and can be easily tackled by measures such as common mode rejection or a classic filter. For filtering, only a specific frequency portion of the signal can be filtered out, e.g., every signal within a frequency range between 49 Hz and 51 Hz is eliminated. This is called a classic notch filter. Luckily, no physiological signals of interest are found in that frequency range. Another, more drastic option is a bandpass filter that cuts off frequencies above e.g., 40 Hz. This is called a lowpass filter and would render the research of higher frequencies above 40 Hz impossible,

but is usually sufficient for the analysis that is presented in this manuscript. In this thesis, more modern approaches were used, such as the plugin *Cleanline* for the EEG processing toolbox *EEGLAB* [35,40]. Those modern solutions stem from advantages in computerized processing of biosignal data and adaptively estimates and removes any sinusoidal frequency that is fed into the software [41]. The mentioned toolbox *Cleanline* utilizes multi-tapering and Thompson F-statistics for the automated removal of sinusoidal line noise with a chosen frequency. After visual inspection of the results, we assumed that *Cleanline* is at least equivalent in performance to a common notch- or bandpass filter. Other oscillatory sources of artifacts include but are not limited to fluorescent lights, screens with a refresh rate of e.g., 60 Hz or 144 Hz for modern monitors or mobile phones, or even the railway system of Deutsche Bahn, which unfortunately uses a very specific frequency of $16\frac{2}{3}\ Hz$ to electrically feed their trains throughout Germany.

Physiological sources of artifacts are e.g., eye blinks or jaw clenching. As both of those processes are carried out by muscles in the facial region and the EEG recording electrodes are right next to them, the artifacts are prominently visible in the EEG data and cannot be fully avoided. In the past years, data portions containing artifacts of physiological nature such as blinking were simply removed, even if they contained data of interest. The only solution was to remove the artifact-ridden portion of the data, as there was no readily available solution to salvage the physiological signatures in that data. In this thesis, more modern approaches such as artifact subspace reconstruction (ASR) were used [1]. This artifact removal routine identifies a noiseless portion of data via principal component analysis and then corrects the remaining data when it exceeds an individually chosen threshold commonly in the range of 10 – 100 standard deviations [1]. Again, explaining the whole removal routine would far exceed the scope of this thesis, as it belongs to the field of computational neurosciences. For now, literature shows that it is a very practical and automated approach for the cleaning of large continuous EEG datasets [1].

2.5 Standardized noxious stimulation to elicit pain

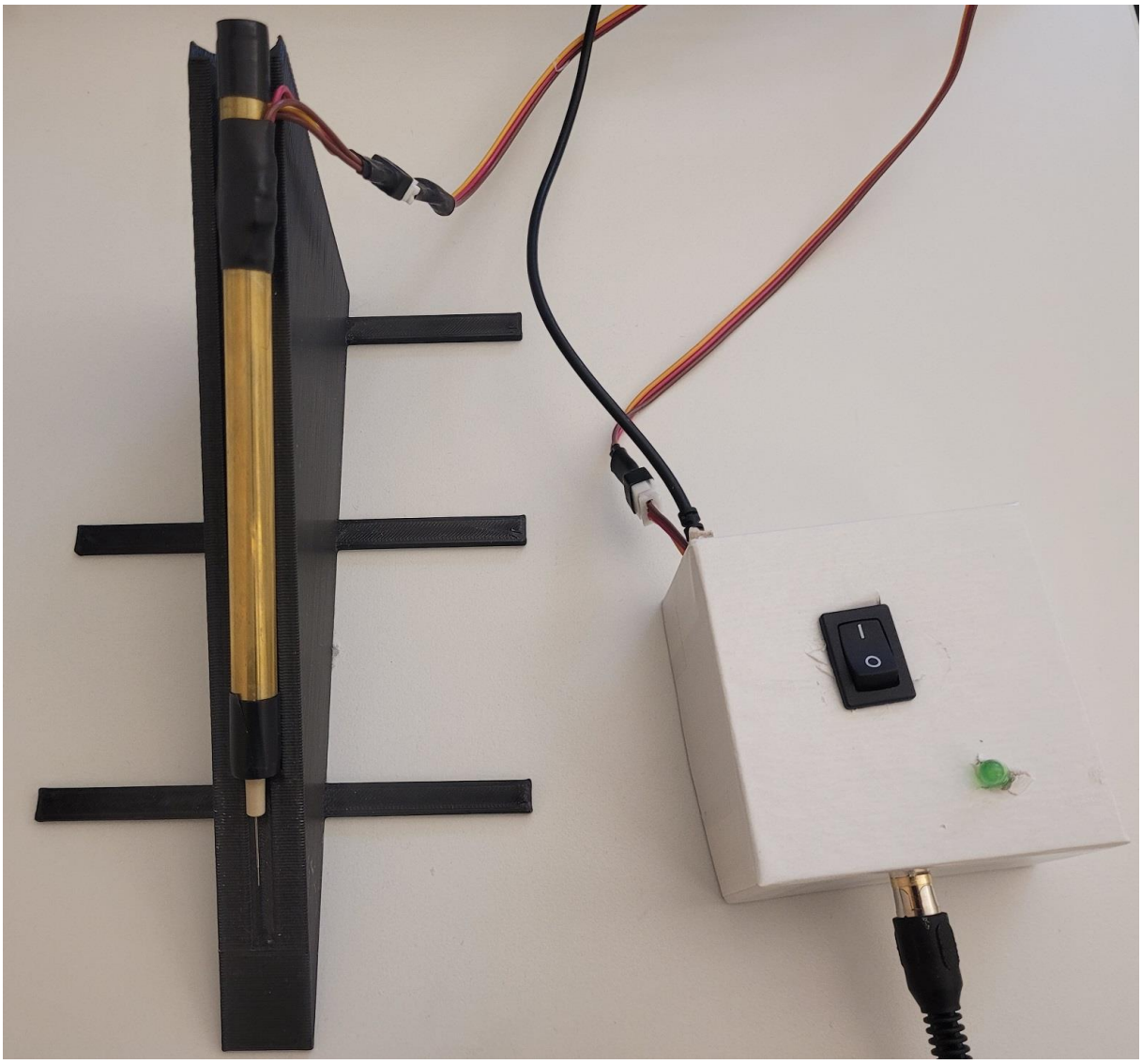

Figure 3: The modified 512 mN pinprick with an Arduino-based 5V pulse generator. A photoelectric sensor is triggered once the weight in the tube is moved upwards, and a +5V transistor-transistor-logic (TTL) pulse is generated by the electronics in the box on the right to synchronize the device with the EEG recording device.

As there is no objective measure of complete pain perception of a human [42], there is a need for experimental pain models that utilize a holistic approach for the standardized noxious stimulation of a subject or patient. Hence, when inducing standardized noxious stimuli, it has to be warranted that we are administering a noxious stimulus that selectively activates pain-specific nociceptors and is then transmitted via pain-specific pathways [43]. It also has to be taken into account that the stimulus has to be above the pain threshold for the given stimulus type [43]. The most prominent examples of those pain-specific nociceptors are C fibers and Aδ fibers, which can be selectively activated by a variety of stimuli [43,44]. Those include, but are not limited to, mechanical (pinprick evoked potentials, PEP, see Figure 3), thermal (contact heat evoked potentials, CHEPS, see Figure 4), or others, such as e.g., chemical stimuli [44]. In our studies, for standardized noxious stimulation, we either used or adapted

mechanical or heat stimuli from the quantitative sensory testing (QST) protocol [9], or applied electrical stimuli (painful cutaneous electrical stimulation, PCES). Especially for electrical stimuli, it is debated that large non-nociceptive-specific fibers are co-activated during the stimulus, making the stimulation non-pain-specific [45,46]. In addition, electrical stimulation bypasses the receptors and activates a variety of nerve fibers directly, making it non-specific for nociceptive stimulation [46], although some authors argue that specific parameters like the stimulus duration or the shape of the stimulation electrode (i.e., a concentric shape) increase the pain-related specificity of the stimulus [47]. Other devices such as a contact heat or mechanical stimulator (pinprick) are designed in a way that they selectively activate the C- and Aδ nociceptive fibers during stimulation [48-50,28,51].

Figure 4: the thermal stimulation device MEDOC PATHWAY Pain and Sensory Evaluation System (Medoc Limited, Ramat Yishai, Israel), with the thermode for the stimulation with contact heat in the top-middle of the picture. The thermal probe of the stimulation device delivers short heat bursts by increasing its temperature at a fixed rate of 70°C/s and selectively activates A- and C fiber nociceptors if an adequate peak temperature is chosen.

2.6 Participants and subjective pain ratings

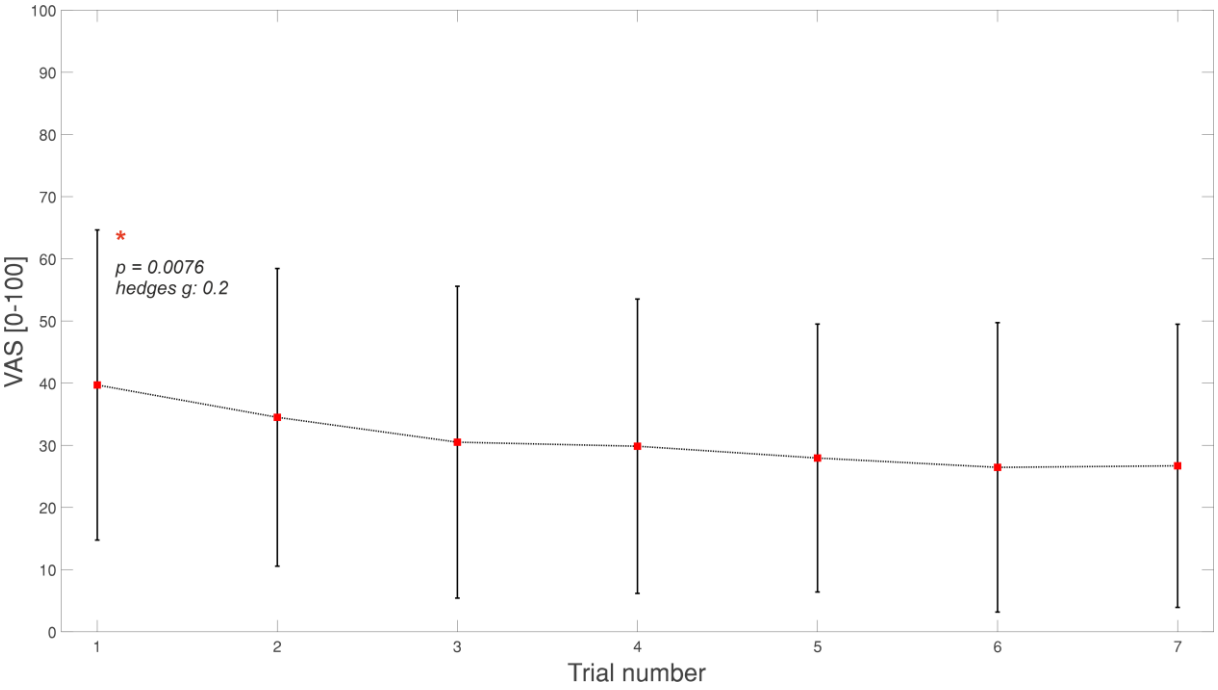

Figure 5: Average subjective pain ratings of the participants in the methodology establishment study [2] after stimulation with noxious contact heat (54 °C peak temperature) over 7 trials. The figure outlines the subjective pain ratings as communicated by the participants for each of the seven trials of noxious contact heat stimulation. The red dots indicate the mean subjective VAS rating for each trial across all participants, the errorbars indicate the standard deviation of the distribution. A significant difference between two adjacent trials is indicated by a red asterisk in between the trials. We show the p value and the Hedges' G effect size for every significant comparison.

As an example on how painful a standardized noxious stimulus is perceived, I included unpublished data from the methodology establishment study [2] in Figure 5. The participants were stimulated with noxious contact heat in 7 trials, with an inter-stimulus interval of 40 seconds and a peak temperature of 54 °C. They rated each trial on a VAS from 0 – 100, with 0 indicating no pain, and 100 indicating the maximum imaginable pain for each individual subject. The mean subjective pain ratings steadily declined from trial 1 to trial 7, with a mean value of 39.7 ± 24.95 across all participants in trial 1, and a mean value of 26.7 ± 22.78 in trial 7. However, only the decline from trial 1 to 2 was statistically significant, with $p = 0.0076$ and a Hedges G effect size of $g = 0.2$, indicating a small effect, i.e., a small decrease. The overall decline from trial 1 to trial 7 was also statistically significant ($p < 0.001$), with $g = 0.52$, indicating a medium effect (statistics not plotted in Figure 5). It is important to notice that we did not move the stimulation thermode after each stimulus, which may indicate that a habituation to the stimulus over time may has occurred.

As far as the EEG is concerned, one study found that CHEPS can also be recorded while not moving the stimulation thermode, with an even lower inter-stimulus interval of 8 – 12 s [52]. The majority of

studies we screened however, repositioned the thermode after each stimulus to avoid fatigue and habituation [53-56]. An early paper from 2007 concluded that human heat perception and CHEPS display signs of rapid habitation, and that the thermode should be moved after each stimulus [57]. They explicitly speak out against keeping the thermode at a fixed location throughout the study. This habituation is also visible in the decline of the subjective pain ratings in Figure 5. Although we have not tested this quantitatively, we found that moving the thermode resulted in more consistent results, a fixed thermode was also good enough to display CHEPS in the average spectrogram with 7 trials. This is further outlined by an example participant that I measured outside of the study presented in the paragraph. The participant was stimulated 12 times with a moving thermode on their volar forearm, and after each burst, the thermode was moved. Right afterwards, the participant was stimulated another 12 times with the thermode at a fixed position. The interstimulus interval (ISI) was set to 8 – 12 seconds in both cases. As it is shown in Figure 6, I was able to record an evoked potential when the thermode was moved, but merely anything was visible in the EEG when the thermode was kept at a fixed position at the same subject. Hence, as a second conclusion from our methodology establishment study, both from the subjective pain ratings as well as from the EEG, we decided to move the thermode after each stimulus from now on, even though we were overall able to record CHEPS.

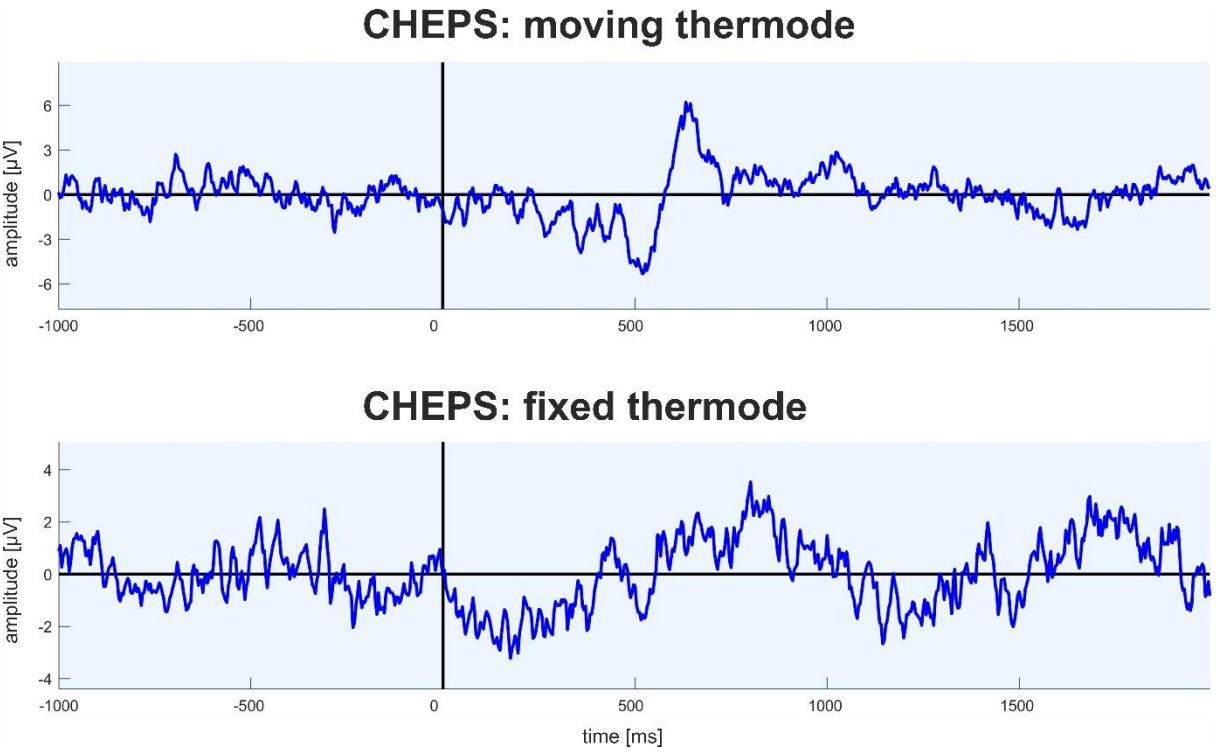

Figure 6: Vanishing of CHEPS in the same participant when the thermode is kept at a fixed position. The data is shown at electrode location Cz, with an average reference.

2.7 Pain thresholds and stimulus energy

During mechanical stimulation, the stimulus energy is defined as the stimulation force with which the pinprick is applied, during contact heat stimulation it is defined as the peak temperature of the stimulus, and during the electrical stimulation it is defined as the amperage/wattage of the stimulus. A common approach to its determination is the pain detection and tolerance threshold of an individual participant. Those thresholds have a huge variability and are different in each participant [58-60]. The pain detection threshold is defined as the threshold where just above it, the stimulus is described as painful by the participant. This translates to the subjective pain rating scale from the QST protocol [9] in the following way: a 0 out of 100 on the scale is described as no pain, mainly only the sensation of the stimulus. Once the stimulus develops an additional painful component which is described as e.g., sharp for mechanical pinprick stimulation, or burning for noxious contact heat stimulation, the value of the subjective pain rating must be increased to a value greater than 0. Just when this transition from a subjective pain rating from a value of 0 to a value greater than 0 happens, the current stimulus intensity is protocoled as the participants' individual pain detection threshold. The pain tolerance threshold increases the stimulus energy even further: it is the maximum stimulus intensity the patient is able to tolerate for a defined period of time [60]. In our case, we usually wanted to stimulate the patient multiple times at their pain tolerance threshold, so going up to a value of 100/100 on the subjective pain rating scale would have likely resulted in problems regarding the participant's compliance. For our observations, we usually defined the pain tolerance threshold as the stimulus intensity where a 60/100 on a subjective pain rating scale was reached, usually aware of the fact that the participant would likely tolerate even more.

Increasing the stimulus energy, i.e., its intensity, just a small notch above the pain detection threshold, or setting the stimulation energy so that pain just at the pain tolerance threshold was elicited ensures that the patient perceives the upcoming stimulus as painful. However, this method has one caveat: due to the high variability between different participants, it is impossible to administer constant stimulus energy to multiple different participants when choosing the method of administering noxious stimulation at a participant's individual pain tolerance threshold. When comparing groups, this would likely result in skewed results: the stimulus energy directly correlates with both the subjective pain perception, as well as the intensity of the response in the EEG [25]. To achieve a robust inter-group comparison of pain and its signatures in the EEG, constant stimulus energy that is perceived as painful by all participants has to be administered. This is a difficult task: even when choosing very high stimulus energies, there could always be participants with medical conditions or abnormal thresholds who perceive the stimulus as non-painful.

3 Summary of the results

3.1 Aims of the cumulative thesis

This thesis investigates the ability of the electroencephalogram (EEG) to measure, quantify, and represent pain and nociception. Pain is a highly subjective sensation and not necessarily the result of nociception. The characterization and treatment of pain is challenging because there are large differences in the described quality and quantity of pain between individuals. For this reason, there have been repeated attempts in recent decades to quantify nociception and, more importantly, pain in order to allow comparability of values between subjects or patients. Currently, the most valid methodology is a simple verbal or visual subjective pain scale, in which the subject or patient estimates his or her individual sensation by means of a number. This works for acute or chronic clinical pain, such as that occurring in response to injury or trauma, or for neuropathic pain. An extension of the simple subjective pain scale is Quantitative Sensory Testing (QST). This uses standardized nociceptive and painful stimuli that are subjectively rated by the subjects or patients. Normative data from a healthy comparison cohort makes it possible to evaluate the reactions of the test persons/patients with regards to the normal range. Due to interindividual variations, this range is quite large. Thus, sometimes only extreme cases are recognized as pathological. Understandably, the scientific community is searching for alternatives that could ideally function as a robust and reproducible biomarker for pain or as a supplement to the established pain scale.

It has long been known that certain somatosensory stimuli evoke a measurable response in the EEG. These stimuli can be of different quality, be it auditory, visual, or nociceptive or painful. They are all subject to certain requirements in terms of duration and intensity: only so-called "time-locked" stimuli can be analyzed, i.e., short stimuli whose onset is synchronized with the recording of the EEG with milliseconds of precision. Independently of the stimulus type, be it noxious/painful or not, the EEG response often has comparable features, with one of the main features being the N2P2 component that is analyzed in this manuscript both in the time domain as event related potentials (ERP) and in the frequency domain as event related spectral perturbation (ERSP). For painful stimuli, this raises the question of how pain-specific the response is, and to what extent it is affected by other non-pain-specific factors. Another requirement is that the stimuli are applied repeatedly. Only in this way is the evoked response visible in the EEG, as the basic physiological cortical activity cancels itself out due to the averaging effect.

The aim of the presented cumulative thesis was to examine under which conditions pain and nociception can be represented in the EEG, and to what extent the evoked signatures measured in the EEG are specific for pain. To this end, three studies were conducted and published, each with its own hypotheses. From the totality of the results, it was possible to draw a conclusion for the question whether the EEG in combination with standardized nociceptive or painful stimuli is a robust, reproducible, and holistic biomarker for pain.

3.2 Summary of the methodology establishment study

In the first study, the methodology of analyzing pain or nociception in the EEG was established. For this purpose, 21 healthy subjects of 18 years of age and older, of all genders, were recruited, who also served as a healthy control group in another study. Furthermore, different parameters were used for both EEG recording and standardized painful stimulation. In summary, all subjects underwent stimulation by means of noxious contact heat stimulation to record contact heat evoked potentials (CHEPS). In this case, the somatosensory stimulus referred to above was a short, noxious contact heat stimulus with a maximum peak temperature of 54°C. The stimulation energy was based on the normative data for QST: it can be assumed that a healthy person, regardless of age or gender, will perceive the stimulus as painful at a contact heat peak temperature of 50°C or higher. In addition, results from in vitro studies have shown that heat-sensitive receptors such as TRPV1 can be activated at temperatures as low as 45 °C. A total of 7 of these stimuli were applied to the underside of the dominant forearm, with an interval of 40 seconds between two stimuli. Subjects rated each stimulus on a verbal pain scale ranging from 0 (no pain) to 100 (individual maximum imaginable pain). The thermode was left at the same location throughout the stimulation. During the entire measurement, an EEG was recorded and the exact time of each stimulus onset was saved as a trigger in the EEG. The primary aim of the study was to find out whether the parameters we chose resulted in a visible evoked response in the EEG. As a secondary goal, we tried to investigate different parameters for EEG recordings as well as EEG analysis. In the methodlogy establishment study, we were able to use the chosen parameters to visualize the evoked response in the EEG to standardized painful contact heat stimuli in the amplitude-time spectrum and compared the amplitude of the response to the subjects' subjective pain ratings. The methodological findings from the study could be incorporated into the design of the other and future studies. This refers in particular to the type of stimulation as well as the recording and evaluation of the EEGs. In summary, 54 °C contact heat stimuli lead to a reproducible evoked response in the EEG in most subjects.

3.3 Summary of the project IMPACE

In the second study under the acronym IMPACE (Intraoperative Monitoring of Pain in the Clinical routine using the EEG), we included 17 patients undergoing a routine surgical procedure at the University Hospital Frankfurt. The aim of the study was to evaluate whether EEG is a suitable non-invasive method for intraoperative monitoring of pain, nociception and analgesia. For this purpose, it was investigated whether the evoked EEG signatures obtained in the methodology establishment study are still reproducible after the administration of clinical doses of the narcotic propofol and the analgesic remifantil (both after sole administration, "mono", and in combination). Instead of the contact heat stimuli from the methodology establishment study, painful constant current stimuli were used in this study, which we titrated until they were rated by the patients in an awake state on a subjective pain scale with a value of 60 out of 100. The patients were recruited as part of routine clinical practice and were all scheduled for low expected risk trauma surgery. The anesthetic regimen was controlled via target controlled infusion (TCI), in which effect organ concentrations of the drugs propofol and remifentanil are modeled based on gender, height, and weight. The target concentrations were based on the clinical standard of the University Hospital Frankfurt and were not adjusted for the outlined clinical observation. The study design differs significantly from that of a controlled clinical trial, as the main goal of clinical anesthesia is the prompt loss of consciousness, protective reflexes, and pain sensation. Overall, the patients were thus administered significantly higher doses of narcotics much more quickly, as this leads to desireable effects in the clinical routine.

The effects of a general anesthesia on the raw EEG, such as the anteriorization of the alphase rythms, are well described in literature. We successfully analyzed the data to confirm that our results align with literature. An excerpt from one sample subject is shown in Figure 7, but as this was a secondary objective, we didn’t include an in-depth analysis into the final manuscript. The figure outlines the anteriorization of alpha oscillations during the induction and maintenance of general anesthesia in an interactive flow between the different stages. It outlines the significant effect of hypnotic drugs such as propofol on the physiological brain oscillations during the awake state especially in the frontal and cortical regions.

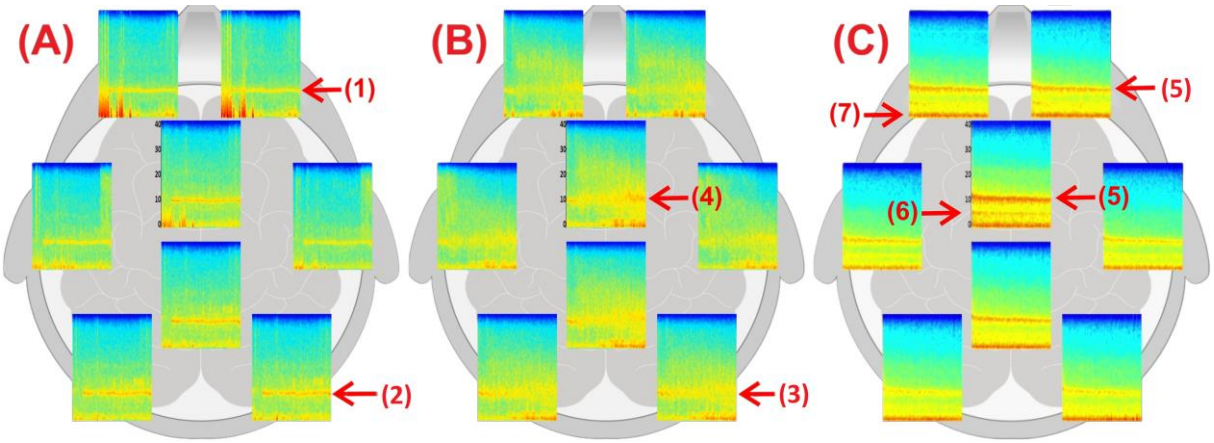

Figure 7: The changes in the EEG from the awake state (A) to the induction process (B) of general anesthesia to the steady state (C) of general anesthesia. During the awake state of a patient as seen in panel (A), the alpha band power in the frontal (1) and occipital (2) regions is rather small. In addition, the alpha band power in the (peri)-occipital regions (2) is dependent on the activity in the occipital cortex, i.e., it increases with the eyes closed. During the induction of general anesthesia in panel (B), the occipital alpha activity in (3) starts to shift towards the front of the head to the cortical regions, indicated by the beginning of a strong alpha band at (4). This process is called anteriorization, i.e., the shift from alpha activity from the occipital regions to the frontal/cortical regions. Panel (C) then shows the steady state of a general anesthesia, and the abovementioned "landmarks" of a good anesthesia: compared to (A), the alpha activity in the frontal/cortical regions is very dominant, indicated by the number (5). In addition, there is prominent theta (6) and delta (7) activity, both similar as important landmarks of a general anesthesia as induced by propofol and remifentanil]. The panels (A), (B) and (C) have been compiled using MATLAB, and the data from one volunteer that has been recorded through his anesthesiologic intervention in the IMPACE project.

As for our standardized noxious stimulation, the administration of remifentanil alone led to a non-significant reduction especially of the evoked N2-component in response to the painful electrical stimulus. Subjective pain scores also decreased nonsignificantly in comparison. As expected, the administration of propofol alone led to loss of consciousness (LOC), so that subjective pain scores could no longer be collected, and more importantly, to the complete disappearance of the evoked response in the EEG. After the administration of the combination of remifentanil and propofol in the context of stable general anesthesia, an EEG-based response could also no longer be derived. Also, the highly painful tetanic stimulus (1500 electric shocks within 30 seconds with a constant current of 50 mA) did not lead to a reproducible and robust change in the EEG, as it would be necessary for a biomarker. It can be concluded that propofol, which has no relevant analgesic properties, prevents the use of EEG as a biomarker for pain after standardized painful tonic stimulation. The derivation of any evoked responses in the clinical patient in both the time-amplitude spectrum and the time-frequency spectrum fails due to application of the narcotic. On the basis of our data, the reason for this effect can only be answered speculatively: the EEG responses presented in the manuscript are derived from the cortex, and a component of the pain response is the transmission of information from the thalamus to the sensory cortex via action potentials. If these action potentials are attenuated or abolished by substances that activate inhibitory neurons, no cortical potential can be derived. It has been shown

that propofol interferes with the communication between thalamus and cortex ("thalamocortical loop"), which, for example, also produces the characteristic frontal alpha oscillation during general anesthesia. However, the exact mechanism of action of propofol on this communication is not fully understoof. Even with an absent or impaired communication between the thalamus and the sensory cortex, a variety of other pain processes are continuously happening throughout the body, both centrally and peripherally. These processes are not recorded by our cortical EEG recordings. They include pain processing at circuit sites upstream of the thalamus or cortex such as the spinal cord, or pain reflexes. From our data, we conclude that the EEG maps a sub-process of pain processing. The EEG-based recording of the sub-process can be impaired by substances such as propofol. Thus, the EEG does not fulfill the requirement of a holistic and reproducible biomarker for pain during general anesthesia using propofol.

3.4 Summary of the project SPINE

After a short literature screening, we initiated the third study under the acronym SPINE (Sports Pain In EEG). We identified elite endurance athletes as a group of subjects whose processing and evaluation of pain is thought to differ from that of a normally/recreationally active (healthy) population. Existing literature suggests that competitive athletes become significantly more resilient to pain over the course of their careers. In recent literature, the pain thresholds of elite endurance athletes at which a stimulus is described as painful are higher than those of normally active controls. We aimed to analyze whether these differences are also revealed in our EEG signatures following standardized noxious stimulation. For this purpose, we recruited 26 elite endurance athletes who participate in one of the endurance leg sports rowing, triathlon, speed skating, or running at a competitive level with at least 15 training hours per week, as well as an age- and gender-matched normally/recreationally active control group with 26 participants, who have never performed more than 9 hours of training per week throughout their lives. As standardized painful stimulation, we recorded PEP and CHEPS, and applied painful mechanical stimuli using a pinprick stimulator in addition to the previously presented contact heat stimuli. This stimulator is also used in the QST test battery. In addition, we compared the endogenous pain modulatory capacities using Conditoned Pain Modulation (CPM) between the groups. In CPM, we applied a painful mechanical test stimulus using the pinprick and analyzed whether a painful conditioning stimulus (8 °C cold water bath) at another body site led to a reduction in the pain rating of the test stimulus. Throughout the study, an EEG was also recorded and analyzed using the parameters outlines in the methodology establishment study.

In summary, the subjective pain perception of the competitive athletes differed from that of the normally active control group only during CPM testing, but not at resting after standardized pain stimulation. In CPM, during the application of the conditioning stimulus (CS), the test stimulus was described as less painful as compared to the baseline only by the control group, but not by the competitive athletes. However, significant differences were revealed by the EEG: in our testing paradigms, the elite endurance athletes showed a significantly stronger evoked response in the somatosensory cortex to our stimuli as compared to the control group. This may be interpreted as a sign of an (early) central sensitization to nociception. However, in analogy to the results from other studies, we also know that the intensity of the evoked response in the EEG is not only determined by pain-specific factors. One of these factors is salience, i.e., the perception of the stimulus outside normal consciousness. The increased activation of the EEG signatures in the group of competitive athletes may indicate that the salience of a painful stimulus is significantly increased as compared to a control group. While this may likely indicate that elite endurance sports has no influence on the subjective pain ratings, we also acknowledged methodological limitations of our study designs as far as our choice of stimuli and statistics are concerned. Furthermore, our data may also indicate that our cohort of competitive athletes has a lower capacity for endogenous pain modulation, but this was most likely also influenced by our methodology: the choice of the pinprick test stimulus is not yet validated in conjunction with CPM testing, and our analysis revealed a significantly higher occurrence of pain in the athletes group in the past. Our data also revealed a difference in the subjective pain rating of the conditioning stimulus which was perceived significantly less painful by the athletes' group, thus triggering the endogenous pain modulatory system to a lesser extent and probably invalidating our CPM methodology. We conclude that the EEG shows early signs of a central sensitization, probably in conjunction with an altered stimulus salience. Our other results were limited by our methodology. Whether this conclusion leads to a role in the development of chronic pain in athletes, which is more common over the course of an athlete's career, cannot be answered from our data. Our data also showed again that the derived signatures cannot be used as surrogate parameters for pain perception, as there is no fully reproducible correlation and, again, factors like stimulus salience also play a role as per the recent literature. Eventually, the EEG may serve as a future tool to unmask signs of a central sensitization, as it is more sensitive than subjective pain testing.

3.5 Summary of the conclusions from the three manuscripts

Based on our results, it can be concluded that the EEG is not a holistic and robust biomarker for all pain-associated processes. In the clinical context, further studies should examine whether other stimulation techniques or even clinically persistent pain can be reproducibly mapped and quantified in the EEG. The short, tonic pain stimulation techniques we administered are determined, among other factors, also by the stimulus salience. Nevertheless, there are proven useful applications for the methods presented here, especially in pharmacological research, as well as in animal models or in the study of nonverbal groups such as newborn infants. For example, EEG in the form presented here can be combined with clinical examination, as reproducible results are obtained in controlled clinical trials of new analgesics. Also, as per the SPINE project, the EEG may unmask early signs of a central sensitization, which may not be captured by subjective pain testing in smaller sample sizes. The EEG can also be used as an indirect marker for the nociceptive system to diagnose conditions such as small fiber neuropathies. Whether increased or altered salience also affects the actual pain perception of a subject in the long term - as in competitive athletes, for example - must be investigated in further studies.

4 Concluding discussion and broader context of the thesis

4.1 On the origin of phase-locked EEG signatures after standardized noxious stimulation

The exact composition of the phase-locked and non-phase-locked signatures in the EEG following time-locked standardized noxious stimulation has been debated up to a certain extent in literature. Some publications see them as some sort of objective marker for the function of the nociceptive system, i.e., as a marker for the function of the small fiber function, and point towards a good correlation between the subjective pain ratings and the strength of the EEG response [61,56,26,62,63,28,64]. Other authors, while still acknowledging the fact that the EEG serves as an indirect readout of the function of the nociceptive system, propose a slightly different model: the strength of the response is not only dependent on the painfulness of the stimulus, but also on the stimulus salience, i.e., the ability/property of the stimulus to capture the participants attention in the surrounding environment [65,25,66]. The propose the EEG as a readout of the function of the somatosensory function of the "pain matrix" [65] that is not only modulated by the painfulness of an event, which is supported by our data. The EEG, in combination with recent advantages in computational analytical tools, also provides (limited) spatial information about the processes in the pain matrix. This is done by deconstructing the EEG into dipoles via independent component analysis as done in the methodology establishment study, and approximating the location of those dipoles via dipole source localization with the *DIPFIT* plugin for EEGLAB. The accuracy of this process is heavily dependent on the quality of the data that is fed to those algorithms: a (very) high-density EEG with at least 64, if not 128 or 256 channels is favorable, an exact electrode placement as per the 10-10 system is obligatory, and the individuals' head proportions need to be considered by creating a custom head model for each participant [67]. All these parameters increase the accuracy of the analysis, but the overall spatial accuracy of the EEG is still heavily inferior to the fMRI [68]. As the hardware that was used throughout the studies only allowed us to place only 64 recording electrodes and we had no hardware for the creation of a custom head model readily available, we refrained from performing a source localization from our data. However, the following figure will give a short insight into the capabilities of the EEG.

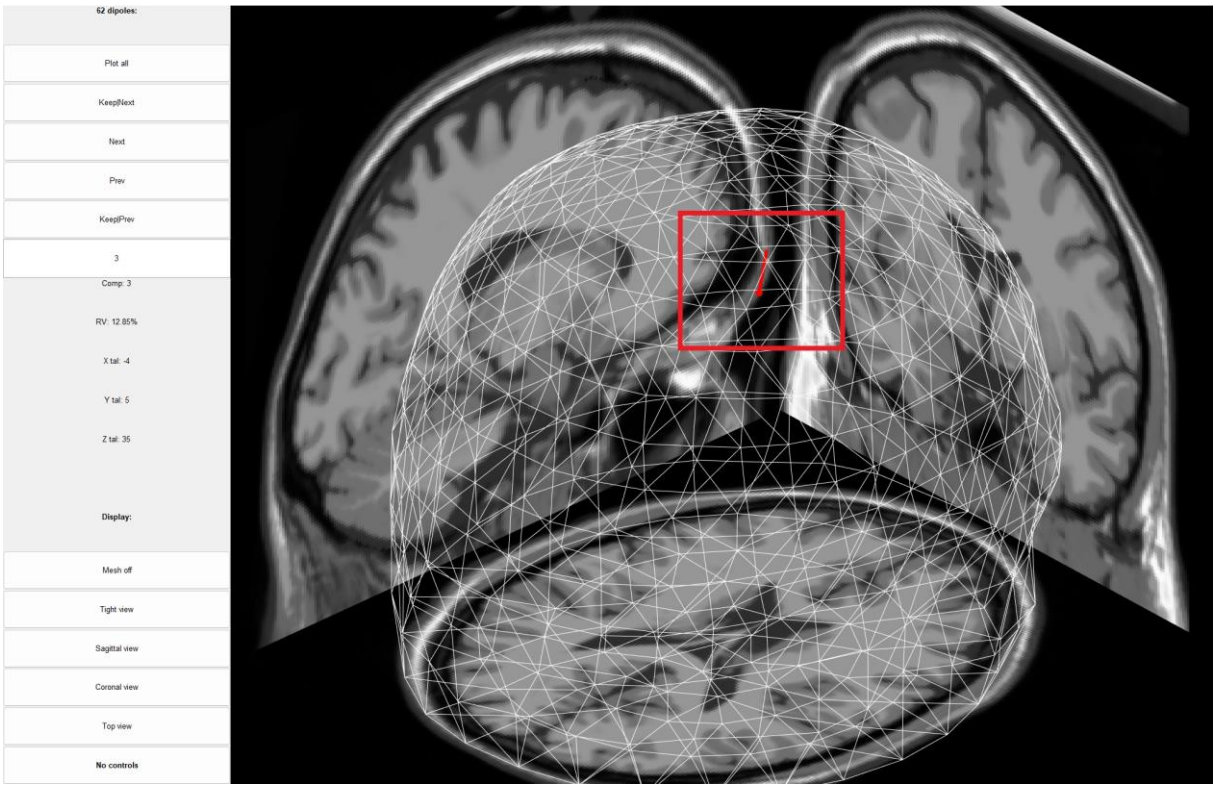

Figure 8: (Extracted from EEGLAB) an example of a dipole source localization of an independent EEG component evoked by standardized stimulation with noxious contact heat, generated in EEGLAB using the DIPFIT toolbox and a sample subject from the SPINE project. The DIPFIT algorithm estimates the dipole to be located in the anterior cingulate cortex (ACC) as per the Desikan-Killiany cortical atlas.

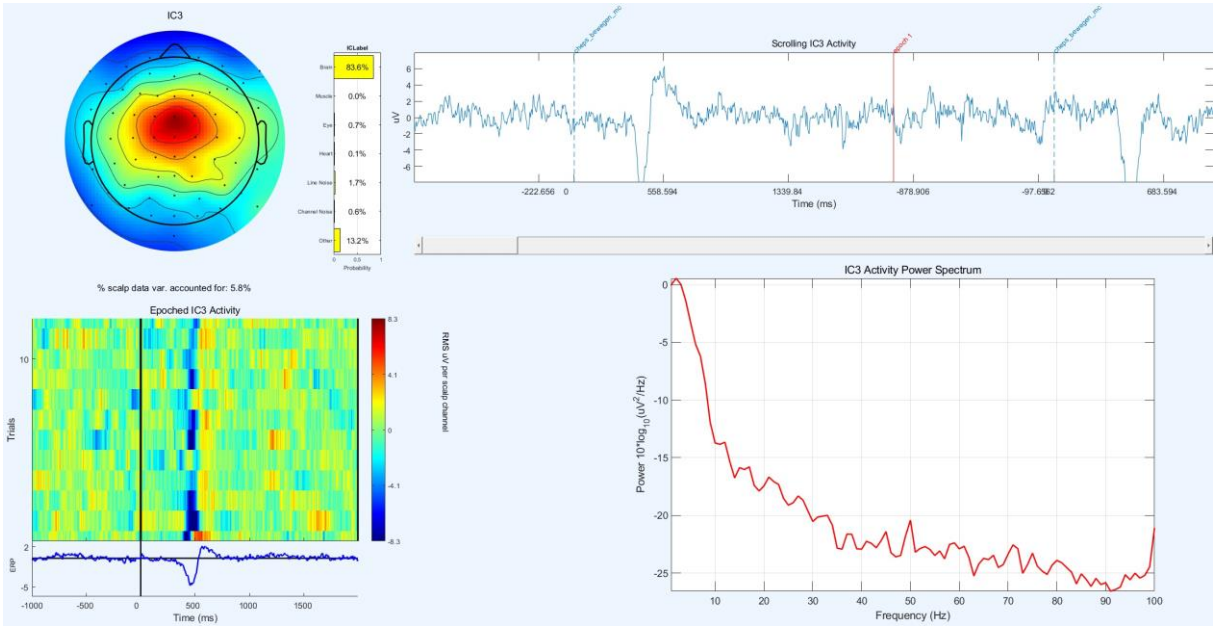

Figure 9: (Extracted from EEGLAB) The EEG characteristics, i.e., the spectral and voltage properties as well as 2D source information of the extracted independent component IC3, whose activation is shown to be directly above the Cz electrode. The ERP characteristics clearly show the N2P2-characteristics of the EEG signature that was chosen, and the EEGLAB plugin ICLabel, which is a deep-learning method for the automatic classification of independent EEG components, estimate the dipole to be generated with a 86.6% probability by the brain.

In Figure 8, I show an example of dipole fitting, i.e., an EEG source localization, of an individual healthy participant's phase-locked EEG response after time-locked noxious stimulation with noxious contact heat. The selection of the independent component was carried out manually with the help of the EEGLAB plugin *ICLabel*, which is a deep-learning assisted alogorithm for the automated classification if independent EEG components. The spectral and 2D-spatial characteristics of the chosen component are outlined in Figure 9, with the ERP information clearly indicating it to be a phase-locked N2P2 component that was evoked by time-locked standardized noxious contact heat stimulation. The participant was stimulated 12 times with a moving thermode and had a unisize 64 channel head cap for EEG recordings attached which was visually aligned to the Cz position as in our publications. The data was pre-processed as outlined in the SPINE publication (from which the data of this healthy control group participant was taken from) [4], deconstructed into individual components using ICA, and fitted to the head model using the EEGLAB plugin *DIPFIT* and its *autofit* function. The dipole location was estimated to be in the anterior cingulate cortex (ACC) as per the Desikan-Killiany cortical atlas, as prompted by the *DIPFIT* plugin. The ACC is part of the pain matrix [69], further underlining that our data indeed shows the processing of sensory information in these cortical/somatosensory parts of the brain. However, the results are somewhat inaccurate as per the limitations listed earlier, so this should be only taken as an indicator of the capabilities of an EEG analysis. We refrained from including that methodologically limited and probably inaccurate analysis into our publications.

Overall, it is likely that the EEG response is not a fully reproducible correlate of the subjective pain perception, i.e., it cannot serve as a surrogate marker, limiting its use cases in the clinical setting, although even spatial analysis confirms that processes in the pain matrix are captured [65]. We concluded the same from our data, as I will outline in the following paragraph.

4.2 Usefulness of the EEG as a biomarker for pain in our data

First and foremost, in the SPINE project, the correlation between the subjective pain ratings and the evoked signatures in the EEG as a response to standardized noxious stimulation is not given. This indicates that the signatures visible in the EEG are not exclusively specific to pain. Admittedly, in the IMPACE project, this was the case – supposedly (we cannot be sure) our patients were not experiencing pain during general anesthesia, and the EEG signatures vanished. However, this was reached using Propofol, a drug that itself has no analgesic effects [70-72]. This means that if we were to trust absent evoked EEG signatures as an indicator that the patient is pain-free, we could conduct major surgical procedures using only Propofol without an analgesic. This is neither advisable nor standard in any clinic [73], although small procedures that only require mild sedation are routinely carried out using propofol mono [74].

Those findings contradict somewhat the narrative of some of the available literature, that outline the correlation between EEG potentials following standardized noxious stimulation and subjective pain perception [62,75-78,61]. One article from 2002 argues that the attention towards the noxious stimulus does not influence the magnitude of the response in the EEG [76]. More recent literature paints the EEG differently. André Mouraux and Gian Domenico Iannetti argue that for being clinically utilizable, a biomarker has to be reliable, robust, and be repetitively recordable in every individual participant [24]. They argue that the EEG response to transient painful stimuli is overstated, as the same response can also be evoked by salient non-painful stimuli such as auditory or visual stimulation, making EEG-based pain biomarkers non-efficient for the clinical context [24]. In our studies, we only performed noxious stimulation, and did not administer other stimulation methods such as auditory or visual stimuli. However, for example regarding mechanical PinPrick stimulation, there are studies available that show that also non-noxious mechanical pinprick stimulation evokes more or less the same responses as a pinprick of a force that should be perceived as painful in participants [50,79]. This alone is an indicator that the EEG signatures after standardized stimulation, be it noxious, auditory, or visual, are not pain-specific, which is also what our data in the IMPACE and SPINE project has shown, and which is also what more recent literature suggests [25,66,24]. Thus, the recent approaches in the last decade to publish normative EEG-based data for CHEPS is an interesting approach for the testing of the nociceptive system in a clinical setting [26,63,28], which does not come without its drawbacks. It cannot replace tools that are based on subjective pain ratings such as QST and subjective pain, which offer a holistic overview of the subjective feeling of pain, whereas the EEG only details one sub-process and is also influences by non-pain-specific factors such as the salience [65]. Hence, rather than seeking a substitute for QST, the EEG may have a niche application when specifically testing the peripheral nociceptive pathways. Given that there are quite a few conditions that alter the way the peripheral nervous system works, ranging from neuroinflammation due to Myalgic Encephalomyelitis [80] to unspecific Small Fiber Neuropathy [81], a targeted use of standardized noxious stimulation and the EEG may be a cheap and quick measure to test the function of the nociceptive system. Then again, it is also influenced by a variety of other factors that are not nociceptive-specific, and research still is sparse on which EEG signatures after standardized noxious stimulation are to be expected from a "healthy and normal" peripheral nociceptive system. This may be tackled by published normative data, which is the same approach as in the QST panel where a big population of healthy volunteers serve as reference data [9,82]. However, some authors such as Mouraux and Iannetti specifically argue against its efficacy, and we have shown that the EEG is not a holistic biomarker of the processing of pain but rather details only the process of the somatosensory processing of pain.

All in all, the EEG and an evoked response to a standardized noxious stimulus is an important tool in characterizing, analyzing, and quantifying the effects of pain in the brain, especially given its upsides as far as the resolution in time are concerned – but only when a specific set of standardized noxious stimuli is used. Due to the lack of high-detail spatial information however, it has to be complemented by tools such as the fMRI to add detailed information about the location of the processing of the noxious information. Recent research has already aimed at combining the fMRI, the EEG, and standardized experimental evoked pain [56]. This combination comes at other costs: as mentioned in the introduction, the EEG itself is already prone to be distorted by artifacts, and adding an fMRI machine to the study setup surely does not help, i.e., a variety of fMRI-based artifacts are introduced into the data, sometimes rendering results unusable [83]. Last but not least, I cannot imagine a doctors' office that combines the EEG, the fMRI, and a CHEPS device to measure pain; in fact, in that case it would be easier to ask the patient how they are feeling, just as stated in the definition of pain by McCaffery in the introduction of this thesis.

4.3 EEG parameters

Our chosen parameters for the EEG analysis throughout the studies resulted in very good overall EEG data. In detail, this was the downsampling of the data to 256 Hz, a bandpass filter between 1 Hz and 40 Hz, and an ASR cutoff parameter of 20. In all other studies except the methodology establishment study, we amended the bandpass filter settings: the higher edge of the bandpass, i.e., the lowpass filter, was moved to 100 Hz to capture higher frequencies as well. To deal with line noise, we decided to utilize the EEGLAB plugin *cleanline* [40]. No statistical comparison was made which of those two options had better results in the way of removing line noise, but the results were still satisfactory without line noise interfering with our data analysis.

4.4 Discussing our methods for EEG analysis: ERSP vs. ERP

While we chose to only evaluate the event-related potentials in the amplitude-time spectrum in the methodology establishment study, we took the analysis of the EEG one step further in the IMPACE and SPINE project and calculated the inter-trial coherence and the event-related spectral perturbation. Those measures basically had the advantage to uncover a high-frequency response in the SPINE project that was not visible when simply analyzing the amplitude-time spectrum of the EEG. As the EEG lacks spatial information, we were not able to determine where this response came from, and only hypothesized on existing literature that it correlates with the attention and distraction from the noxious stimulus. Our CPM testing showed that it correlates with clinical pain in a different way than

the common evoked N2P2 response, i.e., the response that is usually analyzed in the amplitude-time spectrum of the EEG. A future approach to evaluate the origin of that response would be either very high-density EEG (256 channels) with a custom head model to increase the spatial resolution and precision of the EEG analysis or combine the EEG with the fMRI as mentioned in the previous paragraph.

In any case, from our analysis there is no reason to solely rely on a simple ERP analysis, as most of the studies researching noxious stimulation in the EEG do. Then again, analyzing the ERSP is computationally more demanding, and even more parameters have to be chosen. As there is no "standard" protocol for the EEG analysis of nociceptive stimuli, it may be best to rely on the amplitude-time spectrum for normative data [26,63,28], and use the ERSP and the ITC to answer scientific questions about the processes in different frequency bands. I highly doubt that a clinician would spend their time learning about the details of an elaborate frequency analysis of the EEG using the ERSP and ITC. Hence, if the scientific community wants to foster the clinical use of the EEG in pain research as outlined in the previous paragraph, normative data in the amplitude-time spectrum is a good start, and relative frequency-related changes as presented in this thesis should be understood as a scientific, non-clinical approach to the methodology.

4.5 The high frequency ERSP response in the SPINE project

In the SPINE project, the ERSP analysis revealed an additional response that we did not discuss in the published manuscript. Furthermore, this response would also not be visible in a conventional ERP analysis in the amplitude-time spectrum, as higher frequencies usually have a low amplitude and are difficult to quantify in an ERP spectrum. This high frequency response after pinprick stimulation with a low degree of phase locking in the higher frequency regions, approximately 500-600 ms after the stimulus onset, that is mentioned in the results part of the SPINE project, cannot be characterized sufficiently with our analysis as a pain-specific or somatosensory-specific response. The response was significantly affected by the conditioning stimulus in our CPM model in both groups. Another study also evaluated the non-phase-locked EEG response to pinprick stimulation but relied on pinpricks that applied less force (64 mN and 96 mN) than our 512 mN pinprick stimulation device [79]; their results do not show the same oscillations in the higher frequency regions. A different study utilizing painful cutaneous laser stimuli shows a similar response in the lower gamma frequency regions (i.e., regions around 35 – 40 Hz) and address the hypothesis that this gamma-band spectral activity is related to attention vs. distraction from the painful stimulus [84]. Hence, a part of the response to our test stimulus in the higher frequency regions may directly represent the perception, or the awareness, to

a painful stimulus and can be heavily modulated by a conditioning stimulus. Significant differences between our two groups in that frequency region can also be seen during the resting state recordings. A third study utilizing the same electrical stimulation device as ours, but with differences in the stimulation pattern, also examined the same ERSP response and found a decrease of those high-frequency oscillations by applying an ice pack as CS [85]. While they hypothesize that high-frequency responses seem to reflect pain intensity [86], they did not evaluate the exact response approximately 500 ms after the stimulus onset, but at an earlier time point. This leaves us with the possibility that in our data, the said high-frequency response is a muscular response to the stimulus which we would normally expect to observe in the higher frequency regions[87,88]. However, as there is some evidence in our data that the response might be pain-related, future research needs to examine its origin.

4.6 Discussing our models for standardized noxious stimulation

In our study, we repetitively administered brief, tonic noxious stimuli. The reason for this is the easy visibility of those stimuli in the EEG, with the downside being that those stimuli are only somewhat of an adequate measure of acute pain, but not a good measure of chronic, neuropathic, inflammatory, or visceral pain. Recording and analyzing chronic pain, longer noxious stimulation, or deep inflammatory pain in the EEG is a whole base of research for itself [89,86,15,90-93]. The evidence of the correlation between the EEG and "actual" clinical pain is surprisingly limited [94,15]. It has been shown that persistent chronic pain may reduce the evoked response in the EEG after experimentally induced pain, similar to the concept of the EEG being an adequate measure to test for abnormalities in the peripheral nociceptive system as stated in the previous paragraph [15,95-97]. This, however, adds nothing new to the already stated findings: if the peripheral fibers are somehow damaged, "less" noxious information will be transmitted to the brain, and less information is processed, resulting in "less" power in the processing unit and a lesser activation of the corresponding signatures in the EEG.

A second approach is the analysis of certain frequency bands that are affected as a result of chronic pain [15]. Without digging deeper into the available literature, the results about the long-term effects of chronic pain on certain frequency bands have indeed been researched, but the evidence of the EEG being a useful clinical marker to analyze and quantify is sparse, with heterogenous and sometimes contradicting results [15]. This is something we also observed during the IMPACE project: a group of authors has been hypothesizing that alpha dropouts, and delta or beta arousal may be an indicator of intraoperative nociception during clinical anesthesia [98]. We tried to replicate those results in our project using standardized noxious stimulation and failed. Again, this does not mean that things like alpha dropout do not exist. I rather speculate that nociception and pain are simply very heterogenous,

and there is no simple universal rule of how nociception and pain look like in the EEG, especially given different types of noxious stimulation. In our case, we already hypothesized in the discussion of the IMPACE project that our method of standardized electrical noxious stimulation was simply unsuitable to evoke intraoperative nociception.

Given the fact that the baseline EEG itself is already influenced by such a variety of factors, it would be very naïve to assume that the process of nociception, and then the subjective and emotional assessment of the nociceptive event commonly known as pain, can easily and objectively be quantified with such a simple setup as the EEG. There is a reason that the research of the evoked response in all three studies presented here has something in common: the noxious stimulus is repetitively administered. The reason for this is that if the EEG is averaged over our 12 trials in the SPINE project, the background EEG cancels out. Then, and only then, the actual evoked response is visible. If a researcher is somewhat skilled in EEG research (again, like the author of this thesis, of course) and the stimulus is strong enough, they might be able to spot a single trial noxious stimulus in the EEG as well. As this method lacks robustness and the certainty to work "every time", it does not constitute a biomarker.

All in all, there is a need for more research that analyzes clinical pain in the context of the EEG, and if it can be measured reproducibly and robustly. Although there is some heterogenous literature available, the body of literature is not sufficient to establish the EEG as a biomarker for chronic pain, and novel signatures aside from brief tonic noxious stimulation and evoked responses need to be established [15].

4.7 Future outlook: possible use cases of the EEG in pain research

Given the fact that the EEG is an indirect measure of the nociceptive system [25], albeit dependent on a variety of other factors, there are some more use cases to the exact methodology that is presented in this thesis. This is especially true for subject populations who cannot communicate their subjective pain adequately. We researched one of those populations in this thesis (patients undergoing general anesthesia) in the IMPACE project, with little success. However, other populations have been studied in literature where the EEG can be applied as per the methodology outlined in this thesis. One of them are nonverbal, newborn infants [17], where the exact methodology that has been outlined in this thesis has been applied with success. The other big population of interest are animals [99,18,100]. In animal research, a lot of surrogate parameters are used, e.g., for mice, the paw withdrawal reflex to a (noxious) stimulus can be interpreted as a reaction to nociception and thus a result of the unpleasant feeling of pain [101]. I am currently involved in a project at TU Munich that researches the ability of

broiler chicken embryos to react to noxious stimulation before hatching using in-ovo EEG. The project aims to determine in which stage of growth a chicken embryo, which usually takes 21 days after fertilization until it hatches, is able to react to nociception and if the common evoked EEG signatures in this thesis can also be recorded in the animal after standardized noxious stimulation. The main problem is not the stimulation and the recording of the EEG. In fact, it is pretty easy to attach and record an EEG to a chicken embryo, even if it didn't hatch yet [102]. The biggest issue with the project is the differentiation between nociception and pain. We can only roughly estimate which stimulus energy is necessary to evoke a response in the animal and thus, even if we were able to record the evoked signatures presented in this manuscript, we could only be sure that the brain of the growing broiler chicken embryo somehow reacts to the nociceptive-specific stimulus, but we cannot be sure if the chicken embryo is subjected to the feeling of pain.

Of course, our standardized noxious stimulation is paired with well-known methodology in animal research such as the withdrawal reflex. But no chicken would ever be able to give us a number on a pain rating scale – and in this case, this is somewhat not necessary. We can speculate that once the nociceptive stimulus reaches the according area in the brain, and is processed in the respective somatosensory structures, the chicken should be able to receive peripheral stimuli through the pathway to its brain and be able to process those stimuli. Eventually, this still only answers questions about nociception but not pain. But given the fact that billions of chickens are killed each year because they grow up to be male and new methods about the in-ovo determination of chicken embryos are continuously developed, we could answer the question at which stage a chicken embryo would be able to process nociceptive information. We could then assume that before that development stage, killing the embryo in the egg would probably be at least painless, as the nociceptive information either does not reach the brain or the nociceptive event is not processed.

Lastly, the EEG in combination with the evoked responses to noxious stimulation can easily be integrated into the research of drugs that aim at helping patients suffering from pain [21]. There is plenty of literature available that spans back to the 1980s and backs up the EEG as a viable tool to research the effects of analgesics; a reproducible effect of said analgesics on the evoked response in the EEG is a decrease in the power or the amplitude of the N2P2 component [21,103-113]. This is in line with our (non-significant) decrease of the N-wave, or an overall (non-significant) decrease in ERP amplitude, after standardized noxious electrical stimulation, as discussed in the IMPACE project. Hence, the EEG in combination with the methodology of brief, standardized noxious stimulation as presented in this thesis is indeed not a biomarker for pain but can be regarded as a biomarker for the analgesic effect of a drug [21]. Admittedly, in a clinical study for a new analgesic, where healthy participants undergo standardized noxious stimulation, the clinical end point should always be the

reduction in the subjective pain of the patient, as this is what really matters as outlined in the introduction to this thesis. But given the use cases in the previous paragraphs such as special non-verbal cohorts, the EEG may have its raison d'être. Eventually, it can also be used in combination with the QST and other measures of subjective pain testing, when testing new analgesic drugs, as the information extracted from the EEG may help to foster the understanding of the pharmacodynamics of the drugs on the level of central processing. Also, as we learned from the IMPACE and SPINE project, no other CNS-acting drugs should be administered, and environmental influences should be kept to a minimum during EEG testing, but that should be taken as given during a controlled clinical trial of a novel drug.

In another currently ongoing project, I am researching the effects of alcohol on the EEG signatures after standardized noxious stimulation. Alcohol is known to decrease pain and it is speculated that this effect stems from increasing the capacities of the endogenous pain modulatory system [114,115]. While it is currently too early to conclude what effects we can expect in the EEG data, this summarizes the presented use case of the EEG: substances that are known to have effects on the subjective pain perception can further be objectively characterized using the EEG in combination with the well-known stimulation parameters. Then, using the EEG signatures presented in this thesis, further conclusions can be drawn on which signatures in the EEG are affected, and what this implies for the mode of action.

5 Conclusion

I conclude that future research needs to focus more on the clinical applicability and pertinence if the EEG shall be established as a method to objectively quantify acute and chronic pain. The chosen methods of standardized noxious stimulation in this thesis only touch the surface of experimentally induced pain, and do not represent the actual real-world scenario of (chronic) pain, from which millions of people around the world suffer [116]. In this thesis, in the SPINE project, we presented one of the multiple use cases in which brief standardized tonic noxious stimulation in combination with the EEG helped to foster the understanding of nociceptive processing in a specific cohort of participants, i.e., elite endurance athletes. From that, we conclude one of the possible use cases of the EEG: it may serve as an early detection method for central sensitization, at is more robust than subjective pain testing, even in smaller subject groups. Furthermore, it may also be used to evaluate a subject's salience to a noxious stimulus. In the IMPACE project, we deemed the EEG unsuitable to be used in conjunction with a depth-of-anesthesia monitor for the monitoring of intraoperative nociceptive events during clinical anesthesia, which is another use case that has been suggested in the literature. There are a variety of applications where the combination of the EEG and standardized noxious stimulation can be applied, and this thesis gives an overview of the methodological advantages and pitfalls. Eventually, the EEG should not be considered as a holistic biomarker for pain, but the past decades have shown that in pain research, it has and will always have its raison d'être, unless an easier, more cost-efficient, or more precise technology may take its place.

6 Abbreviations

ACC = anterior cingulate cortex

ADC = analog digital converter

ASR = artifact subspace reconstruction

CHEPS = contact heat evoked potentials

CPM = conditioned pain modulation

Cz = central midline as per the 10-20 EEG electrode placement system

DOA = depth of anesthesia

EEG = electroencephalography

ERP = event related potential

ERSP = event related spectral perturbation

fMRI = functional magnetic resonance imaging

IMPACE = Individualized monitoring of pain using a holistic multi-channel EEG approach

ICA = independent component analysis

IC = independent component

IMPACE = Intraoperative Monitoring of Pain in the Clinical routine using the EEG

ISI = inter stimulus interval

ITC = inter trial coherence

LOR = loss-of-responsiveness

MCS = Mental Component Score

n.s. = not significant

n.a. = not applicable

PCES = painful cutaneous electrical stimulation

PCS = Physical Component Score

PEP = pinprick evoked potentials

QST = quantitative sensory testing

RAM = random access memory

SPINE = Sports Pain In EEG

VAS = Visual Analog Scale

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METHODS
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Application of Referencing Techniques in EEG-Based Recordings of Contact Heat Evoked Potentials (CHEPS)

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Evoked potentials in the amplitude-time spectrum of the electroencephalogram are commonly used to assess the extent of brain responses to stimulation with noxious contact heat. The magnitude of the *N*- and *P*-waves are used as a semi-objective measure of the response to the painful stimulus: the higher the magnitude, the more painful the stimulus has been perceived. The strength of the *N-P*-wave response is also largely dependent on the chosen reference electrode site. The goal of this study was to examine which reference technique excels both in practical and theoretical terms when analyzing noxious contact heat evoked potentials (CHEPS) in the amplitude-time spectrum. We recruited 21 subjects (10 male, 11 female, mean age of 55.79 years). We applied seven noxious contact heat stimuli using two temperatures, 51°C, and 54°C, to each subject. During EEG analysis, we aimed to identify the referencing technique which produces the highest *N*-wave and *P*-wave amplitudes with as little artifactual influence as possible. For this purpose, we applied the following six referencing techniques: mathematically linked A1/A2 (earlobes), average reference, REST, AFz, Pz, and mathematically linked PO7/PO8. We evaluated how these techniques impact the *N-P* amplitudes of CHEPS based on our data from healthy subjects. Considering all factors, we found that mathematically linked earlobes to be the ideal referencing site to use when displaying and evaluating CHEPS in the amplitude-time spectrum.

Keywords: electroencephalography (EEG), EEG reference choices, event-related potentials (ERP), independent component analysis (ICA), pain research, contact heat evoked potentials (CHEPS)

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INTRODUCTION

To assess an individual's sensitivity to pain, standardized painful stimuli that activate the A and C fibers in the human body are applied to the surface of the skin. A variety of techniques, including thermal, laser, mechanical (e.g., flat tip probes), or electrical stimulation are commonly used to evoke pain responses in the subject's brain

(Iannetti et al., 2013; Oh et al., 2015; Wulf et al., 2017; Albu and Meagher, 2019; Lefaucheur, 2019). The extent of the response strongly correlates with how "painful" the individual subjectively rates the applied stimulus; a good correlation between a visual analog scale (VAS) pain score communicated by the subject and the amplitude of the noxious contact heat evoked potentials (CHEPS) can usually be detected (Roberts et al., 2008). Parameters of interest are, for example, *N*- and *P*-waves [the lowest negative (N) or highest positive (P) peak in the average EEG amplitude-time spectrum after the stimulus] or the *N*-wave or *P*-wave delay (the latency from the stimulus to the respective peak). From a physiological point of view, the brain response to the noxious contact heat stimulus that is detectable in the electroencephalogram (EEG) is not merely described by a single feature of the subject's brain activity but is somewhat derived from a complex combination of components that, if put together, result in the recorded amplitude (Ploner and May, 2018). Thus, the amplitude or the power of the response is also highly dependent on a variety of other factors which include, but is not limited to: (i) the individual's subjective perception of how "painful" the stimulus is; (ii) the vigilance of the subject; (iii) the stimulation technique; (iv) the time interval between the stimuli and habituation occurring; (v) the exact placement of the recording electrodes; (vi) preprocessing steps (e.g., bandpass filtering) and any artifact rejection in the EEG analysis; and (vii) the reference site. Furthermore, it is feasible that the CHEPS response to the same stimulus by the same subject may vary when measured by different laboratories or by different scientists. After performing the experiments, the EEG data is usually processed for further analysis, hence, decisions regarding preprocessing, artifact rejection, and the choice of reference site need to be made. This study will demonstrate that the reference electrode site influences the CHEPS amplitude in the amplitude-time spectrum of the EEG. Thus, the referencing technique to be used for an EEG-based analysis of CHEPS should be carefully chosen during the study design phase. CHEPS between subject groups or different studies cannot be compared when different referencing techniques have been used. As referencing is a linear step, it can, fortunately, be changed after the EEG recording, irrespective of which reference site was initially defined (Dong et al., 2017). Theoretically, the reference site should be chosen as an "electrically neutral point" somewhere on the subject's body, however, this is practically impossible (Kayser and Tenke, 2010). This study evaluated which reference site—considering the theoretical requirements—provides the best results in the recording and analysis of CHEPS.

MATERIALS AND EQUIPMENT

Parameters of Interest and Selection of Reference Sites

We extracted the following parameters from our EEG-data that can be analyzed after stimulation with noxious contact heat:

(i) *N*-wave: the lowest negative peak in the average EEG waveform in the amplitude-time spectrum across all seven trials for each stimulation temperature found between 250 ms to 550 ms after stimulation onset.
(ii) *P*-wave: the highest positive peak in the average EEG waveform in the amplitude-time spectrum across all seven trials for each stimulation temperature found between 550 ms to 800 ms after stimulation onset.
(iii) *N*-*P*-wave: the difference in amplitude between the *N*-wave and the *P*-wave.
(iv) *N*-wave delay: the latency between the onset of stimulation and *N*-wave.
(v) *P*-wave delay: the latency between the onset of stimulation and *P*-wave.
(vi) *N*-*P*-wave duration: the duration/latency between the *N*-wave peak and the *P*-wave peak.

We identified the following reference sites which we then further evaluated in this study for suitability in measuring CHEPS:

(i) Mathematically linked earlobes A1/A2, where the mathematical average of the earlobe electrodes (A1 and A2), according to the 10-10-system, was calculated and then subtracted from each individual EEG recording electrode (Jurcak et al., 2007).
(ii) AFz, where the single frontal electrode at position AFz, according to the 10-10-system, was used as a reference site (Jurcak et al., 2007).
(iii) Pz, where the central parietal electrode Pz, according to the 10-10-system, was used as a reference site (Jurcak et al., 2007).
(iv) Average reference, where the mathematical average of all EEG recording electrodes was calculated and then subtracted from each individual EEG recording electrode (Nunez, 2010).
(v) Reference Electrode Standardization Technique (REST), or Infinity Reference, where a virtual reference location at infinity was calculated (Yao, 2001). This reference-free approach assumed that the source of the EEG signal at each electrode location was the same, regardless of which reference was used. For REST, a lead field matrix needed to be calculated, that, in a linear relationship, routed the specific source to its measuring (electrode) location on the head. In practice, REST relied heavily on the head model that was used to calculate the reference signal; this could lead to biases and inaccuracies if the head model did not perfectly match the real-world scenario (Nunez, 2010).
(vi) Mathematically linked PO7/PO8, where the mathematical average of the parietal-occipital electrodes PO7 and PO8, according to the 10–10-system, was calculated and then subtracted from each individual EEG recording electrode (Jurcak et al., 2007).

We chose to analyze the above six reference sites and referencing techniques as three of them, A1/A2, average, and REST are commonly used in EEG practice (Yao, 2001), while the other three reference sites and referencing techniques, frontal (AFz), parietal (Pz) and parietal-occipital (PO7/PO8), although they are not commonly used in EEG practice, we thought to evaluate their

suitability for CHEPS as, to our knowledge, they have not been evaluated in the literature.

Subjects

For this study, we included 21 healthy subjects with a minimum age of 18 years: 10 males and 11 females. The data used in this manuscript is a subset taken out of a larger study and was collected between June 2018 and September 2019. The larger study was designed to investigate the level of small fiber neuropathy of patients with rheumatoid arthritis compared to a healthy control group. The EEG data subset used in this manuscript was extracted to answer a question of a purely technical nature (the ideal referencing technique to use when analyzing and displaying CHEPS), whereas the original study tests for a clinical hypothesis regarding differences in the somatosensory profile of patients with rheumatoid arthritis compared to a healthy control group. The clinical hypothesis of the original study does not collide or overlap with the technical hypothesis presented in this manuscript in any form. Additional data from the original study (e.g., the results of conditioned pain modulation or the results of quantitative sensory testing) and the data of the patients with rheumatoid arthritis is not presented in this publication as it is not needed to corroborate the hypothesis in this manuscript. At the time of publication of this manuscript, the original study had not been published.

We obtained written consent from all the participants. The local ethics committee approved the study procedures in a written statement (Ethics Committee Department of Medicine Goethe University Frankfurt, reference number 245/17). Furthermore, we conformed to the standards set by the Declaration of Helsinki. We asked the subjects to avoid taking any pain medication for five days before the commencement of the study visit. Exclusion criteria were the use of antidepressant medication, a history of alcohol abuse, and the presence of chronic pain or neuropathic diseases.

Painful Stimuli

We outlined the study flow and stimulation pattern in **Figure 1**. We used the technique of CHEPS to apply painful stimuli to the left forearm of the subjects; seven stimuli were applied at 51°C and seven stimuli at 54°C using a MEDOC PATHWAY Pain and Sensory Evaluation System (Medoc Limited, Ramat Yishai, Israel). The temperature baseline of the thermal stimulation device was 32°C; this increased to the stimulation temperature of 51°C or 54°C at a rate of 70°C/s. We set the inter-stimulus interval to 40 s to minimize habituation (Bromm and Scharein, 1982). The thermode of the PATHWAY system was circular, with a diameter of 27 mm.

EEG Recordings

Each study took place with the subject sitting, in a quiet room. The investigators equipped each subject with an EEG cap (g.Tec g.GAMMAcap^2; Guger Technologies, Schiedlberg, Austria), which incorporated 21 active EEG electrodes (g.Tec g.SCARABEO) attached to a multichannel amplifier (g.Tec g.HIamp). The electrodes were placed in the standard configuration, following the 10-10 system; these were evenly distributed over the surface of the head. The ground electrode was attached to the subject's forearm. We placed the Cz electrode midway between the nasion (the most anterior point on the nose) and inion (the squamous part of the occipital bone) and midway between both tragi (the small pointed eminence, visible on the external part of the ear). The use of active EEG electrodes guaranteed an exceptionally low output impedance, below 1 Ω, so that artifacts from the movement of the electrode cables were minimized (Metting van Rijn et al., 1990). We recorded the raw EEG using the g.Recorder software from g.TEC with a sample rate of 512 Hz. For visualization purposes only, we applied a high-pass filter at 1 Hz and a low-pass filter at 30 Hz. We set AFz as the initial reference site and stored the raw EEG recordings electronically.

METHODS

EEG Analysis

We used EEGLAB, a MATLAB-based toolbox (The MathWorks Inc., Natick, MA, USA) for data preprocessing (Delorme and Makeig, 2004). We down-sampled the EEG to 256 Hz for data reduction using the EEGLAB's *pop_resample* function. This function utilizes the resample-function from the MATLAB signal processing toolbox that automatically applies the necessary low-pass filter. Moreover, we high-pass filtered the data at 1 Hz (cutoff frequency: 0.5 Hz) and additionally low-pass filtered with a passband-edge at 40 Hz (cutoff frequency: 45 Hz) to eliminate 50 Hz line noise. For both bandpass filters, we used the EEGLAB *pop_eegfiltnew* function that applies a zero-phase bandpass filter to avoid phase shift. For preprocessing, we referenced the datasets to mathematically linked earlobes (A1/A2) by using the mathematical average of electrodes A1 and A2 as the reference signal for all other electrodes.

After adding the electrode locations to the datasets, we used Artifact Subspace Reconstruction (ASR) to detect and remove malfunctioning channels and to clean noisy data (Chang et al., 2018). We removed EEG channels if they did not correlate by at least 80% with their neighboring channels, for example, as a result of electrode displacement. In our case, our parameter selection led to a rejection of an average of 0.2 channels per dataset; the channel of interest for data inspection (Cz) was not rejected. We then interpolated all removed channels using spherical spline interpolation for the sole purpose of avoiding bias in the datasets that were later re-referenced to average or subjected to REST. For artifact rejection, we set the tolerance parameter for ASR to 20 (Chang et al., 2018). Thus, the variance of large-amplitude artifactual components (defined by PCA via the algorithm) is allowed up to a value of 20, compared to "clean" calibration data (i.e., the cleanest part of the recorded EEG data as defined by the algorithm; Mullen et al., 2015). This setting appeared to present a reasonable point of balance between rejecting data frames and correcting artifacts and eye-related components. The EEG dataset was then split into epochs using the 5V trigger from our thermal sensory testing device with a time range from −1 to +2 s around every event containing

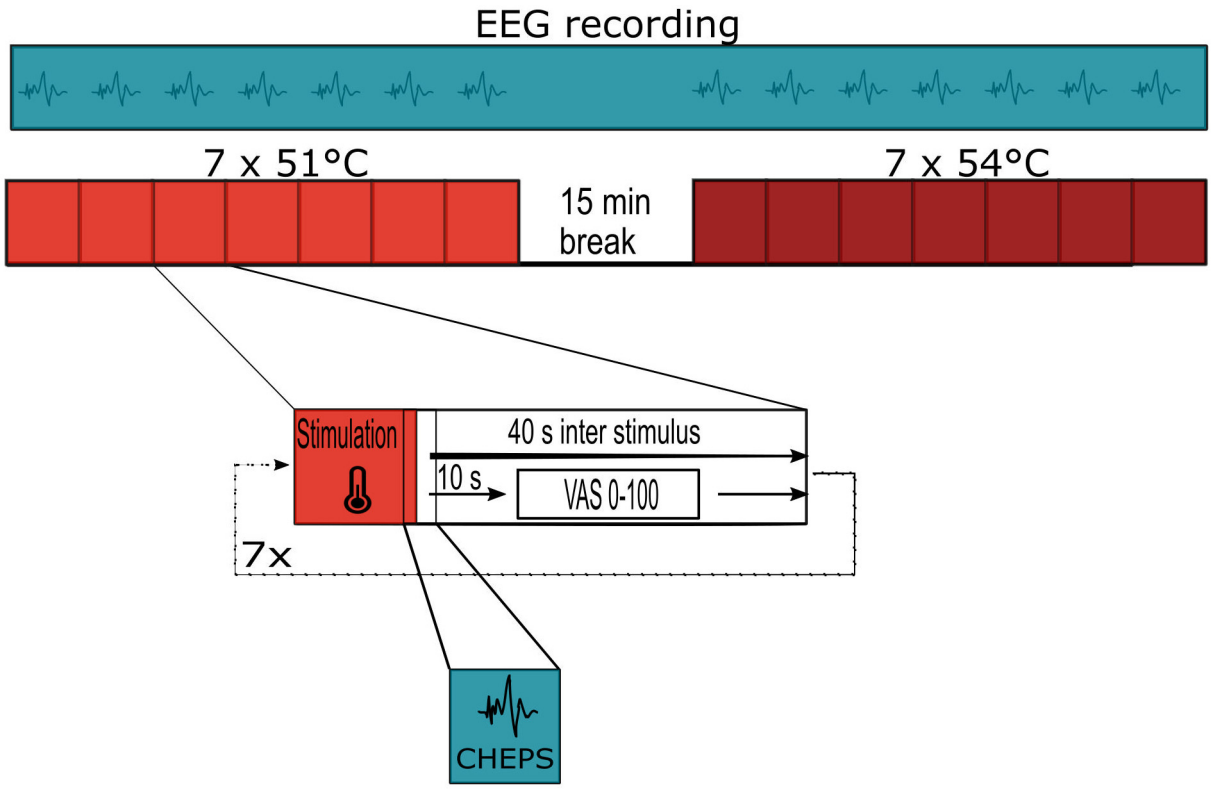

FIGURE 1 | Study flow and stimulation pattern for each subject.

painful stimuli. This epoch range covers the whole duration of the stimulation; this takes approximately 629 ms from the baseline temperature (32°C) to the peak temperature (54°C) and back to the baseline temperature (32°C), with the peak temperature (54°C) being reached approximately 315 ms after stimulation onset.

For comparison, we created six different groups of datasets that solely differed in the definition of the reference. We kept one group of datasets at the A1/A2 reference while re-referencing the other datasets to AFz, Pz, average reference, REST (Dong et al., 2017), or mathematically linked PO7/PO8 (i.e., the mathematical average of electrodes PO7 and PO8). We then visualized and post-processed the epochs using MATLAB's built-in functions. For calculation of the power frequency values, we used the EEGLAB *spectopo*-function that utilizes the *pwelch*-function of the MATLAB signal processing toolbox.

Independent Component Analysis

We applied Independent Component Analysis (ICA) on the datasets with the reference set to AFz, A1/A2, average, and REST, using the integrated EEGLAB-function *runica* (Makeig et al., 2004a,b; Delorme et al., 2012; Pion-Tonachini et al., 2019). The rank for ICA decomposition was decreased by 1, accordingly, when we rejected an electrode or changed the reference of the dataset to REST or average.

Clustering approaches using EEGLAB's *kmeans()* algorithm yielded in highly variable results. Hence, we decided to manually select ICs according to their event-related activity patterns to obtain functionally consistent clusters. We determined if a component was caused only by the painful contact heat stimulation according to its ERP waveform across trials with the help of the EEGLAB plugin *ICLabel* (Pion-Tonachini et al., 2019). Selection criteria were a visible deactivation and/or activation of the component with suitable latency around 500 ms, a visible peak in the delta region (<6 Hz) in the frequency diagram, and a visible *N*- and/or *P*-wave in the ERP spectrum. Scalp maps of the components were ignored during selection to avoid statistical double-dipping (Kriegeskorte et al., 2009). The independent components were evaluated in a blinded fashion: the IC images were provided with a four-digit code by author BA and then evaluated by author MA according to the mentioned criteria. MA was blinded to the subject code, IC scalp maps, and the IC number. We then clustered the manually selected components using EEGLAB's *STUDY* function. If we selected more than one component per subject, we assigned the component with the highest amplitude to the cluster and discarded all others for further evaluation.

Statistical Analyses

We applied different statistical approaches to fully describe our data. To present visually the EEG reaction to the stimuli,

we used a five-point moving mean to smooth the response. Because of our rather small sample size, we decided to apply non-parametric tests for inferential statistics. For an overview of group differences between the different referencing approaches, we applied the Friedman test using the MATLAB *friedman* function. For the inference statistics, the significance level was set to $p < 0.05$. For *Post hoc* analysis, we calculated the Wilcoxon signed-rank test with a Bonferroni correction. For comparison of the average scalp maps, we used the Wilcoxon rank-sum test with significance thresholds of 0.05, 0.01, and 0.001. We have only reported results as being significant if we observed a cluster of significant results, similar to the idea of cluster-based permutation tests (Sassenhagen and Draschkow, 2019).

RESULTS

Overview

One female subject expressed discomfort while wearing the EEG cap throughout the study; as the study protocol was not finished, we excluded this subject's data for analysis. All remaining 20 subjects (10 male, mean age 56.30 ± 14.66 years; 10 female, mean age 54.90 ± 14.40 years) showed visible *N*- and *P*-waves at both stimulation settings (51°C and 54°C stimulation temperatures) in one or more stimulation epochs. We visually compared the average CHEPS signal obtained with the 51°C and 54°C stimulation temperatures. We observed comparable reactions in terms of *N*-wave delay, *P*-wave delay, *N*-*P*-wave duration, and overall ERP waveform, but lower magnitudes for the *N*-*P*-wave at 51°C when compared to the 54°C stimulation temperature. Therefore, we have presented the findings from the 54°C experiments in the main text. For completeness, we have presented the average ERP waveforms at the 51°C stimulation temperature in **Supplementary Figure 1**.

Influence of the Referencing Technique on the EEG Stimulus-Response

We observed the highest average *P*-peak amplitude with the A1/A2 electrodes set as the reference site, while the lowest average *N*-peak amplitude was observed with the AFz electrode set as the reference site. Both the average reference and REST showed smaller average *N*- and *P*-wave amplitudes with smaller standard deviation windows (gray areas in **Figure 2**) compared to the A1/A2 reference. The PO7/PO8 reference electrodes showed rather small visible *N*- and *P*-peaks with an overall distorted waveform, increased standard deviation, and visible alpha waveforms (i.e., a visible EEG signal with a frequency between 8–12 Hz) compared to the EEG signals with other reference electrode sites or techniques. The use of the Pz reference led to no identifiable *N*- and *P*-peaks. **Figure 2** shows the CHEPS waveforms at our designated Cz electrode site with the applied moving mean for the different EEG reference settings. **Supplementary Table 1** contains detailed amplitude information.

After visually inspecting **Figure 2**, we discarded Pz and PO7/PO8 as possible reference sites for the evaluation of CHEPS and so these were not considered for further analyses. With regards to Pz as the reference site, we could not properly identify N and P peaks because the EEG signal appeared too close to the baseline. Concerning PO7/PO8 as the reference site, although we calculated *N*- and *P*-values for every subject in **Table 1** and the *N*- and *P*-wave delays in **Table 2**, we did not evaluate the reference site further, as the CHEPS waveform in **Figure 2** appeared distorted, overall.

When analyzing the amplitudes in the EEG response, we found significant differences between the average reference, A1/A2, REST, and AFz reference settings, as displayed in **Figure 3**. The AFz setting led to significantly higher amplitudes in the *N*-peak when compared to the average reference. We also observed a significantly higher *P*-peak with A1/A2 as the references when compared to the average or REST referencing technique. Interestingly, the *P*-peak of the CHEPS waveform with the REST referencing technique was significantly higher than the *P*-peak with the average reference, although the difference in absolute terms is scarcely noticeable. Thus, although our testing revealed a "statistically significant difference", this should not be taken as advice that there is indeed a relevant difference in CHEPS waveform between those two referencing techniques. In practical terms, both average and REST may perform equally in terms of the observed response (Amrhein et al., 2019).

The spectral power of the response also heavily depended on the choice of the reference point. We have presented the spectral power for the five chosen reference sites from 0–20 Hz in **Figure 4**. We do not show spectral graphs for the Pz reference site due to the reasons outlined above. For comparison, we present in **Figure 4** the spectral graph for PO7/PO8 set as a reference site to emphasize the alpha noise (visible parts of the EEG signal with a frequency of 8–12 Hz) that occurs when choosing occipital reference sites. The CHEPS signal with PO7/PO8 set as the reference site shows the highest average peak in the alpha region (8–12 Hz) that statistically differs from all other reference sites, except for the earlobes reference point ($p < 0.05$). This statistical difference in the power spectrum is also observable in the amplitude-time spectrum via visual inspection.

In **Figure 4**, the Friedman test indicated a significant difference in the comparison of the five plotted graphs for all frequencies between 0 and 20 Hz. The *Post hoc* testing revealed that the power of the distribution between the A1/A2 reference and the PO7/PO8 reference, as well as between the REST and the AFz reference, was not significantly different in most frequency ranges ($p > 0.05$).

Scalp Maps of Independent Components

For the different reference sites, we found the following numbers of independent components (ICs) that could be directly allocated to our painful stimulation:

- A1/A2 reference: 14 independent components, see **Figure 5**.
- average reference: 12 independent components, see **Figure 6**.
- REST reference: 11 independent components, see **Figure 7**.
- AFz reference: 12 independent components, see **Figure 8**.

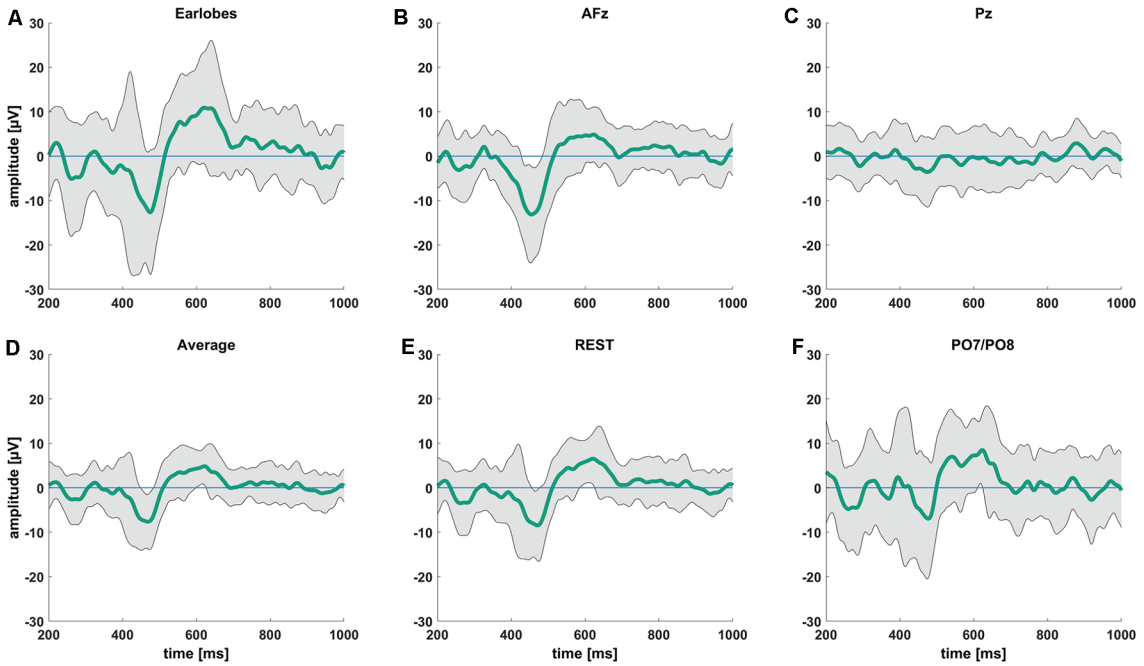

FIGURE 2 | Average contact heat evoked potentials (CHEPS) waveforms from 200 ms to 1,000 ms following the stimulus at 54°C stimulation temperature. The green line indicates the average amplitude at the Cz electrode location with **(A)** mathematically linked earlobes A1/A2, **(B)** AFz, **(C)** Pz, **(D)** average, **(E)** REST, and **(F)** mathematically linked PO7/PO8 as the reference sites.

We present the scalp map of every selected component for each reference site and their average scalp map in **Figures 5–8**. We statistically compared the average scalp maps in **Figure 9** in a binary fashion, using the Wilcoxon rank-sum test with significance thresholds of 0.05, 0.01, and 0.001; we did not observe any clusters of statistical differences at any thresholds between the REST, average, and AFz reference sites. Consequently, we have not shown the statistical comparison

TABLE 1 | *N*-, *P*-, and *N-P*-wave amplitudes for every reference site for the 54°C stimulation temperature and their respective standard deviations.

	***N*-wave amplitude [μV]**	***P*-wave amplitude [μV]**	***N-P*-wave [μV]**	**Percentage of amplitude compared to A1/A2 (assigning 100% to A1/A2) [%]**
A1/A2	−25.13 ± 15.08	21.83 ± 7.81	46.96 ± 19.25	100
AFz	−17.58 ± 9.42	13.12 ± 5.70	30.71 ± 12.38	68.87 ± 29.23
Pz	No value	No value	No value	No value
Average	−13.11 ± 6.33	10.09 ± 3.80	23.20 ± 8.40	49.96 ± 9.76
Rest	−15.50 ± 8.52	12.78 ± 4.50	28.28 ± 10.98	60.32 ± 7.08
PO7/PO8	−19.46 ± 10.59	19.38 ± 8.32	38.85 ± 14.41	88.10 ± 25.69

The data have been calculated for every subject individually, using a window from 250 ms to 550 ms following the stimulus to detect the lowest peak (N-wave) and a window from 550 ms to 800 ms to detect the highest peak (P-wave). The amplitude of the N-P-wave, as a percentage of amplitude at the earlobes (assigned 100%), is also presented. The N-P-wave value for every subject is presented in the supplement.

TABLE 2 | *N*- and *P*-wave delays and *N-P*-wave duration for every reference site for the 54°C stimulation temperature and their respective standard deviations.

	***N*-wave delay [ms]**	***P*-wave delay [ms]**	***N-P*-wave duration [ms]**
Earlobes	431.25 ± 74.19	641.80 ± 77.52	210.55 ± 98.85
AFz	432.42 ± 73.92	661.52 ± 92.81	229.10 ± 114.43
Pz	No value	No value	No value
Average	418.75 ± 80.87	666.99 ± 83.07	248.24 ± 105.57
Rest	433.20 ± 66.12	641.60 ± 77.92	208.40 ± 94.89
PO7/PO8	376.76 ± 87.75	622.46 ± 76.46	245.70 ± 106.08

The data have been calculated for every subject individually, using a window from 250 ms to 550 ms following the stimulus to detect the lowest peak (N-wave) and a window from 550 ms to 800 ms to detect the highest peak (P-wave).

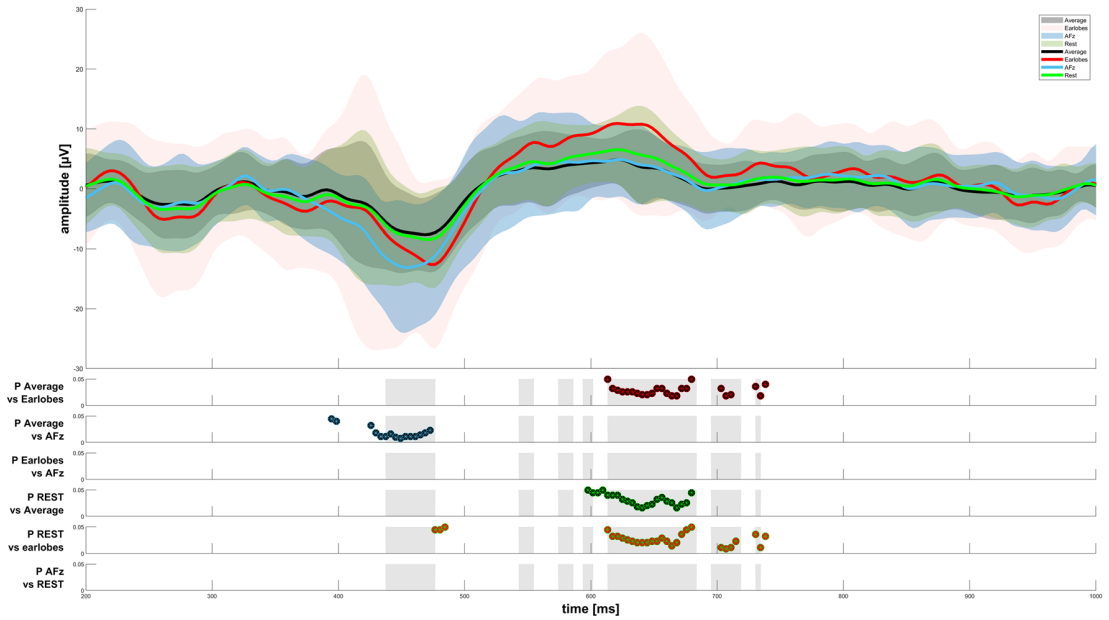

FIGURE 3 | CHEPS graphs for four reference sites. The results of the Friedman test were plotted as gray areas in the second half of the figure. *Post hoc* tests with a significance level $p < 0.05$ have been plotted for every reference site comparison.

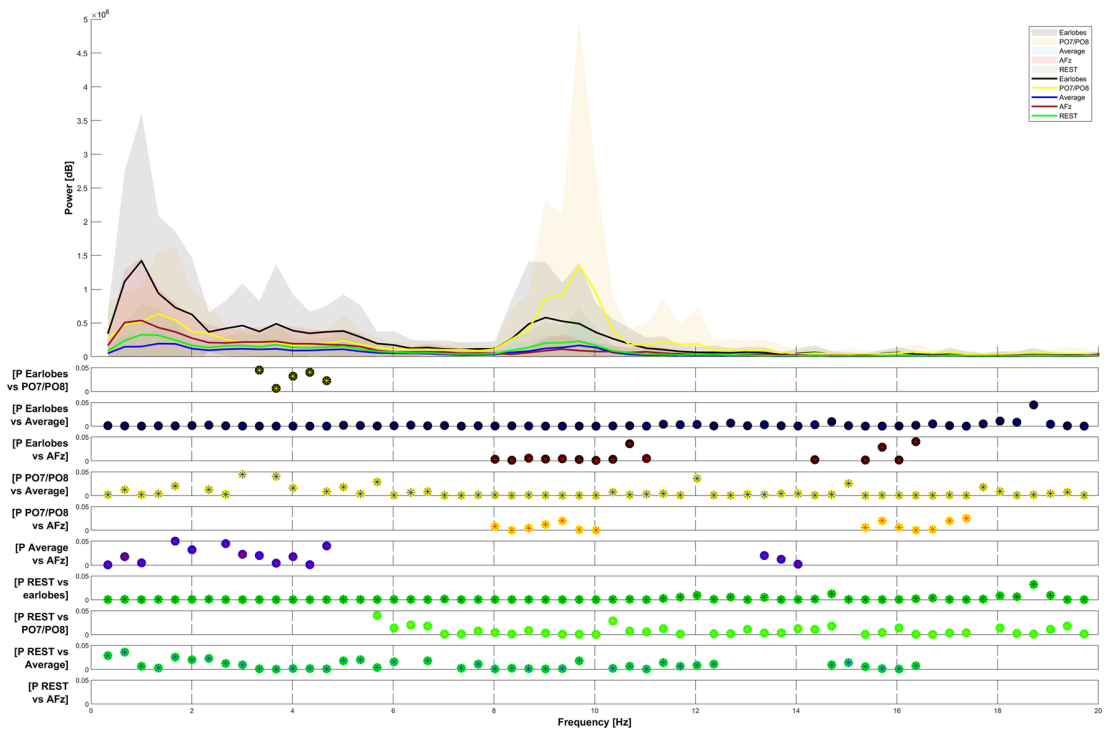

FIGURE 4 | Frequency-power plots for five selected reference sites. The Friedman test shows significance ($p < 0.05$) for all observed differences. *Post hoc* analysis (Wilcoxon signed-rank with Bonferroni correction) has been plotted for data pairs in the lower half of the figure.

of these reference sites. Concerning the A1/A2 reference, we observed that this reference site exhibited significant differences at all thresholds compared to the other three reference sites. While the activation of the noxious contact heat-related component between A1/A2 and AFz as the reference sites only exhibited significant differences in the frontal head region,

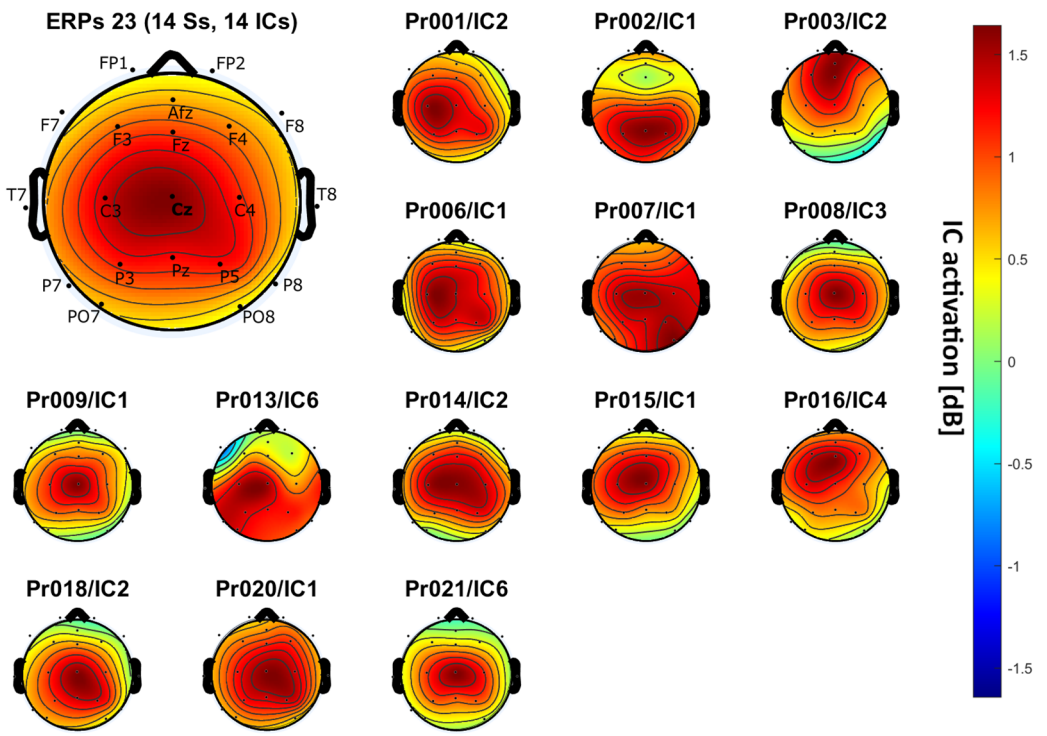

FIGURE 5 | Scalp activation for the selected independent components (ICs) for 14 subjects with A1/A2 as the reference site. The color-coding indicates the activation of the components in dB. The average activation is plotted in the top-left corner, including detailed electrode locations.

significant differences between A1/A2 and REST or average, respectively, are particularly evident in a circular fashion around the whole head.

As far as the average and REST referencing techniques are concerned, the extent of noxious CHEPS (i.e., the magnitude of the *N*- and *P*-waves) depends on the number of electrodes used for measurements and where they are placed. According to **Figures 6**, **7**, the CHEPS amplitude would rise if more electrodes were placed on the outer regions of the head (as those electrodes do not pick up the specific CHEPS activation). Subsequently, if more electrodes were placed next to Cz, a region, where still a reasonable level of CHEPS IC activation is picked up, then setting the reference to the average or REST referencing technique would result in lower amplitudes of the CHEPS waveform. Hence, when measuring noxious CHEPS following our experimental setup and selecting the REST or average as referencing technique, the exact positioning and the number of electrodes used are key factors for the strength of the resulting response (mainly the *N*-wave and *P*-wave magnitudes).

We have presented the spectral power of the pain-related clustered ICs with the A1/A2 referencing point in **Figure 10**. On average, we observed the highest absolute power in the lower frequency regions (<6 Hz) with peaks around 1 Hz and 3 Hz. Based on this fact, our settings for high-pass filtering (which was set to 1 Hz) probably also influence the CHEPS waveform and amplitude. Our results are, thus, only applicable to our chosen filter settings (1 Hz passband-edge). We wish to highlight that other common physiological EEG characteristics, such as alpha and beta waves above 6 Hz, can be seen as physiological artifacts that tend to distort waveforms if those signals are recorded either at the reference site or the measurement site.

DISCUSSION

We hypothesized that single (AFz or Pz) or dual (PO7/PO8 or A1/A2) reference electrode locations would have practical advantages over referencing techniques that require more electrodes that are evenly distributed over the head surface. Examples for the latter are both average and REST, with REST also requiring the computation of a lead field matrix. High-density layouts are, however, not necessary requirements for measuring CHEPS, so we evaluated if one of the single or dual-electrode locations excels when analyzing CHEPS in the amplitude-time spectrum of the EEG.

As described in the results, we observed poor responses for the PO7/PO8 reference site and the Pz reference setting. Regarding Pz as the reference site, **Figures 5–8** show that the Pz electrode always records a high amount of the pain-related independent component, regardless of which reference was chosen. This has led to the conclusion that Pz also appears to record a certain

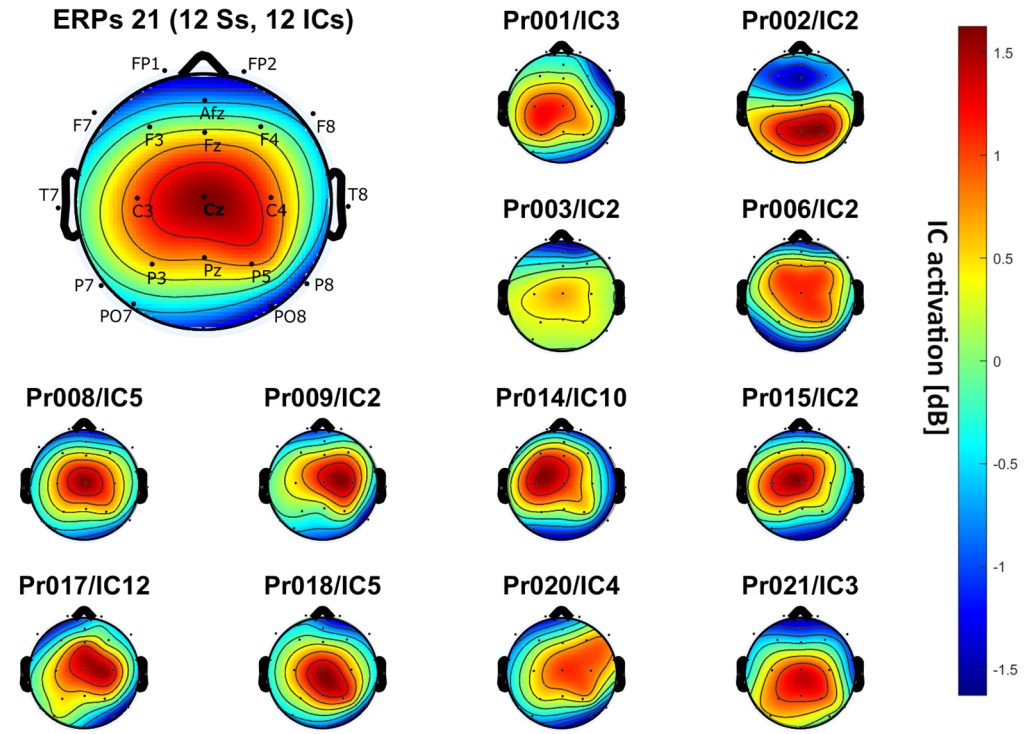

FIGURE 6 | Scalp activation for the selected ICs for 12 subjects with average reference as referencing technique. The color-coding indicates the activation of the components in dB. The average activation is plotted in the top-left corner, including detailed electrode locations.

amount of the CHEPS amplitude so that when referencing Cz vs. Pz, the overall CHEPS amplitude is mathematically canceled out. As a conclusion, Pz should not be chosen as a reference site in studies that aim to record painful CHEPS.

In **Figure 4**, we outlined that the reference site PO7/PO8 leads to the greatest amount of alpha power in the data. This alpha power is visible, even in the average EEG amplitude-time spectrum in **Figure 2** (graph F), and distorts the CHEPS waveform in a manner that renders identification of the *N*-wave and *P*-wave demanding. The alpha power increases due to activity in the primary visual cortex when subjects close their eyes (Britton et al., 2016). In conclusion, PO7/PO8 should not be used as a reference site to avoid having increased amounts of physiological alpha noise present in the data.

AFz as a frontal reference site commonly picks up a high amount of eye blink artifacts that share the same frequency characteristics as the CHEPS waveforms in the regions below 6 Hz (Dimigen, 2020). In our study, AFz worked reasonably well as a reference site as we used ASR to clean up the ocular artifacts to a certain extent. However, no artifact rejection mechanism is perfect. In the average CHEPS waveform with AFz as the reference, we were not able to determine how much noise by eye blinks was still present in the data, even after artifact rejection. Therefore, we would not recommend AFz as a frontal reference site for the CHEPS experiments.

Following visual analysis of **Figures 2**, **3**, we concluded that the average and REST referencing techniques both worked reasonably well to display noxious CHEPS in the averaged EEG amplitude-time spectrum. These referencing techniques require an electrode layout that covers the whole head and is evenly distributed, however, the extensive layout of electrodes is not a mandatory requirement when investigating CHEPS. On the other hand, for further analysis, such as Dipole Source Localization, the REST or average referencing techniques might be necessary (Trujillo et al., 2017). There have been attempts to converge protocols to reference-free techniques in recent literature, although it has been highlighted that no reference can be ideal for all EEG recordings (Kayser and Tenke, 2010). As far as our test sample showed, modern techniques such as REST are not necessary for a simple research of noxious CHEPS in the amplitude-time spectrum, as other options may perform better. Practical reasons (such as the number of electrodes required) and the ease of the intercomparison of results between studies should be considered when designing an experiment and selecting a referencing technique; nonetheless, the theoretical advantages of some techniques have been highlighted in recent studies (Yao et al., 2019). Overall, although the use of the average and REST referencing techniques result in visible CHEPS amplitudes, their pitfalls, in terms of practicability and ICA performance, at least in our case, mean that they cannot be generally recommended

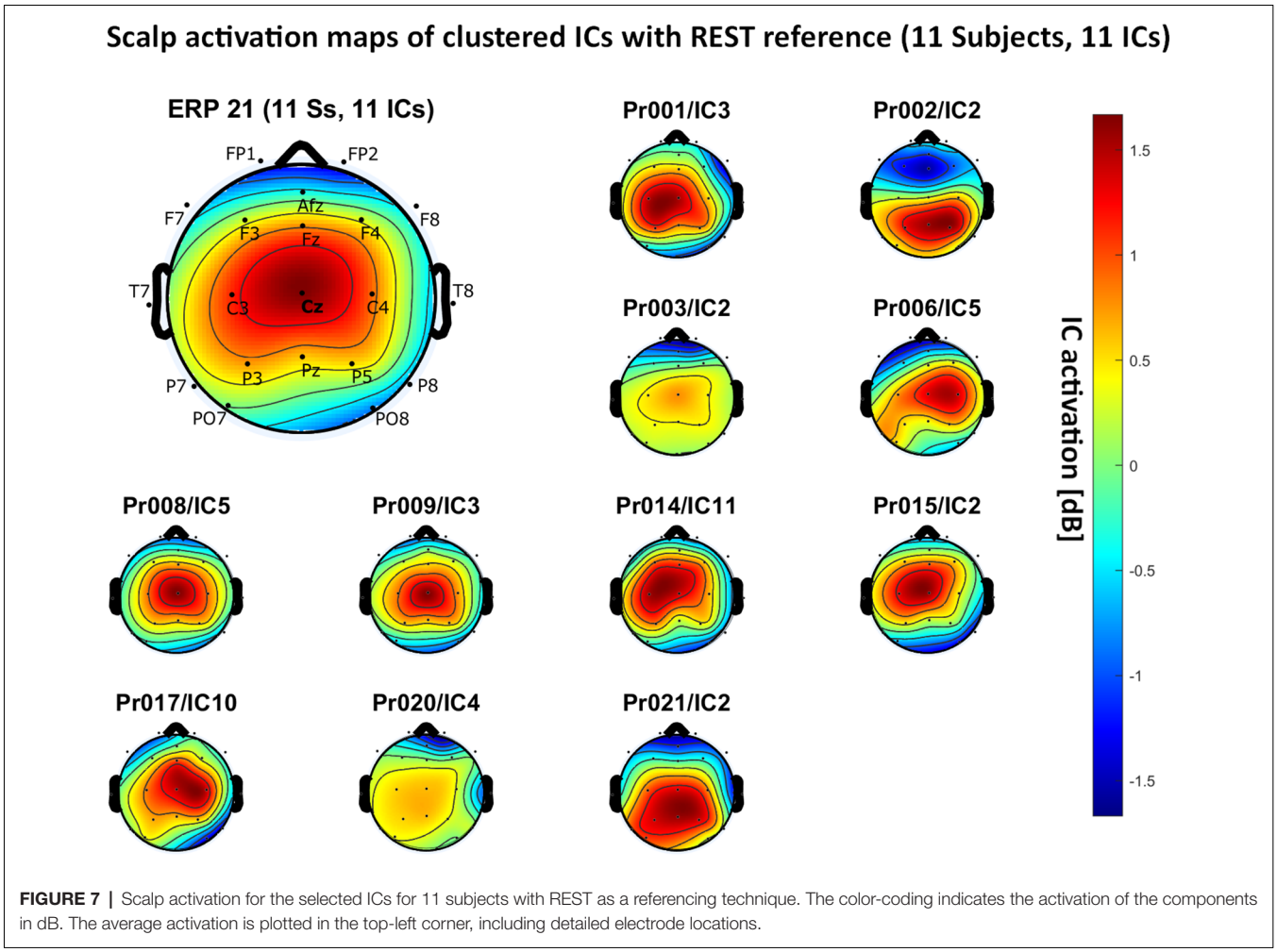

FIGURE 7 | Scalp activation for the selected ICs for 11 subjects with REST as a referencing technique. The color-coding indicates the activation of the components in dB. The average activation is plotted in the top-left corner, including detailed electrode locations.

if the aim of the study is simply to analyze the amplitude-time spectrum of the EEG.

Figures 5–8 also point out that Cz is the electrode location where, on average, the signal of the noxious contact heat-related ICs is the highest, regardless of which reference site is chosen. A visual interpretation of the average scalp heat map in those figures reveals that the average IC activation power at the Cz electrode location has the highest magnitude (around 1.5 dB), compared to the IC activation power at every other electrode location. For example, the power at electrode location Fz, Pz, C3, and C4, which in our electrode layout were the locations closest to Cz, tended to fluctuate around 1 dB. As higher IC ERP activation power correlates with a higher visible CHEPS amplitude, Cz should be chosen as the measurement site.

Concerning the ICA performance, A1/A2 as a reference site enabled us to identify pain-related ICs in 14 out of the 20 subjects, with other reference settings resulting in inferior performances. By its nature, ICA has a bias towards high amplitude data and cannot recover the exact amplitude of the dipole generator which is in our case responsible for eliciting the CHEPS waveform in the amplitude-time spectrum of the EEG (Debener et al., 2010). As the A1/A2 reference site resulted in the highest overall CHEPS amplitudes, the ICA performed best in our test data. However, ICA should not be used to compare the CHEPS amplitude, as no component would fully include the whole amplitude that is generated by the dipole that outputs the pain-related EEG information in the head. In our example, ICA only served as a technique to identify the frequency regions that the CHEPS amplitudes appeared in the spectrogram and at which electrode sites the signal can be visualized.

We also wish to highlight that the three recent studies that published normative data for CHEPS all used A1/A2 as the reference (Granovsky et al., 2016; Jutzeler et al., 2016; Rosner et al., 2018). Study (Granovsky et al., 2016) evaluated the same body region with the same stimulation pattern as our study. Study (Jutzeler et al., 2016) and (Rosner et al., 2018) evaluated different body regions with a slightly different stimulation pattern and a different baseline temperature. All three studies analyzed the same CHEPS parameters as we did in this manuscript, such as *N*-wave delay, *P*-wave delay, and *N*-*P*-wave. Hence, the results of CHEPS studies (i.e., between different body regions or different baseline temperatures) can only be compared with published normative data if the reference site

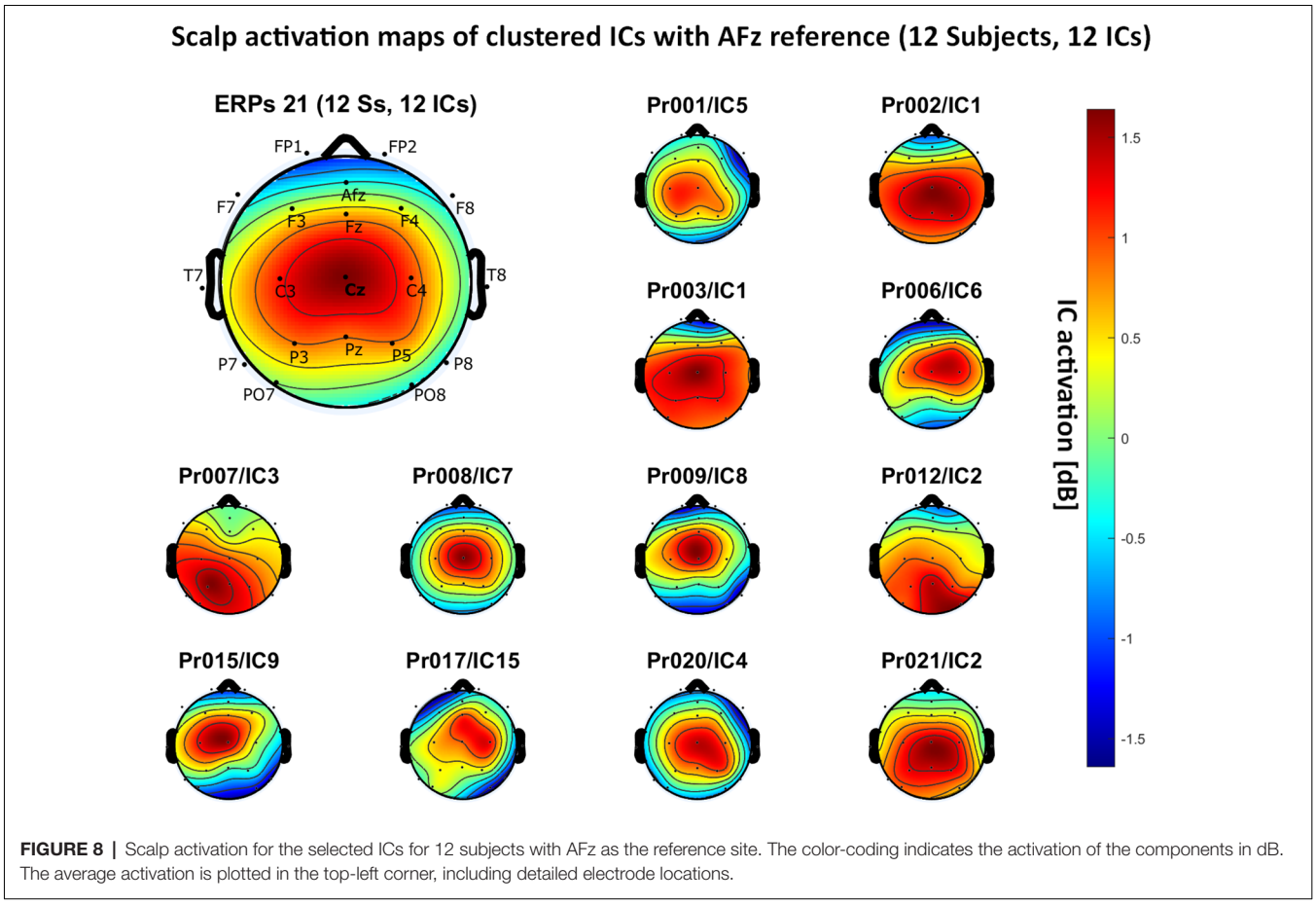

FIGURE 8 | Scalp activation for the selected ICs for 12 subjects with AFz as the reference site. The color-coding indicates the activation of the components in dB. The average activation is plotted in the top-left corner, including detailed electrode locations.

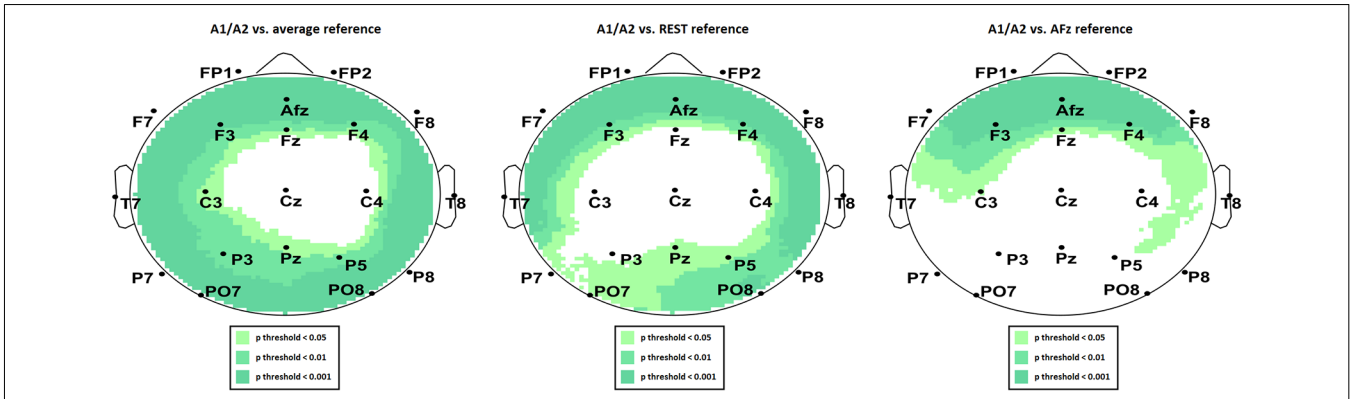

FIGURE 9 | Statistical comparison of average component activation between A1/A2 as the reference site and average (left), REST (middle), and AFz (right) as referencing technique/site.

in future CHEPS studies is the same (i.e., A1/A2). We strongly recommend that future studies additionally record data from both earlobes during data collection; this would allow for the data comparison of their data with published normative data, even if the reference is subsequently changed for further analysis. This would pave the way towards a more standardized use of the EEG in the research of noxious CHEPS.

In conclusion, there is no optimum reference point for all EEG studies. The results of this research are, thus, only applicable to common pain-related brain dynamics (CHEPS). By using A1/A2 as the reference site, we found the *N*- and *P*-wave amplitudes in every subject to be higher than in all the other referencing settings. Also, the limited requirements of using A1/A2, in terms of practical implementation, meant that it was

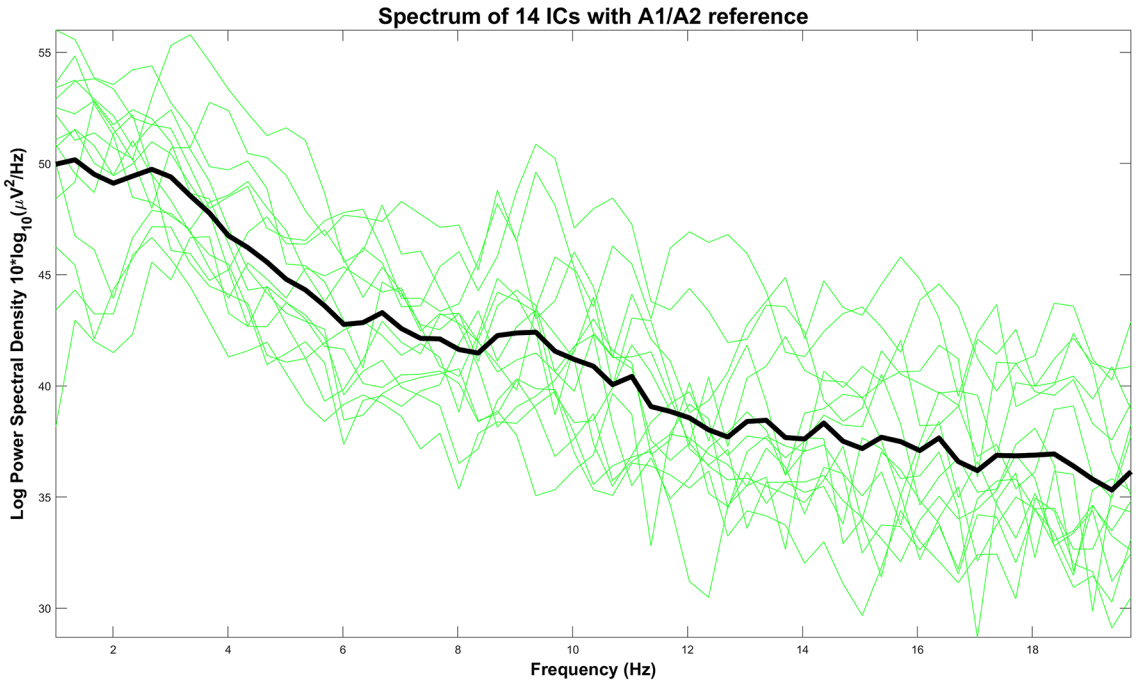

FIGURE 10 | Power spectrum of every IC with A1/A2 as the reference site (green lines) and their average (black line).

easier to identify and evaluate the *N*- and *P*-waves and, thus, improve the performance of the ICA. Future studies should agree on A1/A2 as the reference site, as methodological standardized recordings will foster the role of CHEPS in pain research. The technique can then be incorporated into clinical research that tests for differences in pain profiles between groups, i.e., patients with small fiber neuropathy vs. healthy control subjects.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee Department of Medicine Goethe University Frankfurt Theodor-Stern-Kai 7 Haus 1, 2. OG, Zimmer 207-211 60590 Frankfurt am Main Reference number: 245/17. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MA was responsible for the design of the study, data collection, data analysis, creation of data plots, statistical analysis, and writing of the first draft. BA was responsible for the design of the study, subject recruitment, and data collection. MK was responsible for data analysis, creation of data plots, and statistical analysis. SZ was responsible for image processing, data analysis, and creation of data plots. CW was responsible for the design of the study, data management, and funding. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnhum.2020.559969/full#supplementary-material.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

EEG trajectories of standardized noxious stimulation during general anaesthesia in real patients – a pilot study

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During copy editing, the title was changed to: "EEG responses to standardised noxious stimulation during clinical anaesthesia: a pilot study", and some orthographic changes were introduced. In this thesis, the paper is included with the old title and without the orthographic changes that are present in the published version.

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Abstract

During clinical anaesthesia, the occurrence and management of nociceptive stress varies from patient to patient. Previous studies in healthy volunteers under controlled conditions revealed nociceptive activity in the EEG even at high doses of remifentanil and propofol whereas in patient care, the administration of analgesics before, during, and after a surgical procedure mostly relies on empirical knowledge and the observation of the patient's reactions. To investigate the transferability of these standardized nociceptive stimuli into routine clinical practice, we included 17 patients during post-injury orthopaedic surgery into our clinical observation. We evaluated if the EEG could track the standardized noxious phase-locked electrical stimulation as well as tetanic stimulation, a time locked surrogate for incision pain, before, during, and after the induction of general anaesthesia in the theatre. Subsequently, we analysed the effect of the tetanic stimulation on the surgical pleth index as peripheral, vegetative nociceptive marker. We found that the phase-locked evoked potentials following noxious electrical stimulation vanished after the administration of propofol, but not at low concentrations of remifentanil. After noxious tetanic stimulation under general anaesthesia, there were no consistent spectral changes in the EEG, but the vegetative response in the SPI was statistically significant. We conclude that our standardised nociception stimulations are not optimised for obtaining consistent EEG responses in patients during clinical anaesthesia. To validate and sufficiently reproduce EEG-based standardized stimulation as a marker for nociception in clinical anaesthesia, other pain models or stimulation settings might be required to transfer preclinical studies into clinical practice.

Running title: Nociception in EEG clinical anaesthesia

Keywords: EEG; general anaesthesia; nociception; pain; pain-related evoked potentials; Pilot Study; tetany

Abbreviations: AUROC = area under the receiver operating characteristics; EP = evoked potential; ERP = event-related potential; ERSP = event-related spectral perturbation; LOR = loss of responsiveness; NMB = neuro muscular blockage; SPI = surgical pleth index, TCI = target-controlled infusion; TIVA = total intravenous anaesthesia

Introduction

Adequate patient monitoring during surgical intervention under general anaesthesia is crucial to reduce complications, including pain, which impairs the quality of life.[1-5] In order to improve patient outcome, the goal is a targeted, personalized concept of general anaesthesia with optimized drug dosing.[6, 7] Existing electroencephalography (EEG)-based monitoring devices, such as the bispectral index (BIS), generate a dimensionless index scale to reflect the anaesthetic level.[8, 9] The outcome utility of processed EEG (pEEG) information is still under discussion. The values obtained by proprietary algorithms heavily compress the large information content of the information in frequency, amplitude and phase of the raw EEG.[10] Specific physiological, pathophysiological, and pharmacological signatures in the EEG are lost. Also, the administration of pharmaceutical drugs sometimes affects the pEEG values in contradictory ways, e.g., the hypnotic ketanest can lead to falsely high values or a muscle relaxants without any hypnotic properties can lead to a decrease in the index.[11] However, specific patterns can be distinguished by observing the raw EEG during anaesthesia, which is not yet a routine task for an anaesthesia provider.[12] In addition, the usual pEEG indices do not specifically track the nociceptive component, i.e., the brain's reaction to a noxious stimulus.[13] At moderate levels of general anaesthesia or during deep sedation, noxious stimulation, such as endotracheal intubation or skin incision, may cause an increase in EEG beta power (beta arousal).[14] Some EEG-based monitoring systems can detect these arousals,[15, 16] which may also be accompanied by movement of the patient.[17, 18] At deeper levels of anaesthesia, the EEG can show a different set of changes to a noxious stimulus, which is either a decrease of prevailing alpha oscillations caused by a thalamocortical loop absent of afferent input[19] that may reflect adequate anaesthesia,[20] or an increase in amplitude of delta oscillations. These changes are not reliably tracked by the monitors and can even lead incorrectly low indices.[12]

Non-cortical, vegetative biomarkers, which are used to track nociception, are sensitive, but not very specific to noxious events. Only routine parameters like blood pressure and heart rate are used to assess nociceptive stress. Some objective nociceptive biomarkers such as the Surgical Pleth Index (SPI, GE Healthcare, Helsinki, Finland) [21, 22] which are a haemodynamic surrogate of the autonomic response, support the monitoring of the balance between nociception and antinociception.[23-25]

EEG studies on pain in awake, healthy subjects use highly standardized, time-locked, painful stimulations to obtain insights into nociceptive processes. In contrast, EEG studies of nociception during clinical anaesthesia in a heterogeneous patient population often consider

only the invasive, intense noxious stimuli during the routine, like skin incision, which are more difficult to compare.[15] While noxious events may alter the EEG in different ways during general anaesthesia [26] and are still being researched, the somatosensory processing as an marker for the perception of noxious stimulation can be tracked using the EEG in healthy, awake participants and is extensively described in literature.[27-30]

With the study presented in this manuscript, we aimed to determine to what extent (1) conventional evoked responses in the EEG after standardized noxious stimulation can still be identified in patients undergoing general anaesthesia with propofol and remifentanil in the clinical routine and (2) if more intense and prolonged standardized tetanic stimulation alters the cortical function in the EEG or the vegetative reaction in the SPI in a reproducible fashion.

Materials and methods

Study protocol and patients

The local ethical committee ("Ethik-Kommission des Fachbereichs Medizin") at the Goethe University Hospital Frankfurt approved our study protocol in a written statement under the processing number 6/19. We registered the study with the German Clinical Trials Register under the trial-ID DRKS00017829 on 03/02/2020. Furthermore, this study conformed to the standards set by the Declaration of Helsinki. We explained the study protocol to the patients during the standardized anaesthesia informed consent interview. If the patients expressed willingness to take part in the study, we obtained their written consent. The study was carried out at the Goethe University Hospital in Frankfurt, Germany. We enrolled patients who were scheduled for a standardized orthopaedic surgery with a low risk of complications (ASA I & II). Our patients were required be at least 18 years old, to not suffer from any known chronic pain diseases, and to not take any opioids within 24 hours prior to surgery. We also excluded patients with polyneuropathies, current ongoing drug abuse, neuro-psychological disorders, and pregnant women. Our results were collected during anaesthetic management according to clinical routine but before the first surgical incision.

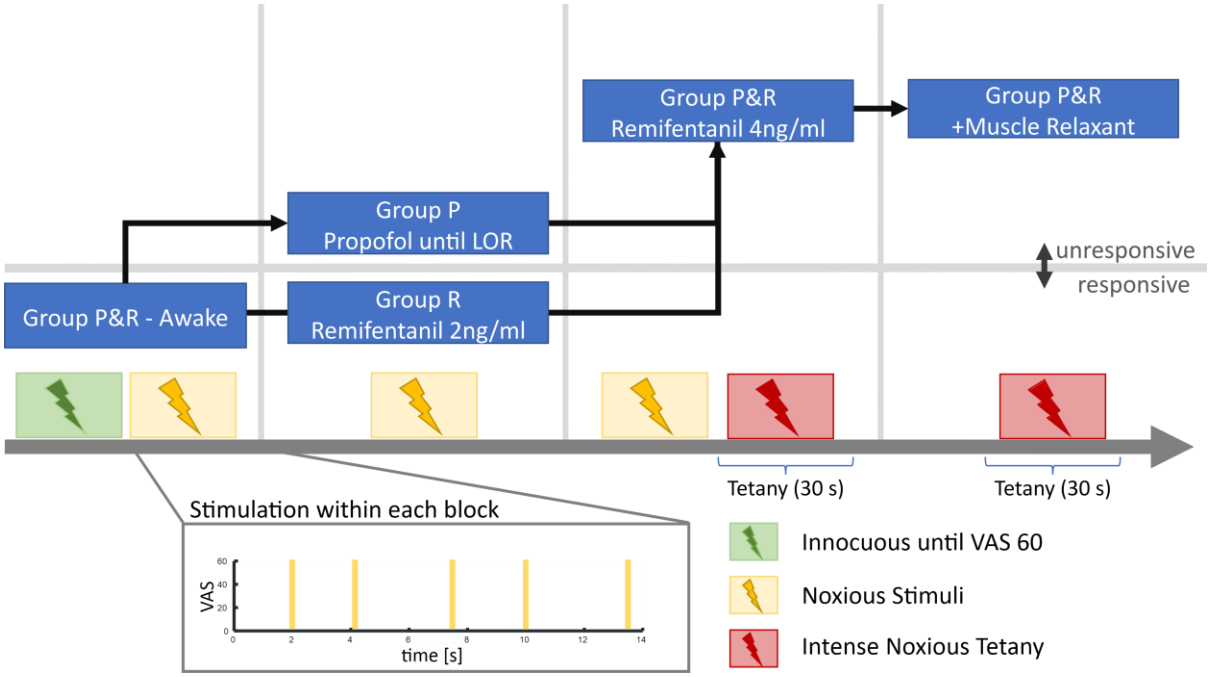

Figure 1: Study flow. *phase locked stimuli were applied in awake patients (1), at induction with propofol alone or with remifentanil alone (2) and at steady state in combination of propofol with remifentanil (3). Tetany was performed in our patients during stable anaesthesia.*

Induction and maintenance of general anaesthesia

We induced and maintained the total intravenous general anaesthesia via target-controlled infusion (TCI), using a B. Braun Space® pump system (B. Braun SE, Melsungen, Germany) and propofol and remifentanil. In every case, we titrated propofol until the rapid loss of responsiveness (LOR) was achieved. LOR was determined by testing the absence of a visible ocular reaction to a trapezius muscle squeeze. Our target effect-site concentrations for maintenance were 3 µg/mL for propofol (Schnider model)[31] and 2 – 4 ng/mL for remifentanil (Minto model).[32,33] A swift induction and an adequate level of anaesthesia as per clinical standard were prioritized in every case, resulting in the necessity for higher initial propofol doses (table 1). During the induction, six patients in the study first received propofol until LOR followed by remifentanil; the remaining 11 patients received remifentanil at a low dose (2 ng/mL) followed by propofol up to LOR and a subsequent increase in remifentanil concentration up to 4 ng/mL as well as rocuronium (0.6 mg/kg bw) for neuromuscular blocking (NMB). No other CNS-acting drugs were administered during the induction phase.

EEG/SPI recordings and pre-processing

We equipped our patients with 32 active EEG electrodes (g.Tec g.SCARABEO, Guger Technologies, Schiedlberg, Austria) attached to a g.Tec amplifier (g.HIamp), arranged in the 10-20 system. We chose AFz as the reference and grounding point during the recording phase and changed it to an average reference over all 32 electrodes during off-line pre-processing. For EEG analysis, we utilized MATLAB 2021a (Natick, Massachusetts, United States of America) and EEGLAB v2021.1.[34] Using EEGLAB for pre-processing, we downsampled the data from 512 Hz to 256 Hz (*pop_resample*), bandpass filtered the data (1-100 Hz, *pop_eegfiltnew*), filtered the line noise (*pop_cleanline*) and performed artifact rejection (*pop_clean_rawdata*). The latter toolbox applies an automated process called artifact subspace reconstruction to filter out artifacts in continuous EEG data; our threshold was set to 20 standard deviations from the cleanest part of the data.[35-37] We extracted the epochs containing the painful stimuli from -1 s to +2 s around the stimulus onset and the epochs containing the tetanic stimulation from -10 s to +40 s around the stimulus onset.

For the analysis of the spectrogram, we utilized the MATLAB function *pwelch* with a 5 s window length and a 0.5 s window shift from the signal processing toolbox. For the analysis of the common frequency bands, we averaged delta (1-4 Hz), alpha (8-12 Hz) and beta (13-25 Hz) oscillations over the respective frequency range [38]. For the analysis of the event-related potentials (ERP), we relied on custom-made MATLAB scripts. We also analysed the event-related spectral perturbation (ERSP), a measure that evaluates the relative spectral changes versus time against an individual baseline for each patient. For the ERSP we applied the EEGLAB *newtimef* function. For normalization, we used a divisive baseline from -1 s – 0 s pre-stimulus,[39] a resolution of 200 frequency points and a resolution of 400 points in time for the phase-locked data. For the tetanic stimulation, we set the baseline to -10 s – 0 s pre-stimulus, the frequency resolution to 1596 and the resolution in time to 6000 time points. *newtimef* incorporates both a short-term Fourier transform and a wavelet transform; the wavelet transform was applied with 3 cycles at the lowest frequency (3 Hz for the ERP data and 1 Hz for the tetanic stimulation) and 100 Hz for the highest frequency. In this manuscript, we show the data at the Cz electrode location.[28] For analyses of the frontal region, we show density spectral array (DSA) for the average of frontal electrode positions Fp1, Fp2, and F9.[20, 40]

The SPI was recorded using an additional GE Carescape B450 monitoring system (GE Healthcare, Solingen, Germany). The data was extracted using the open source software Vital Recorder and stored off-line.[41]

For the objective comparison of the respective anaesthetic level, the BIS was subsequently calculated (Medtronic GmbH, Meerbusch, Germany). We replayed the original EEG to a BIS Monitor with an NI USB-6343 DAQ card (National Instruments, Austin, TX, USA) which converts the EEG into a continuous signal.[42] We extracted trend data with 1/s from the BIS via a .spa file generated during playback via USB.

Painful stimuli

All patients received painful stimuli during consciousness before anaesthesia using a Digitimer DS7A Constant Current Stimulator (Digitimer Limited, Hertfordshire, United Kingdom) synchronized to our EEG device via the +5 V TTL output.

One electrical shock consisted of four consecutive single electrical stimuli with a pulse width of 200 µs and a maximum voltage of 400 V. The inter-stimulus interval between those four stimuli was 5 ms. Although we administered four concurrent stimuli, due to the short overall duration, they were perceived as one long electrical shock. To determine the required stimulus current, we increased it from 1 mA to a value where the patient rated the subjective pain as being approximately 60/100 on a verbal scale.

We administered a train of five single electrical shocks with a pseudo-randomized inter-stimulus interval of 3 - 5 seconds at different stages of anaesthesia induction after reaching stable target concentrations. These stages were while the patient (1) was awake, (2) had received either propofol at levels required to be non-responsive (group P) or remifentanil at a target concentration of 2 ng/mL (group R) and (3) had received propofol and remifentanil combined, at propofol levels that were required to maintain unconsciousness and a remifentanil target concentration of 4 ng/mL.

Tetanic stimulation

For tetanic stimulation, we applied 1500 electrical stimuli with a current of 50 mA, an inter-stimulus interval of 20 ms (50 Hz), a maximum voltage of 400 V, a pulse width of 200 µs and a total duration of 30 s. Tetanic stimulation after LOR was only carried out if the following conditions were met: remifentanil was used at a concentration of 4 ng/mL, the patient belonged

to the group that received remifentanil first and if the timetable during the clinical routine allowed for it.

Statistics

Here we investigate the applicability and transferability of noxious stimulations during routine clinical practice in patients.[43] In preclinical studies with comparable stimulation patterns, 10 patients were included.[26] To compensate for uncertainties in effects due to the clinical anaesthetic regime, our exploratory study included at least 15 patients who received the weakest stimulus awake and 10 patients who received the strongest stimulus, as is done in preclinical studies.[26, 44]

For the intra-subject ERSP and ITC analysis, we calculated effect size using the area under the receiver operating characteristics (AUROC) by the MATLAB toolbox MES.[45] We applied a 1000-fold bootstrap to the 95% confidence intervals and only reported results as being significant if the intervals did not include 0.5.[45] For dichotomous data, this approach is equivalent to the non-parametric Wilcoxon-Mann Whitney test or the prediction probability (pk).[46] We compared the changes between the two conditions (awake vs. fully sedated) versus a fixed value of 1 with the *auroc* function of the MES toolbox. An AUROC value of 0.5 indicates a completely random relationship between the conditions, whereas a value of AUROC = 0 or AUROC = 1 indicates a perfect separation. We further ranked our AUROC values according to a traditional point system with an AUROC value of 1 – 0.9 / 0 – 0.1 being excellent, an AUROC value of 0.9 – 0.8 / 0.1 – 0.2 being good, an AUROC value of 0.8 – 0.7 / 0.2 – 0.3 being fair, an AUROC value of 0.7 – 0.6 / 0.3 – 0.4 being poor and the remaining AUROC values as being fails.[47] For comprehensibility, we only extracted the maximum ERSP, ITC and AUROC values from our data as they were not dependent on the chosen size of the window. To avoid multiple comparisons over time in the cases of ERSP and ITC analysis, we only reported results as being significant if they occurred in a cluster of at least 4 x 4 pixels in size. For the AUROC values, we have shown the 95% confidence intervals in square brackets. To calculate the effect size of index values before and after tetanic stimulation, we used paired tests with the hedges g function of the MES toolbox.

To evaluate statistical differences without previous power calculation, we applied a paired non-parametric Wilcoxon signed-rank test and reported results as being significant if their p value was less than 0.05. For the mean values, we have shown the standard deviations in brackets, whilst for the median values, we have shown the 25% and 75% percentiles in square brackets.

To avoid false-positive results due to multiple comparisons over time in the ERP analysis, we only reported results as being significant if at least three adjacent time points had a *p* value less than 0.05.[48, 49]

Results

Patients

25 patients categorized as ASA I or II agreed to take part in the study and gave their written consent. Due to organizational constraints in the clinical routine (e.g., alterations of the surgical timetable at short notice), we were only able to record data from 17 of the 25 patients. None of the patients took opioids within the last 24h or had a chronic pain history. In group P, all patients required elective surgery due to a traumatic injury (3x osteosyntheses, Danis-Weber C fracture, 1x osteosyntheses ulnar fracture, 1x removal of osteosynthesis material tibia). In group R, elective surgeries were required as a result of trauma in 8 patients (2x osteosyntheses Danis-Weber C fracture, osteosyntheses radius fracture, 1x removal femoral osteosynthesis material,2x anterior cruciate ligament reconstruction, 2x meniscus repair, 1x knee arthroscopy and as a result of chondropathy in a further 3 patients (3x knee arthroscopy/synovectomy). At median, patients reported pain on the visual analogue scale (VAS) during the resting state at median of 5 with a wide variance [min 0, max 80] (table 1). 17 patients received painful stimuli for the calculation of the event-related spectral perturbation (ERPS) before the administration of anaesthetics, while being fully conscious. 6 patients first received propofol until LOR (group P, table 1) and then received noxious stimuli for ERPS. 11 patients received remifentanil as the first drug (group R). 10 of these patients were paced with noxious stimulation for ERPS while under the influence of a remifentanil target concentration of 2 ng/ml. Subsequently, the patients from group R were infused with propofol until LOR (table 1). In both groups, remifentanil concentration was increased to 4ng/ml for the last measurement. Patients first received the stimuli for ERPS in this phase. 7 patients subsequently received tetanic stimulation before, and 10 of 11 patients after NMB. We were able to record an SPI in nine patients in group R. Besides the expected drop in blood pressure and heart rate, no patient in the P or R group experienced further, clinically relevant hemodynamic impairment after LOR that required the administration of catecholamines.

Table 1: Patient demographics. *Propofol concentration in the effector organ after Schnider during induction and maintenance of anaesthesia. Subjective pain ratings [VAS] before and after general anaesthesia.*

	GROUP P			GROUP R		
	mean	**min**	**max**	**mean**	**min**	**max**
AGE	41	23	67	36	18	66
SEX		5x male 0x female			7x male 4x female	
WEIGHT [KG]	84	65	97	81	65	105
HEIGHT [M]	1.79	1.68	1,88	1.79	1.66	1.98
BMI	26.1	21.2	28.0	25.3	18.4	34.5
EFFECT SITE CONC.PROPOFOL AT INDUCTION [µG/ML]	6.60	4.99	8.70	3.38	2.90	4.00
EFFECT SITE CONC.PROPOFOL FOR MAINTENANCE [µG/ML]	3.78	3.19	4.70	3.03	2.55	3.37
BASE LINE PAIN	0	0	0	18	0	80
POST SURGERY PAIN	28	0	40	29	0	60
PAIN AT TOF STIMULATIONS	49	20	70	59	35	95

Evoked response after phase-locked noxious electrical stimulation

All 17 patients tolerated the painful cutaneous electrical stimulation during the awake state. The overall median current (obtained in the awake subject before the administration of propofol and remifentanil) for an indicated subjective pain rating of 60/100 was 17 mA [11.38; 22.0]; in the propofol group (group P), this was 20.5 mA [10.5; 22.0], whilst in the remifentanil group (group R), it was 16.5 mA [11.63; 21.88]. The overall median subjective pain rating for a consecutive stimulation of five painful bursts was 55 [50; 60] in group R and 50 [40; 60]; 40 [30; 50] in group P. In group R, the subjective pain ratings for the five consecutive electrical bursts decreased to a median value of 50 [42.5; 60.0] after the administration of only remifentanil (target effect-site concentration of 2 ng/mL), the difference as compared to pre-remifentanil was not statistically significant ($p = 0.094$). No other subjective pain ratings could be obtained as the patients were unresponsive during the other conditions.

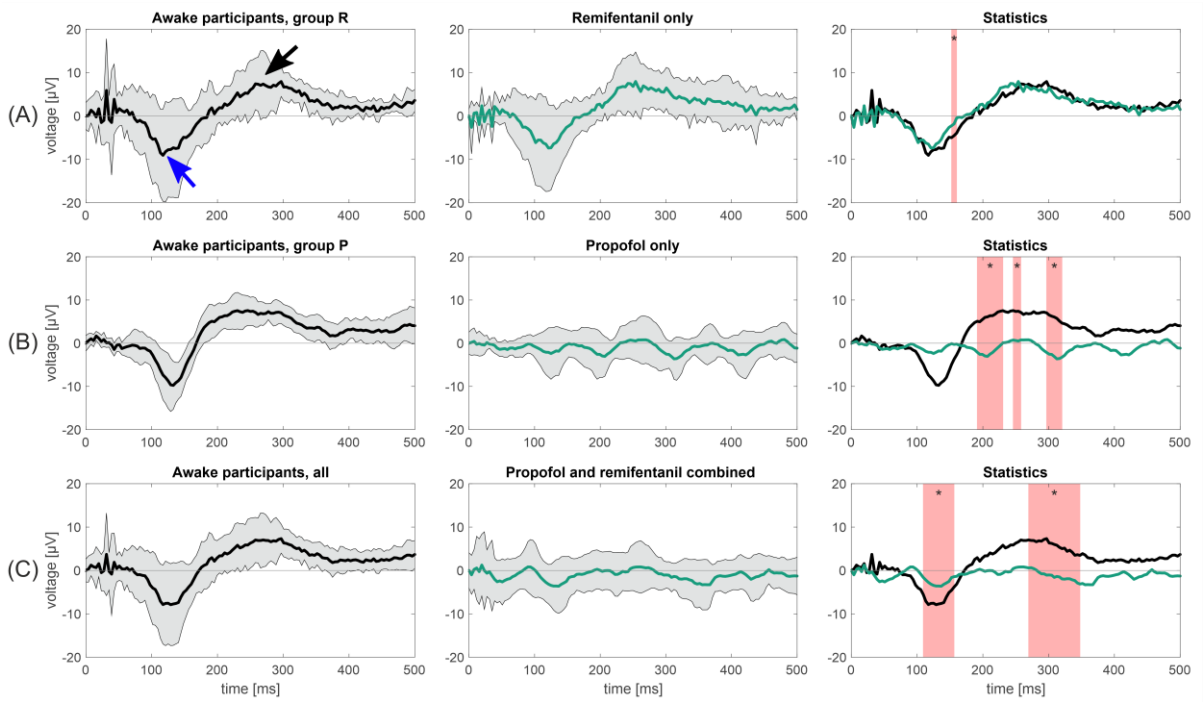

Figure 2: Evoked response after painful noxious electrical stimulation in awake and sedated patients. *The top row (A) shows group R, i.e., the group that started the induction of anaesthesia with remifentanil. The middle row (B) shows group P, i.e., the group that started the induction of anaesthesia with propofol. The bottom row (C) shows all patients of group R and P combined in an awake state and after the administration of both propofol and remifentanil later in time after a steady state general anaesthesia using both drugs was achieved. Similar to (B), the evoked response vanished and only some alpha waves were visible under this condition. The right-hand side column shows the statistical comparison between the left and middle columns, while a red box indicates a statistically significant difference in at least three adjoined points in time. The blue arrow indicates the N-wave, the black arrow indicates the P-wave.*

All awake patients in group P, R and P&R showed a visible evoked response to the noxious electrical stimulation in the EEG (Panel (A, B & C) **Figure 2**). In group R (A), the N-wave increased from -9.06 µV (± 10.63 µV) around 120 ms post-stimulus to -6.49 µV (± 10.92 µV) at the lowest point from the awake state; this difference was not statistically significant (p = 0.106). The magnitude of the P-wave increased slightly between conditions. The maximum value for the P wave of 7.94 µV ± 3.46 µV at 297 ms in the awake state increased to a maximum value of 7.95 µV ± 6.8 µV at 254 ms after remifentanil administration, without significant changes.

In group P (Panel (B) **Figure 2**), the evoked potential completely vanished after the administration of propofol. After loss of consciousness, only alpha waves were visible in the

averaged EEG. The increase around 130 ms of the N-wave from -9.75 µV ± 5.55 µV to -1.78 µV ± 5.65 µV was not statistically significant (p = 0.156, max AUC effect size 0.083); the decrease of the P-wave at 250 ms from the maximum value of 7.55 µV ± 3.43 µV to -0.73 µV ± 2.85 µV was statistically significant (p = 0.031, max AUC effect size (0.94)).

All patients (Panel (C) in **Figure 2**) lost their N- and P-waves during a stable general anaesthesia with propofol and remifentanil. The disappearance of the N-wave at 130 ms, with minimum values increasing from -7.88 µV ± 9.44 µV to -3.58 µV ± 5.54 µV was statistically significant (p = 0.002) and showed a max effect size of 0.20. The decrease of the P-wave around 300 ms from a maximum value of 7.34 µV ± 3.35 µV to -1.06 µV ± 3.27 µV was also statistically significant (p = 0.001) with a max effect size of 0.93.

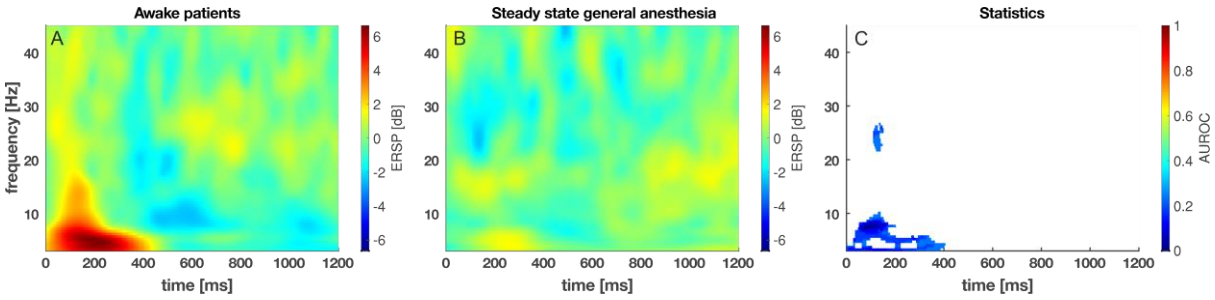

Figure 3: Event-related spectral perturbation (ERSP) and statistical comparison including AUROC effect sizes of the event-related data of all patients during wakefulness and general anaesthesia*. The panel shows the event-related spectral changes after the phase-locked noxious stimulation. The graph on the right shows the statistical comparison; a pixel is only coloured red or blue according to the colour bar if the difference is statistically significant. The Colour then depicts the value of the AUROC effect size.*

For more detailed view in the time frequency domain, we also looked at the phase-locked response as spectral perturbation **Figure 3** at the same conditions for all the patients during wakefulness and steady general anaesthesia, and employed a statistical comparison using our AUROC model. The ERSP value of the phase-locked response between 1 and 10 Hz from approximately 0 to 400 ms, decreased significantly from 6.63 dB ± 5.70 dB to 0.79 dB ± 0.19 dB at different time points (203 ms during wakefulness, 266 ms during steady general anaesthesia) and different points in frequency (4.46 Hz during wakefulness, 3.49 Hz during steady general anaesthesia). The minimum AUROC value in the same region was 0 [0; 0] at 7.39 Hz and 74 ms and, thus, rated as excellent on the traditional scale.

Tetanic stimulation

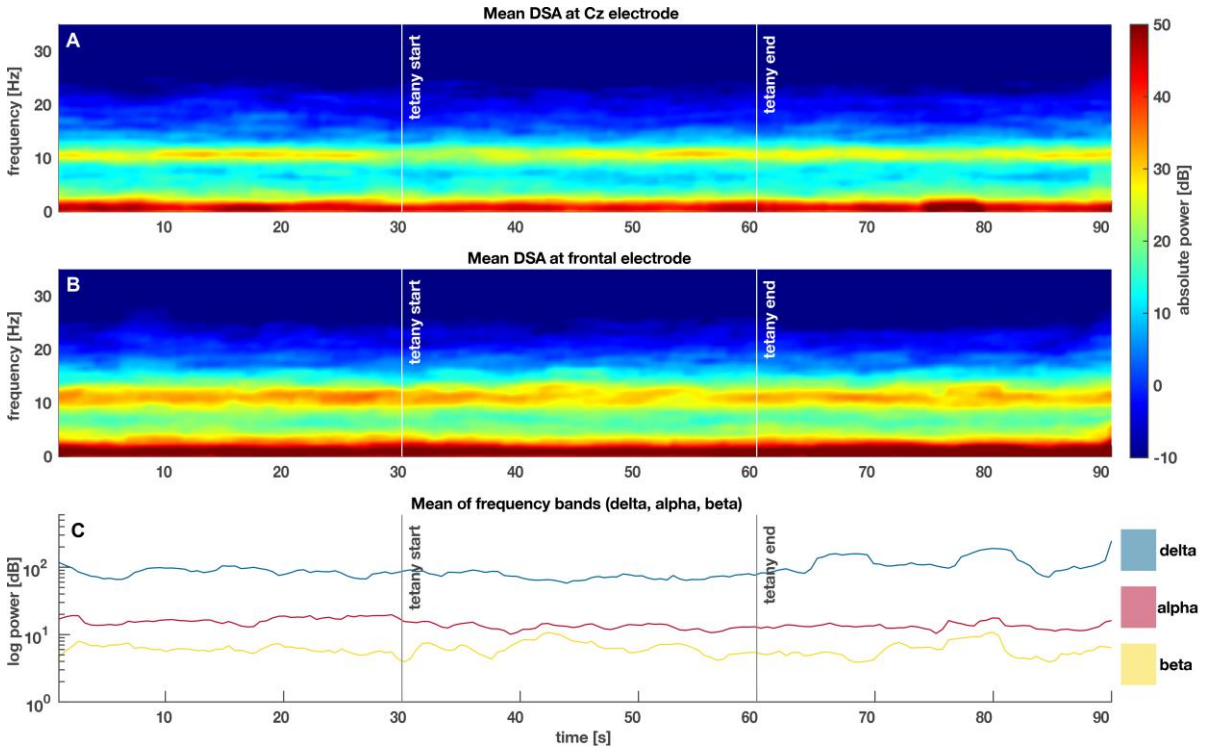

Figure 4: Spectral changes before, during and after painful tetanic stimulation in absolute terms. *The upper panel shows the changes in the absolute power in density spectral array (DSA) for electrode Cz across time averaged over 7 patients, the lower panel same at average frontal electrodes Fp2, Fp2 and F4, averaged over the 17 patients. The black lines in all three panels indicate the start and end of the tetanic stimulation*

As ERSP and ERP vanished under clinical anaesthesia, we applied tetanic stimulation as a more power full noxious stimulus and described as a proxy for surgical pain.[50] We looked at spectral changes at central and frontal electrodes as well as reaction of SPI as peripheral nociceptive indices. The average spectrogram across 10 patients in ***Figure 4*** shows strong delta and alpha oscillations at electrode Cz that fluctuated in intensity over time. We visually inspected each of the 7 patients for the alpha power trend (8 – 12 Hz) before, during and after the tetanic stimulation. No patient showed a visible alpha drop-out[40] (a decrease of oscillatory power). As the frontal EEG is of high interest in the clinical setting, we present the corresponding average spectrogram for the frontal EEG in **Figure 4 B**. The average spectrogram shows that a decrease in the absolute alpha oscillation power occurred after beginning of the tetanic stimulation at this electrode location. Changes in the EEG changes due to nociceptive stimulation during general anaesthesia are described for the delta, alpha and beta frequencies [15]. We show the average power of these frequency ranges in **Figure 4 C**. We did not observe any consistent changes during the ongoing tetanic stimulation. At the end of the

tetany, the volatility in power of the slow delta and beta bands appeared to increase, but we refrain from drawing conclusions for our population. We did not observe any visible alpha dropouts either [40]. We also found no signatures of burst suppression [51] in any of the 17 individual DSA plots.

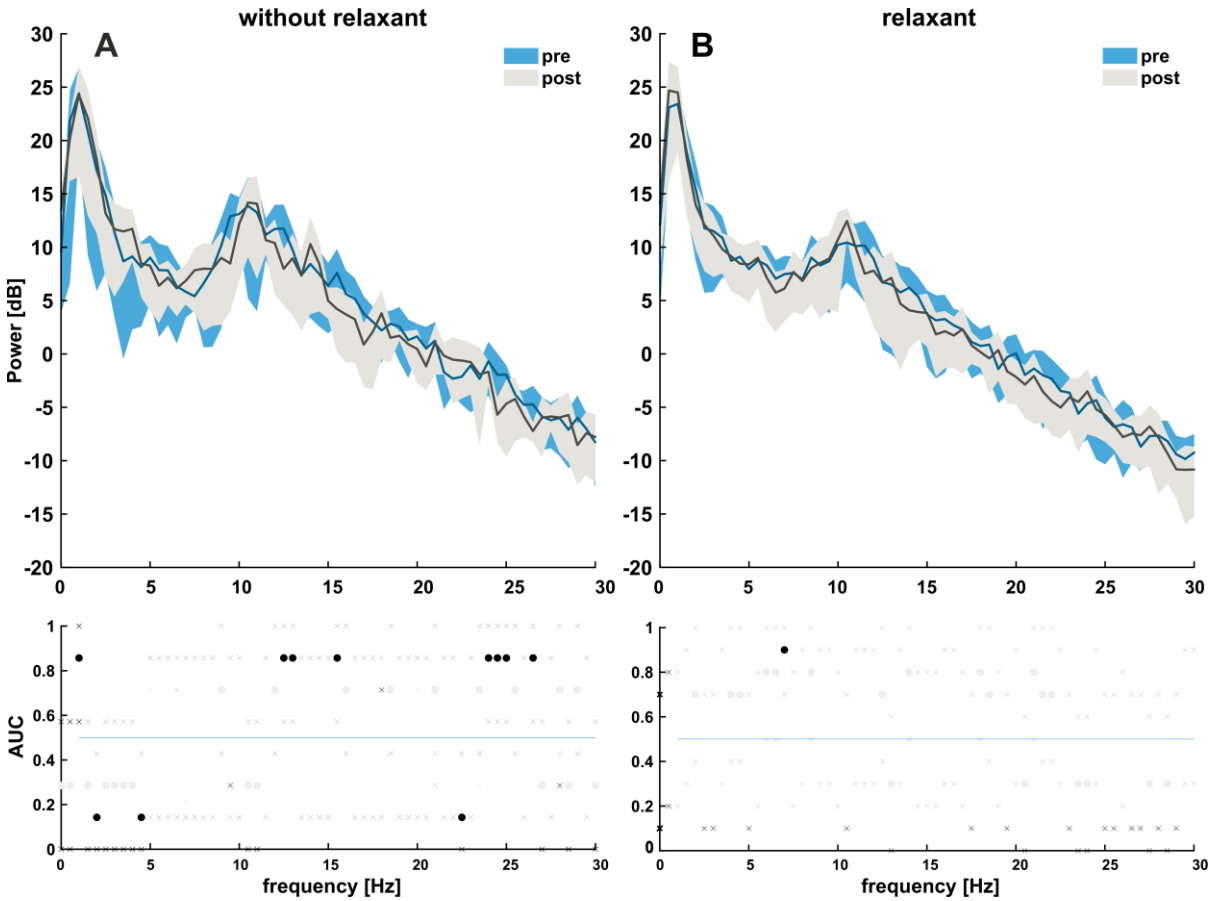

Figure 5: The spectral power changes either with or without neuromuscular blockage (NMB) after tetanic stimulation. *Panel A shows the mean power spectrum of seven patients within 10 s before (blue) and within 10 s after (grey) tetanic stimulation before NMB. Panel B is the same for ten patients with complete NMB. Statistic is shown in the lower panel. AUC is calculated as individual absolute change between the pre- and post-stimulation. black dots indicate AUC effect size greater 0.75, grey AUC effect size smaller 0.75.*

Since neuromuscular blockade (NMB) has been discussed as an influencing factor on EEG during tetanic stimuli, we considered the subgroups without and with NMB [52]. Seven tetanic stimulations occurred without and ten with complete NMB (**Figure 5 A & B**). We compare the power spectrum of the frontal EEG 10s before and last 10s of tetanic stimulation. Both subgroups show dominant delta and alpha oscillations. AUC analysis shows no consistent changes between pre- and post-stimulation. Only in the group without NMB (A) before tetanic stimulation showed a small cluster of AUC values > 0.75 in the beta region at 25 Hz is visible witch is not statistically significant. The group with NMB visually shows a lower alpha peak.

Surgical Pleth Index and bispectral index

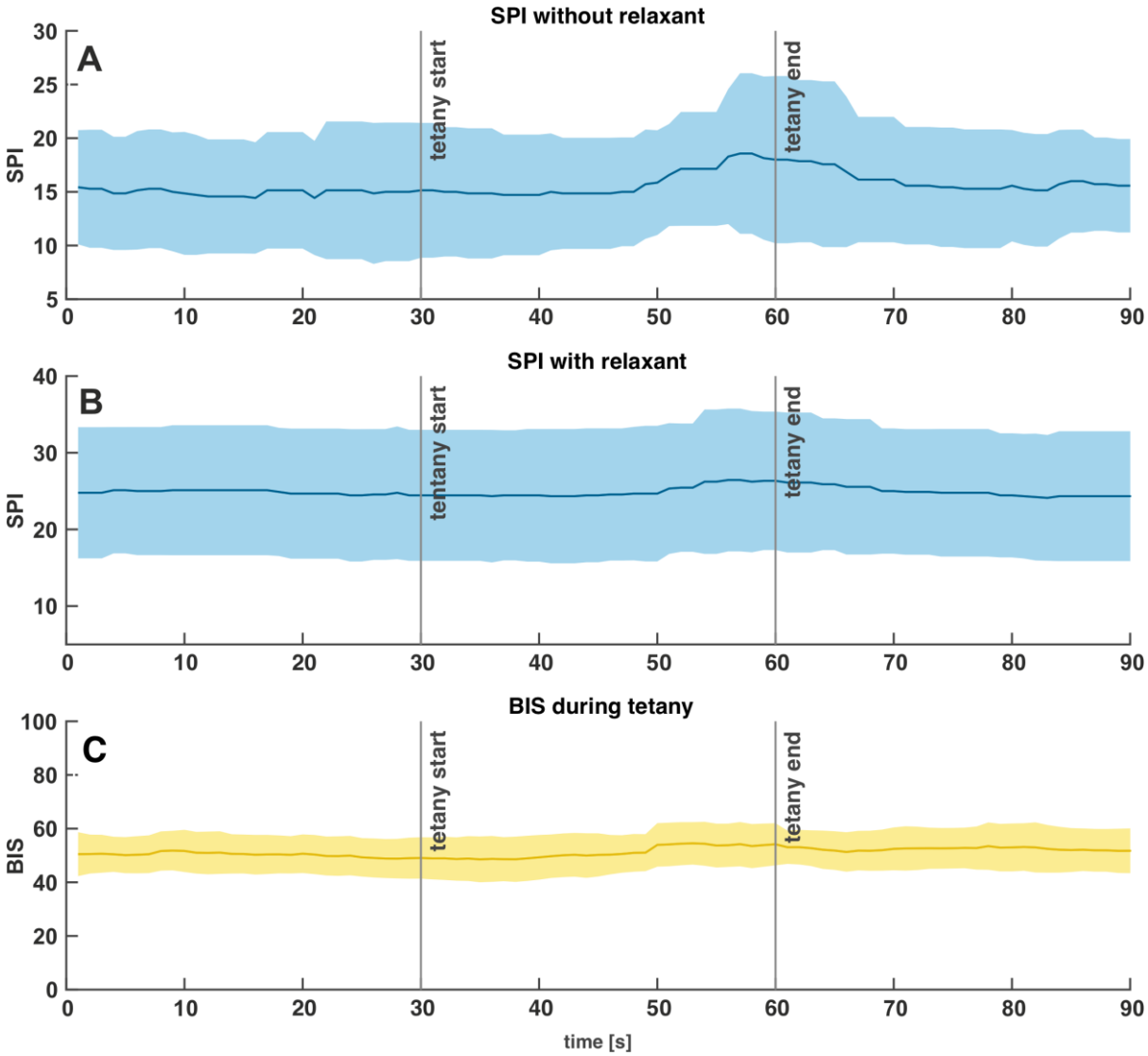

Figure 6: The average SPI during tetanic stimulation changes in patients without NMB. *The lines indicate the average SPI or BIS values across time, while the shading indicates one standard deviation. The black bars indicate the start and end of the tetanic stimulation. As there are no absolute target values established for the SPI, normalized Values are shown. Panel A shows SPI response of 7 stimulations before NMB, panel B the same of 10 stimulations at full NMB and panel C the average BIS of all 17 patients.*

Last, we examined the influence on processed index values mapping frontal cortical (BIS) response and noncortical vegetative responses (SPI). The average SPI value increased after approximately 20 s of the onset of the tetanic stimulation without neuromuscular blocking, as shown in **Figure 6**. The mean SPI before tetanic stimulation was found to be 14 while the mean SPI at the end was 18. The difference was statistically significant (p = 0.035, Hedge's g effect

size 0.32 [CI 0.12 – 0.77] low to medium effect). After neuromuscular blockage the mean SPI was 25 before tetanic stimulation and 26 at the end of the stimulation. We found no statistically significant difference ($p = 0.11$, hedges g effect size 0.11 [CI 0.01 - 0.42], no effect).

During tetanic stimulation, all patients had a clinical adequate and stable level of anaesthesia with a BIS value in the recommended target range for general anaesthesia of between 40 - 60, as shown in **Figure 6 C**. During tetanic stimuli, no statistical significant difference in BIS values was observed in either relaxed or non-relaxed patients. Individually, we saw an increase of BIS values in 2 out of 17 patients during tetany, whereas all others showed no visible change.

Discussion

Here, we demonstrate the integration of standardized electrical phase-locked noxious stimuli and tetanic stimulation into patient care during routine general anaesthesia. While the EEG is a promising tool for the non-invasive analysis of pain,[29] the reproducibility of the EEG trajectories as biomarkers for pain and the pain specificity of the EEG signatures is still under discussion.[30, 53] Nonetheless, phase-locked evoked potentials in the amplitude-time perspective and time frequency analysis provide a practical, highly standardizable approach to readout of the function of the nociceptive system on a cortical level.[53, 54] We focused on the question of whether EEG assessment of pain processing in the somatosensory system can be integrated into patient care during routine anaesthesia. Furthermore, we wanted to answer the question whether these standardized nociceptive signatures measured in volunteers can also be detected in patients during general anaesthesia procedures.

The effects of propofol on the evoked and the phase-locked response

The administration of remifentanil up to an effect-site concentration of 2 ng/mL reduced the painful evoked response slightly at the N-wave, but not statically significant. Furthermore, our data shows that once propofol is administered to the patient, either before or after remifentanil, the common evoked potential in the EEG after standardized noxious stimulation vanishes. Propofol, itself, has no long-lasting decreasing effects on the subjective pain ratings,[55] hence, it is unlikely that the elimination of these potentials stems from an analgesic effect. It has also been shown that spinal transmission and a regional brain activity persist during intense noxious stimuli, even during deep propofol anaesthesia.[44]

Nevertheless, as there was no identifiable response in the perturbation spectrogram or the amplitude time spectrum, we conclude that we were not able to extract any identifiable response in the EEG after repeated phase-locked noxious stimulation during routine general anaesthesia, at least with our stimulation parameters.

Tetanic stimulation as an intraoperative pain model

A recent study showed that intense noxious stimulation can lead to cortical activation, even at deep levels of anaesthesia which could cause burst suppression in the EEG.[26, 44] We could not

reproduce the same somatosensory response in the EEG with our slightly lower current setting. In contrast to our stimulation setting, the investigators of the aforementioned study applied tetanic stimulation with a higher current of 80 mA (50 mA in our study). Nevertheless, a tetanic stimulation with 50 mA is a strong, intense noxious stimulus as also shown by the response of the SPI during anaesthesia and as reported before.[50] This was also confirmed by an increase of SPI in a significant manner to the tetanic stimulus in patients without NMB. In patients with NMB, this effect was not significant, raising the question of whether the effect is caused by the direct nerve stimulation or the corresponding muscle contraction. Since the SPI is determined from the optical pulse oximetry, we exclude a direct electrophysiological artifact. With values below 30, our SPI indicated an adequate balance of nociception-antinociception during both conditions.[24] Normative SPI reference values for the tetanic pain model are not established and individual, relative change over time seems to be more important.[24] It is likely that the SPI is influenced by the age of a patient, as the index depends on the reactivity of the cardiovascular system. Thus, age as a factor should be considered in future studies,[24] but in our study, the age range of our patients is relatively narrow. Out of the many other nociceptive indices, we chose the SPI because it is widely used, easy to apply and well validated for TIVA anaesthesia.[21] Further detailed examination of other parameters such as heart rate spectra after noxious stimulation may provide further insights.[50, 56] Other commercially available nociceptive indices measure other parameters such as changes in pupil width, skin conductance, or changes in reflex responses, which we are not reporting in our study. However, we believe that the choice of index used should be made with careful consideration of the patient client, the applicability and the nociceptive stimulus used.[24] All our patients maintained a stable anaesthesia during and after tetanic stimulation, with BIS values of between 40 to 60. The BIS may be affected by beta arousal [15], which we observed to some extent in a non-significant fashion in the non-NMB group. This was also shown by the presence of ongoing slow delta and alpha oscillations, which predominantly serve as landmarks for a deep and adequate anaesthesia,[57] and the absence of burst suppression, which would be prominently visible in the raw EEG and spectrogram.[51, 58] Although we have avoided disturbing environmental influences such as noise and different times of day as far as possible, our conditions are not comparable to the laboratory conditions of a study with volunteers. We assume that pre-existing pain, as it occurred in some of our subjects, as well as the perceived stress of an upcoming surgery, influence the nociceptive sensation and lead to an activation of the antinociceptive system.[59] This could strongly influence the response to pain stimuli as compared to healthy subjects.

In our case, patients were induced with high initial doses of propofol and received remifentanil either immediately before, or immediately after, which is a different setting than in the preclinical studies.[26, 44] The aim of our anaesthesia protocol was to achieve LOR an maintain anaesthesia in accordance with the clinical routine, hence, we needed a wide range of effect-site concentrations of propofol as each patient required different doses. The order in which remifentanil and propofol were administered also played an important role as the combination of both drugs leads to synergistic effects.[60] Although tetanic stimulation is discussed as a pain model for noxious incision during general anaesthesia,[50] our data shows that with our stimulation settings it is unlikely to be a suitable pain model for analysing the EEG as a nociceptive marker in the clinical practice of the operating room, outside of the standardized setting of a clinical study.

With our approach, strong tetanic stimulation may not be a general proxy of noxious stimulation as needed for studies in a clinical setting. It is possible that our tetanic stimulus intensity was too weak in the context of clinical injury in our patients undergoing traumatological/orthopaedic surgery. Our results apply to procedures during clinical routine prior to incision. Due to the small number of heterogeneous patients, the transferability to large collectives is open. As the SPI depends on the reactivity of the cardiovascular system, age influences on the SPI are likely.[24] The age range of our patients is relatively narrow. In future studies, however, age should be taken into account, especially because the influence of age on the pEEG as well as on parameters of the raw EEG has been described many times.[61 62, 63] Furthermore, no statements can be made about the research of different intraoperative pain types, e.g., somatic incisional pain, visceral pain or chronic postoperative pain.

Summarizing the limitations, we can imagine some changes in our stimulation parameters that could lead to a positive result:

1) Increase the number of trials for ERSP from e.g., 5 to 100. This would ensure that the background EEG features common to general anaesthesia (dominant slow-delta and alpha oscillations) would be more likely to cancel out.

2) Increase the target effect-site concentration of propofol more slowly and, thus, achieve LOR later in the protocol. This would highlight if there were a cut-off concentration for propofol at which it eliminates the evoked potential in the EEG, as shown in studies on healthy subjects.[44]

3) Greater consideration of NMB in electrical pain models.

4) Increase current intensity for intense stimulation, titrated using a peripheral nociceptive index.

Conclusion

Our data revealed that anaesthetic agents affect the cortical processing of noxious stimuli. Low doses of remifentanil alone decreased the ERSP response to noxious stimulation in our patients less than what would be expected from volunteer studies. During the alpha-dominant EEG rhythm induced by propofol, the ERSP response to noxious stimulations is not uncovered. Strong nociceptive tetanic stimulation would more likely be detectable peripherally than in the cortical EEG of our patients. We argue that stimulation settings optimized for translation into clinical practice need to be further adapted to obtain reproducible responses to noxious stimulation in spectral EEG as found in preclinical studies. These properties are a prerequisite for a biomarker of nociception during general anaesthesia in a heterogeneous clinical patient population. We found that the peripheral nociceptive index is more sensitive to intense stimulation and could help to find standardized stimulation settings combining comparability of biomarkers and clinically relevant response. An adjustment of the stimulus intensity to the external circumstances of clinical practice and patient characteristics should be considered in future studies.

Authors' contributions

MA: Study design, Patient recruitment, data collection, data analysis, and writing up of the first draft of the paper; BA: Study design, revising the manuscript critically; ED: interpretation of data, revising the manuscript critically; DH: data analysis, interpretation of data, revising the manuscript critically; MK Study design, data analysis, interpretation of data, revising the manuscript critically; CW: Study design, Patient recruitment, data collection, revising the manuscript critically; SZ Study design, Patient recruitment, data collection, data analysis, and writing up of the first draft of the paper; All authors: Final approval

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Competing interests

The authors report no competing interests.

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EEG-based sensory testing reveals altered nociceptive processing in elite endurance athletes

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During copy editing, some orthographic changes were introduced. In this thesis, the paper is included without the orthographic changes that are present in the published version.

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Abstract

Increased exercise loads, as observed in elite athletes, seem to modulate the subjective pain perception in healthy subjects. The combination of electroencephalography (EEG) and standardized noxious stimulation can contribute to an objective assessment of the somatosensory stimulus processing. We assessed the subjective pain ratings and the electroencephalogram (EEG)-based response after standardized noxious mechanical and thermal stimuli as well as during conditioned pain modulation (CPM) in 26 elite endurance athletes and compared them to 26 recreationally active controls. Elite endurance athletes had consistently stronger somatosensory responses in the EEG to both mechanical and thermal noxious stimuli than the control group. We observed no significant group differences in the subjective pain ratings, which may have been influenced by our statistics and choice of stimuli. The CPM testing revealed that our conditioning stimulus modulated the subjective pain perception only in the control group, whereas the EEG indicated a modulatory effect of the conditioning stimulus on the spectral response only in the athletes group. We conclude that a higher activation in the cortical regions that process nociceptive information may either be an indicator for central sensitization or an altered stimulus salience in the elite endurance athletes' group. Our findings from our CPM testing were limited by our methodology. Further longitudinal studies are needed to examine if exercise-induced changes in the somatosensory system might have a critical impact on the long-term health of athletes.

Keywords

EEG, pain, nociception, elite endurance athletes, conditioned pain modulation, event-related spectral perturbation

Statements and Declarations

The authors have no relevant financial or non-financial interests to disclose.

Introduction

Elite athletes experience pain with some regularity. They have a very high lifetime prevalence of up to 84% for chronic pain syndromes including lower back pain (Fett et al. 2017; Farahbakhsh et al. 2018), with a broad variety of biopsychosocial factors playing a role even at early stages in their careers (Bumann et al. 2020). One of the possible risk factors for the chronification of pain may be an altered nociceptive processing (Roussel et al. 2013). To evaluate if endurance exercise influences the nociceptive processing and pain perception of elite athletes, it is important to understand how pain is defined, assessed, and quantified.

Pain by its definition is a personal experience depending on biological, psychological, and social factors and, thus, is influenced by subjective factors (Raja et al. 2020). The assessment of nociceptive processing is tricky; it aims to objectively quantify pathophysiological changes besides assessing psychosocial variates (Sommer 2016; Treede et al. 2019). Subjective pain testing is the gold standard in research, e.g., via questionnaires such as the McGill pain questionnaire (Main 2016), via quantitative sensory testing (QST) (Rolke et al. 2006), or with paradigms testing the conditioned pain modulation (CPM) (Nir and Yarnitsky 2015). Additionally, electroencephalography (EEG)-based cortical evoked potentials in response to noxious stimuli have been introduced as promising tools (van den Broeke et al. 2015; Özgül Ö et al. 2017; Hüllemann et al. 2019; Fabig et al. 2021). Nociceptive testing using EEG can be carried out in a non-verbal population such as newborn infants (Hartley et al. 2017) or in animals (Murrell and Johnson 2006), and advances in computerized analytics of the EEG, like the analysis of the event-related spectral perturbation (ERSP) and the inter-trial coherence (ITC), allow for an in-depth analysis of event-related EEG data. While subjective pain ratings give an insight into the subjective sensory response of the body to nociception ("pain perception"), certain methods of neuroimaging e.g., high-density, multi-channel EEG recordings combined with standardized noxious stimulation, enable a different and not invariably correlated analysis: the activation of the cortical regions in the brain, which are called the "pain matrix", to noxious stimuli. The activation of these cortical structures as measured by the EEG is not only dependent on the perceived painfulness of the stimulus, but also on the stimulus salience of an individual, i.e., the significance the participant is directing towards the stimulus (Iannetti et al. 2008; Legrain et al. 2011). It is thus usefully extending the conventional approach of only testing the subjective pain ratings.

The differences in pain perception between elite athletes and a normally active population have been studied extensively, although no studies have integrated the EEG into their testing paradigm. In literature, a higher pain tolerance of elite athletes is concluded and higher pain thresholds are suggested (Tesarz et al. 2012) and recent research seems to further manifest these findings (Geva and Defrin 2013; Tesarz et al. 2013; Pettersen et al. 2020). Furthermore, the type of sports, e.g., strength versus endurance, does seem to play a major role in the exact changes in pain perception (Assa et al. 2019). Two studies evaluated the acute effects of exercise on nociceptive processing in trained athletes using functional magnetic resonance imaging (fMRI) (Scheef et al. 2012; Geisler et al. 2021), with different aims: the first study researched the acute short-term effect of endurance exercise on the pain response as measured in the fMRI (Scheef et al. 2012), while the other researched the long-term neuronal alterations as a result of heavy endurance exercise (Geisler et al. 2021). Scheef et al. concluded that acute endurance exercise in elite athletes reproducibly suppressed the activation of pain-induced processes in different cortical brain regions that are responsible for nociceptive processing, alongside with elevated levels of antinociceptive endogenous opioid neuropeptides. Geisler et al. concluded that in the long term, high training levels of endurance sports also seem to suppress the activation of these cortical structures, compared to a sedentary control group. Although the fMRI excels in providing such detailed spatial information, its temporal resolution of these processes is significantly inferior to the EEG (Cohen 2011).

As the EEG provides information with a high-temporal-resolution about the neural processing of nociception, we analyzed fast-acting time-locked nociceptive-related processes after standardized noxious stimulation of trained endurance athletes, as compared to non-elite, recreationally active controls. We also examine if there are differences in the endogenous pain modulation capacities as assessed by CPM, both via the EEG and subjective pain scores. We aimed at analyzing if our groups differed in their conventional subjective pain ratings to our standardized nociceptive stimuli and if those differences were represented in a similar fashion in their activation of the pain matrix as expressed by our EEG data. In contrast to the existing neuroimaging studies of elite endurance athletes, we aimed at capturing short-term processes after brief noxious stimulation in the range of milliseconds, which cannot be reliably captured by the fMRI. We hypothesize that there are long-term modulatory effects of elite endurance sports on the cortical regions that process nociception, which can be uncovered by using neuroimaging tools but not by subjective pain testing alone.

Results

Participants: Total numbers and anthropometric data

We recorded and analyzed data from 26 elite endurance athletes and compared them to 26 normally active controls. Their anthropometric and sport-specific data as well as data regarding the pain history of the groups are outlined in Table 1. The athletes engaged in rowing (12 participants), triathlon (9 participants), speed skating (3 participants) and running (2 participants). The hourly weekly training load included endurance, weight training and circuit training. Our athletes had a significantly higher training load, a significantly lower resting heart rate and reported more frequent suffering from pain levels in the past 3 months. Both study groups did not differ in their quality of life as assessed by the Veterans RAND 12 Item Health Survey (VR-12) global health questionnaire, a questionnaire for the self-evaluation of one's health-related quality of life.

Visual Analogue Scale (VAS): Subjective pain ratings to standardized noxious stimuli

Both groups did not differ in their subjectively perceived pain intensity following mechanical or heat noxious stimuli (see Table 1).

The subjective pain ratings of the test stimulus were affected differently by the conditioning stimulus in both groups (see Table 1). In the controls' group, the conditioning stimulus significantly lowered the subjective pain ratings to the test stimulus (see Table 2). After the conditioning stimulus was removed, the pain ratings significantly increased back to baseline levels. We did not observe a significant decrease or increase of the subjective pain ratings to the test stimulus because of the conditioning stimulus in the elite endurance athletes' group between any of the conditions.

Table 1: Anthropometric data of the participants included in our study, as well as the VAS score for standardized noxious mechanical and heat stimulation. The data are presented as median values with the 25% and 75% interquartile ranges stated in square brackets. An asterisk in the statistics column depicts a p-value smaller than 0.05, "n.s." depicts a p-value greater than 0.05 and "n.a." depicts that we did not calculate statistics for the comparison.

	Active controls	**Elite endurance athletes**	**Statistics**
Number and sex of participants	26 (15 male, 11 female)	26 (15 male, 11 female)	n.a.
Age	25.5 [24.6; 27.1] years	26.1 [23.5; 29.7] years	n.s. $p = 0.71$
Weekly training load	4 [2; 6] hours	20 [18; 24] hours	* $p < 0.001$
Question: for how many years have you been training for more than 15 hours/week?	0 [0; 0]	8 [5; 10]	* $p < 0.001$
Heart rate after the submaximal endurance test	136 [120; 148]	90 [80; 108]	* $p < 0.001$
Resting heart rate	64 [60; 72]	48 [44; 56]	* $p < 0.001$
Pcs-12 scores	61.8 [59.0; 62.2]	61.7 [57.0; 63.2]	n.s. $p = 0.93$
Mcs-12 scores	39.8 [36.0; 41.6]	41.0 [38.7; 43.5]	n.s. $p = 0.17$
Mechanical pain during PEP	12 [5; 20]	11 [7; 18]	n.s. $p = 0.95$
Heat pain during CHEPS	21 [13; 34]	17 [9; 33]	n.s. $p = 0.30$
CPM + pinprick: before water bath	19 [11; 27]	16 [6; 22]	n.s.
CPM + pinprick: during water bath	13 [9; 18]	14 [5; 24]	n.s.
CPM + pinprick: after water bath	20 [8; 31]	16 [4; 23]	n.s.
Conditioning stimulus (8° c water bath): initial pain intensity after inserting the foot	28 [15; 40]	15.5 [6; 29]	* $p = 0.026$
Vas: average pain intensity in the past 3 months (0-100)	2 [0; 5]	14 [3; 34]	* $p = 0.007$
Vas: current pain level at rest	0 [0; 0]	1 [0; 3]	* $p = 0.007$
Question: experienced pain that persisted / recurred for more than 3 months (ICD-11 definition of chronic pain)?	Yes: n = 2 No: n = 24	Yes: n = 5 No: n = 21	n.s. $p = 0.22$

Question: have you experienced a sports-related injury in the past year, and can you specify the body region?	Yes: n = 4 No: n = 22	Yes: n = 13 No: n = 13	* $p = 0.007$
Question: is your current pain sports-associated?	Yes: n = 4 No: n = 22	Yes: n = 19 No: n = 7	* $p < 0.001$
Question: are you doing sports while suffering from pain?	Yes: n = 6 No: n = 20	Yes: n = 19 No: n = 7	* $p < 0.001$

PCS: Physical Component Score; MCS: Mental Component Score; VAS: Visual Analog Scale; n.s.: not significant; n.a.: not applicable; CHEPS: Contact Heat Evoked Potentials

Table 2: Intra-group statistical testing of the relative change in subjective pain ratings of the test stimulus difference between the CPM conditions before vs. during, and during vs. after the conditioning stimulus. For the intra-group testing, we show the Friedmans/multcompare statistics and the p value. An asterisk depicts statistical significance.

	Recreationally active controls	**Elite endurance athletes**
Intra-group testing: Friedman's test: CPM + pinprick before vs. during vs. after	* $p = 0.001$	n.s. $p = 0.13$
Intra-group posthoc testing: before vs. during	* $p = 0.007$	Not tested
Intra-group posthoc testing: during vs. after	* $p = 0.003$	Not tested
Intra-group posthoc testing: before vs. after	n.s. $p = 0.96$	Not tested

CPM: Conditioned Pain Modulation; n.s.: not significant; n.a.: not applicable

Mechanical pain: Pinprick-evoked potentials (PEP) as the spectral perturbation

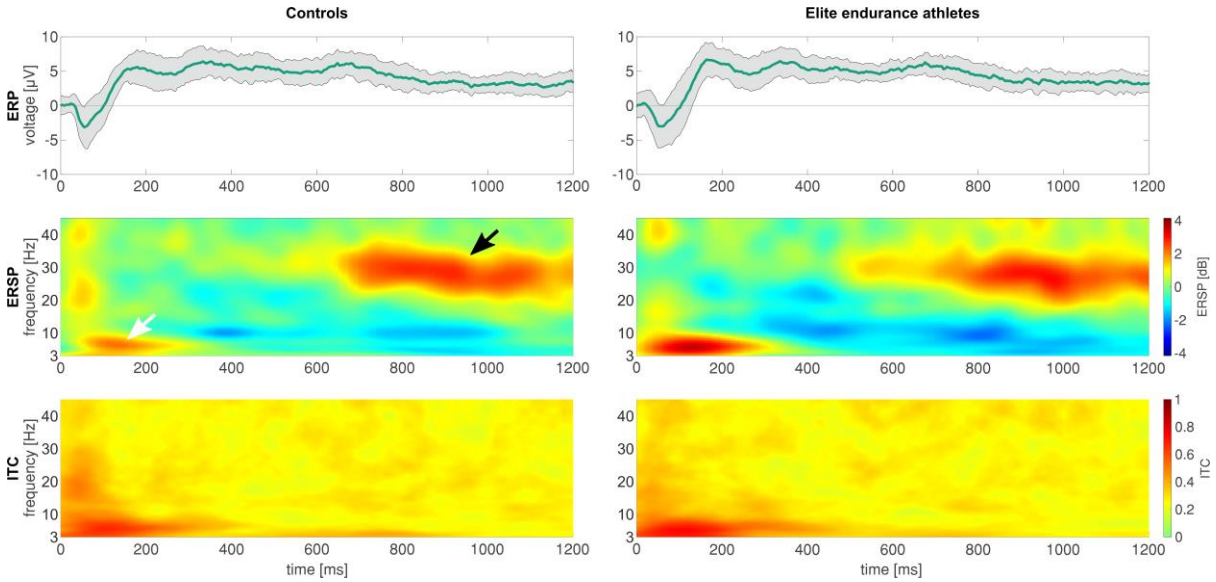

Figure 1: Raw EEG data and spectral changes of the pinprick-evoked potentials in the controls and elite endurance athletes at electrode Cz. Upper row: event -related potential (ERP). Original EEG data in the amplitude-time spectrum after pinprick stimulation for both the controls and the elite endurance athletes. Traces are the means across all 12 trials per participant and all 26 participants. The shaded area indicates the standard deviations of the 26 participants. Middle row: Average event-related spectral perturbation (ERSP), i.e., the spectral changes over time. The white arrow indicates the N2P2 response, while the black arrow indicates a response not visible in the ERP analysis. Lower row: inter-trial coherence (ITC) or phase locking factor (PLF), i.e., the spectral synchronization among the trials.

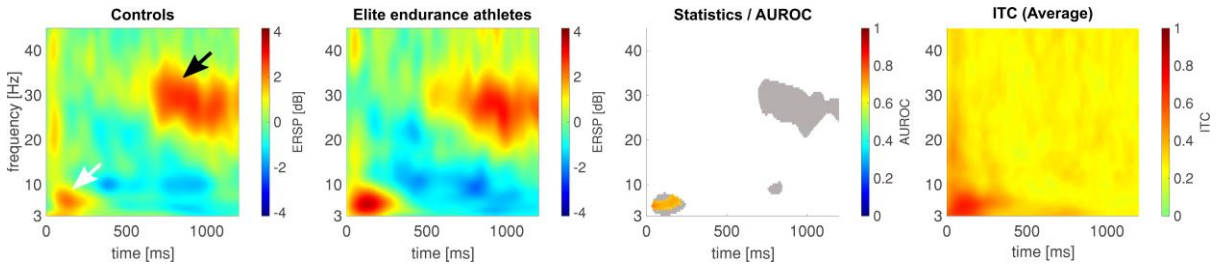

Figure 2: Elite endurance athletes show stronger pinprick-evoked potentials at electrode Cz, compared to controls. Panel 1 shows the event-related spectral perturbation of the controls, likewise, panel 2 shows that of the elite endurance athletes. Third panel: The statistical comparison includes both a common non-parametric statistical testing with a cluster-based correction for multiple comparisons, and the AUROC effect size. Pixels that are significantly different are colored red/orange or blue, according to the c-axis next to the image, which indicates the AUROC effect size of the comparison. The gray shaded area in the statistics image indicates that the accompanying pixel in the ERSP in either one of the groups exceeds a [2 dB; -2 dB] range; areas of interest for further analysis are highlighted in panel 1 with a white (early low-frequency response) and black arrow (late high-frequency response). The fourth panel shows the average ITC calculated for all 52 participants to help the reader to identify the area where the N2P2 component is commonly found.

We show the raw event-related EEG in the amplitude-time spectrum and the event-related spectral perturbation (ERSP) in Figure 1 and our ERSP-based group comparison of the pinprick stimuli in Figure 2. On average, athletes had a significantly higher response to mechanical stimulation in the area with the highest degree of phase locking (white arrow in Figure 2) than

the control group, indicating that they elicited a higher EEG-based response to noxious mechanical stimuli. The maximum average ITC value of 0.71 was found in that area. The maximum ERSP values were 3.60 dB (athletes at 5.44 Hz and 129 ms) and 2.18 dB (controls at 5.92 Hz and 129 ms). The ERSP values between both groups were significantly different. The maximum AUROC value was 0.74 [0.61; 0.87] at 27 ms and 5.44 Hz. We classified our AUROC values according to a traditional point system with a value of 0.74 indicating a fair effect.

There was no significant difference in the later high-frequency response with a low degree of phase locking between the two groups (black arrow in Figure 2). The maximum ERSP value was 3.06 dB (at 26.40 Hz and 969 ms) in the athletes' group vs. 2.81 dB (at 28.35 Hz and 910 ms) in the controls' group.

Heat pain: Contact heat-evoked potentials (CHEPS) as the spectral perturbation

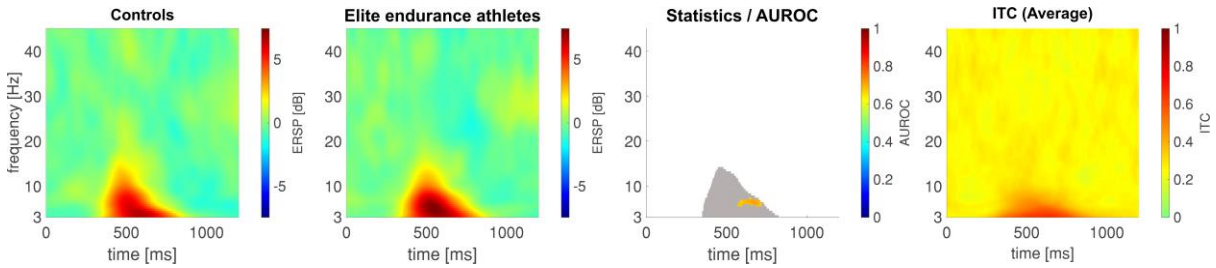

Figure 3: Elite endurance athletes show stronger contact-heat evoked potentials at electrode location Cz, compared to controls. Panel 1 shows the event-related spectral perturbation of the controls, likewise, panel 2 shows that of the elite endurance athletes. Third panel: The statistical comparison includes both a common non-parametric statistical testing with a cluster-based correction for multiple comparisons, and the AUROC effect size. Pixels that are significantly different are colored red/orange or blue, according to the c-axis next to the image, which indicates the AUROC effect size of the comparison. The gray shaded area in the statistics image indicates that the accompanying pixel in the ERSP in either one of the groups exceeds a [2 dB; -2 dB] range; areas of interest for further analysis are highlighted in panel 1 with a white (early low-frequency response) and black arrow (late high-frequency response). The fourth panel shows the average ITC calculated for all 52 participants to help the reader to identify the area where the N2P2 component is commonly found.

In Figure 3, elite endurance athletes elicited a higher EEG-based response to noxious contact heat stimuli in the EEG. The maximum ERSP value for this response was 6.71 dB at 3.49 Hz and 582 ms in the control group and 7.45 dB at 4.46 Hz and 547 ms in the elite endurance athletes' group. This response had the highest average ITC, with a maximum value of 0.67. There was a significant difference with a maximum AUROC value of 0.73 [0.57; 0.87] at 6.41 Hz and 656 ms, indicating a fair effect between both groups.

Conditioned pain modulation (CPM): assessing the endogenous pain inhibition mechanisms

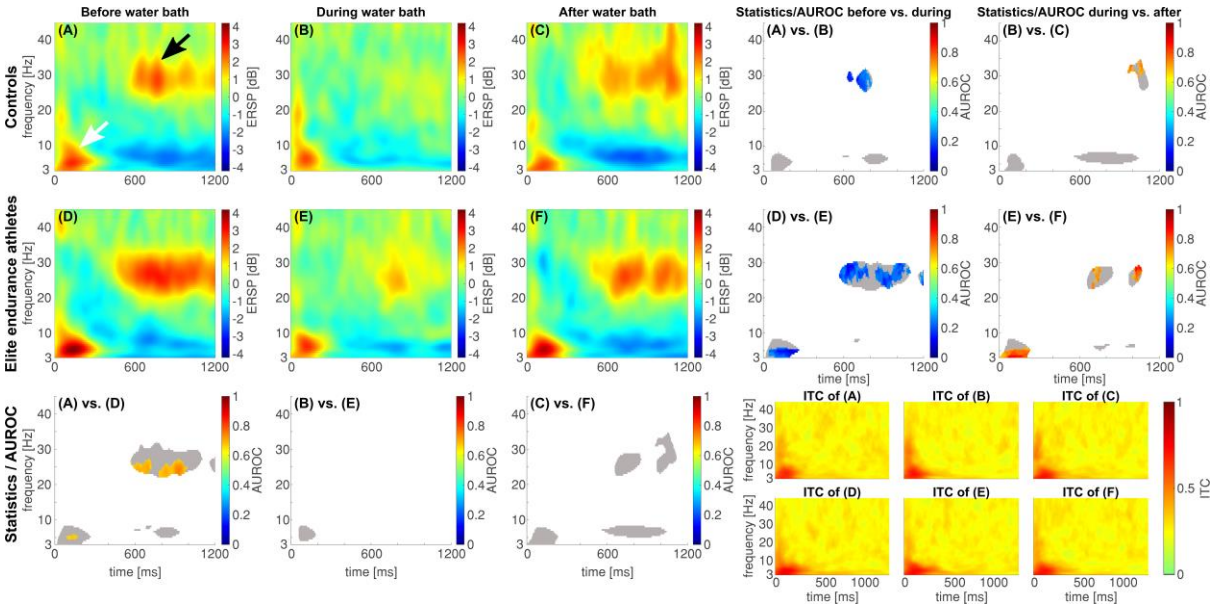

Figure 4: The conditioning stimulus during conditioned pain modulation testing affects the N2P2 component only in the elite endurance athletes, but not in the controls. A noxious cold water bath at 8°C was used as the conditioning stimulus (CS). The event-related spectral perturbation (ERSP) of pinprick-evoked Potentials (PEP as the test stimulus) plotted before, during and after the conditioning stimulus for the controls (A, B and C) as well as for the elite endurance athletes (D, E and F). The right panels show a statistical comparison between the conditions before vs. during and during vs. after for both groups; the bottom panel shows a group comparison between the groups for each condition. The gray shaded area in the statistics image indicates that the accompanying pixel in the ERSP in either one of the groups, or conditions, exceeds a [2 dB; -2 dB] range, and is considered an EEG response to the stimulus. Furthermore, our areas of interest are highlighted in panel 1 with a white and black arrow (white arrow for the early low-frequency response, black arrow for the late high-frequency response). The statistical comparison includes both a common non-parametric statistical testing (paired for intra-group testing and unpaired for inter-group testing) with a cluster-based correction for multiple comparisons and the AUROC effect size. Pixels that are significantly different are colored red/orange or blue, according to the c-axis next to the image, which indicates the AUROC effect size. The lower right panels show the respective inter-trial coherence (ITC) for each condition and group.

The low-frequency, highly phase-locked response to the test stimulus was only significantly affected by the conditioning stimulus in the elite endurance athletes' group, but not in the control group, as shown in Figure 4. During the conditioning stimulus in the elite endurance athletes' group, the maximum value of this highly phase-locked response was significantly reduced from 4.19 dB in (D) to 2.81 dB in (E) and increased to 3.76 dB thereafter (F). The minimum value of the AUROC was 0.08 [0; 0.19] at 4.95 Hz and 187 ms (D vs. E), while the maximum AUROC value was 0.92 [0.81; 1] at 3.0 Hz and 74 ms (E vs. F), both indicating an excellent effect between the two conditions.

In the control group, the changes in the ERSP of the low-frequency, highly phase-locked response were not statistically significant during our CPM testing. The maximum ERSP value

decreased from 2.84 dB in (A) before the application of the cold pressor task to 2.55 dB in (B) during the cold pressor task and increased to 2.58 dB in (C) thereafter.

The maximum ERSP values of the low-frequency, highly phase-locked response during the before-condition were 2.84 dB at 4.95 Hz at 129 ms in the control group, and 4.19 dB at 4.95 Hz and 133 ms in the elite endurance athletes' group. The difference between these was significant; the maximum AUROC value was 0.68 [0.53; 0.82] at 4.95 Hz and 133 ms between the groups (A vs. D), indicating a poor effect.

Our conditioning stimulus affected the response in the higher frequency regions in both groups (black arrow). The maximum ERSP values before the water bath were 2.62 dB for the controls and 2.99 dB for the elite endurance athletes. The difference between these was significant; the maximum value of the AUROC was 0.75 [0.61; 0.89] at 23.96 Hz and 930 ms between the groups before the cold pressor task (A vs. D), indicating a fair effect.

The maximum ERSP values of the high-frequency response during the ongoing cold pressor task decreased to 0.75 dB in the controls and 1.73 dB in the elite endurance athletes. The decrease of both responses was significant in both groups, with a minimum AUROC effect size of 0.12 [0; 0.23] at 29.80 Hz and 672 ms in the control group (A vs. B, good effect) and a minimum AUROC effect size of 0.08 [0; 0.19] at 28.83 Hz and 625 ms in the elite endurance athletes (D vs. E, excellent effect).

One minute after the cold pressor task, the ERSP of the high-frequency response increased to a maximum value of 2.36 dB in the controls and 2.62 dB in the elite endurance athletes. This increase is significant in both groups, with a maximum AUROC effect size of 0.77 [0.58; 0.92] at 31.76 Hz and 973 ms in the controls (B vs. C, fair effect) and a maximum AUROC effect size of 0.88 [0.77; 1] at 27.86 Hz and 1020 ms in the elite endurance athletes (E vs. F, good effect).

Discussion

Subjective pain perception

The consensus in literature suggests that with growing age, the pain thresholds of elite athletes increases and pain is increasingly tolerated, i.e., the subjective response to a noxious stimulus is silenced (Pettersen et al. 2020). From our data and for our noxious stimulation methods, we

could not conclude this in our cohort of elite athletes with a median age in their mid-twenties. The subjective pain perception of noxious events in our cohort of 18 – 35-year-old participants did not differ between participants with a recreational training level and the elite level when using brief, noxious, tonic stimulation. As this contradicts the findings of most of the available literature, other factors may have played a role in our study: our small sample size per group, a high interindividual variability, and our choice of stimuli may have prevented us from unmasking differences in the subjective pain perception. After all, a significant difference in the initial pain rating of the conditioning stimulus indicated that our elite athletes are, at least in the case of a noxious cold water bath, more pain-resilient than the controls. Hence, our brief, tonic, noxious stimulation may be a good way to research the nociceptive processing in the EEG, but not a suitable way to test for differences in the subjective pain ratings, for which a full somatosensory testing panel such as quantitative sensory testing (QST) would be the more appropriate research method.

Differences in EEG-based processing of standardized noxious stimuli during the resting state

A higher activation in an area with a high degree of phase locking in the elite endurance athletes' group may be interpreted as a sign of a central sensitization to noxious stimuli, i.e., the stimulus activates the central processing units in the S2 region of the brain in a stronger fashion in the elite endurance athletes' group than in the control group. Pinprick evoked potentials (PEPs) have been demonstrated to be an objective tool to quantify the effect of an experimentally induced secondary mechanical hyperalgesia and have been suggested to be a viable diagnostic tool for mechanical hyperalgesia for patients with a presumed central sensitization (Iannetti et al. 2013; van den Broeke et al. 2015; van den Broeke et al. 2017). From our data, a central sensitization or a hyperalgesia could not be concluded from the subjective pain ratings. Thus, although the theory of a central sensitization cannot be confirmed by the subjective pain ratings, there are some methodological aspects regarding the type of stimuli, the sample size, and the interindividual variability that need to be accounted for as discussed in the previous paragraph. Hence, we still propose a central sensitization of our elite endurance athletes as a possible reason for the significant increase in the EEG response, which may not be unmasked by our subjective pain testing due to methodological limitations. This theory is also backed up by the pain history in Table 1: although there was no difference in the recurrence of chronic pain in both groups, our athletes still suffered from sport-associated pain

states more frequently. Recurring pain has been shown to induce neuroplastic changes in both the brain and the spinal cord, and literature clearly proves that it leads to central sensitization (Latremoliere and Woolf 2009; Nijs et al. 2021).

Another possible explanation for the higher EEG-based activation of the athletes is that ERPs or the corresponding N2P2-related component in the ERSP, as elicited by transient nociceptive stimuli, are mostly determined by two factors: the painfulness of the stimulus, and by the stimulus salience (Iannetti et al. 2008; Ronga et al. 2013). The salience is the property of a stimulus of how much it can capture attention, i.e., how much focus the participant will pay to the stimulus. If we account for the fact that elite endurance athletes perceived the stimulus as equally as painful as the control group, another likely explanation would be an increased salience to noxious mechanical and heat stimulation, as represented by the increased activation of the N2P2-representing component in the ERSP of the EEG. This may indicate as some sort of "priming" of the athletes to noxious events and subsequently, pain, to which they are somewhat used to due to their sports career (Fett et al. 2017; Farahbakhsh et al. 2018; Bumann et al. 2020). Eventually, this process may lead to coping strategies such as suppressing the subjective pain response, as shown by other studies (Pettersen et al. 2020).

Interestingly, a recent study that researched the pain perception of elite endurance athletes using the fMRI seems contradicting to our data (Geisler et al. 2021), as for elite endurance athletes compared to a sedentary control group, their data revealed a significantly reduced response to a noxious heat stimulus in cortical regions such as the insula and the anterior cingulate cortex. These regions are usually also captured by our EEG methodology. However, not only is there a difference in taking either a sedentary versus a normally active control group and some individual researchers even recommend using only physically/normally active control groups (Booth and Lees 2006; Buford and Manini 2010). A sedentary lifestyle has been shown to be a factor in the development of chronic pain (Senba and Kami 2017), which would further add confounding factors to the interpretation of our data. As stated in the introduction, the fMRI and the EEG also excel in different areas regarding spatial and temporal resolution. Using the fMRI, a width of the BOLD response of ~3 s and a peak that occurs ~5-6 s after the onset of a brief stimulus are common (Kim et al. 1997; Glover 2011), so that the very early processes in the ranger of milliseconds after a brief noxious stimulus that we captured in this manuscript cannot be analyzed. Unsurprisingly, the researchers thus also relied on long-acting noxious thermal stimuli (20 s), which are vastly different from the brief stimuli applied in this manuscript. A combined fMRI/EEG approach is an interesting approach for future research

about the differences in processing of short- and long-acting noxious stimuli in endurance athletes.

For the brief noxious stimuli in our data, both a presumed central sensitization and/or an altered stimulus salience were a likely explanation for the observed statistical differences. Our conclusion is limited by the methodological choices, as the exact reason for the altered response cannot be answered by only using EEG data. Hence, future studies may consider our postulated explanations in their research, e.g., by incorporating the combination of the EEG and fMRI into the study design.

Conditioned Pain Modulation

A recent meta-analysis about conditioned pain modulation in elite endurance athletes (McDougall et al. 2020) concluded that aggregated results, despite a higher nominal number of studies reporting higher CPM capacities in athletes, do not favor a significant difference. While a possible correlation between training hours and CPM capacities is suggested, the meta-analysis also points out the methodologically low quality of several studies. The conclusion is, however, further supported by preclinical studies (Sluka et al. 2018), but it is not in-line with our findings. We will now discuss that this is probably also due to methodological issues but also offer a hypothetical explanation for our data. Overall, as stated in the meta-analysis, higher quality CPM data will be needed to analyse the full extent of the modulatory effects of endurance sports on endogenous pain inhibition. Our following discussion about our own methodological issues may be considered by future studies to achieve the necessary higher quality in CPM testing of elite endurance athletes.

In our data, the conditioning stimulus significantly reduced the subjective pain ratings of the test stimulus only in the controls' group. By design of the CPM paradigm, this is the expected effect: the pain rating of a test stimulus is significantly reduced due to the activation of the endogenous pain inhibitory system by a conditioning stimulus (Nir and Yarnitsky 2015). We observed no such significant effect in the elite endurance athletes' group. This may at least partially be explained by the perceived painfulness of the conditioning stimulus: our data in Table 1 shows that, although perceived as painful by both groups, the stimulus is significantly less perceived as painful in the elite endurance athletes' group. The implies that their endogenous pain inhibitory system is less activated. Our analysis may thus be methodologically constrained by this factor, as we did not equally activate the endogenous pain inhibitory system.

By using a constant stimulus energy, i.e., the same water bath temperature for both groups, this result may be expectable in elite endurance athletes: the available literature about pain thresholds in elite endurance athletes reports higher pain thresholds than in normally active controls (Pettersen et al. 2020). To achieve a comparable level of conditioning pain levels, an adaption of the temperature of the water bath to a target pain score may have been necessary. Another possible methodological limitation could also be the use of a 512 mN pinprick as a test stimulus, as this has not been reliably tested in literature.

A different possible explanation is the pain history of our athletes' group: our data in Table 1 indicated that our athletes, as compared to our controls, are significantly more affected by sports-related injuries and pain and show a significantly higher current pain intensity as well as a significantly higher average pain intensity in the past 3 months. In addition, as discussed above, a central sensitization may be one possible explanation for our elevated EEG response to noxious stimulation in the athletes' group. Although the recurrence of chronic pain is not significantly higher in the athletes' group, it has been shown that ongoing pain impairs the response to a conditioning stimulus during CPM testing (Lewis et al. 2012). This increased recurrence of pain may thus be a facilitating factor that leads to a loss of descending pain inhibitory function or an increase in descending facilitation of spinal nociceptive pathways (Bannister and Dickenson 2017), which would explain the absence of a CPM effect in our athletes' group.

Limitations

Due to our conservative statistical approach, we may have only captured strong effects between the groups and more subtle differences may need to be further investigated. As this was an explorative translational study, we did not perform a conventional a-priori sample size calculation (Bacchetti et al. 2011), as no appropriate preliminary data has been published yet. In combination with our small sample size, this may have limited us to capture statistical differences between the groups especially regarding the subjective pain ratings. Furthermore, we only analyzed the pain perception using a standardized VAS to two different stimuli and did not assess a complete somatosensory profile. For our analysis, we separated our groups into elite endurance athletes and non-athletes in a binary fashion, without taking the exact individual performance level into account. Studies that aim at determining the exact nature of this "dose-response" relationship in the future should rely on cardiopulmonary exercise testing. Furthermore, we only assessed the activity level of our control group by means of a subjective

self-report in predefined categories (e.g., strength, endurance), which did not reveal the exact type and intensity of activity. Our results from the conditioned pain modulation testing are limited by our methodology.

Methods

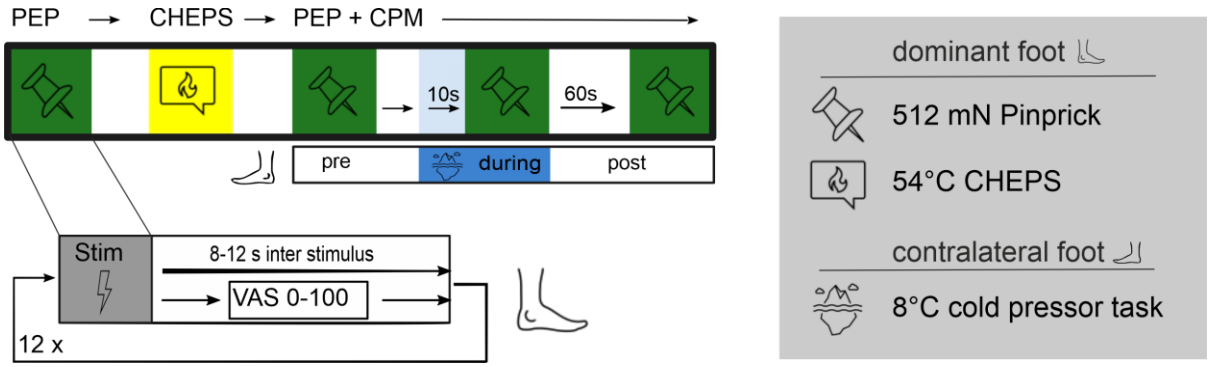

Figure 5: Study flow for each participant.

Participants

The local ethics committee approved the study procedures in a written statement on 14/07/2020 (Ethics Committee of the Faculty of Psychology and Sports Sciences at Goethe University Frankfurt, reference number 2020-40). The participants received a compensation of 30€ for their successful participation. Furthermore, we conformed to the standards set by the Declaration of Helsinki and prospectively registered the study on 24/07/2020 with the WHO-approved German Clinical Trials Register (DRKS) (DRKS-ID: DRKS00022349). The study was carried out in an institution certified for QST assessment.

Two groups of competitive elite endurance athletes and regularly active, non-elite controls were included in this study. To define the characteristics of an elite athlete, we followed the criteria published by Swann (Swann et al. 2015). Each participant underwent the same study flow as outlined in Figure 5. The main inclusion criterion for the elite endurance athletes was a regular training for national or international competitions in their respective type of endurance sport with a training load of at least 15 hours per week for the past 2 years. This was based on the average total training time of a German elite athlete (Breuer and Wicker 2010). In contrast, participants in our control group did not engage competitively in elite endurance sports on a national or international level. We required them to have never partaken competitively in any type of sports at elite level with a training load greater than 15 hour per week. Participants in

both groups reported their weekly hours of training load in terms of the American College of Sports Medicines' definition of exercise (Pescatello and Wilkins 2014).

General inclusion criteria were a minimum age of 18 years and a maximum age of 35 years, as we relied on the QST reference values for that age group (Magerl et al. 2010). We further required no regular intake of pain medication, the absence of sensory disorders (peripheral neuropathies or neuropathic pain), the absence of depression and no intake of antidepressants, no current intake of antipsychotics and no known autoimmune diseases or pregnancy. We recruited an equal number of males and females in each group and matched those participants by age. In addition, we asked all the participants to refrain from excessive physical activity (e.g., taking part in competitions) and the intake of pain medication 24 hours prior to the study. Written informed consent was sought from all the participants prior to enrollment.

Visual analog scale (VAS)

For subjective pain ratings, we used a tablet (Apple iPad mini) with a visual analog scale app (Apple App Store: "VAS - Visual Analog Scale" by Herve Kasparian D.O. and Ghislaine Signoret D.O., Cabinet d'ostéopathie Kasparian-Signoret, France). The app consisted of a slider with a red triangle underneath and a scale ranging from no pain (left, "0") to worst pain (right, "100"). Visual ratings corresponded to ratings from 0 (no pain) to 100 (worst pain) with graduations of 1. We presented the tablet to the participants with the slider in the left position. The numerical expression recorded could only be viewed by the examiner. According to the QST protocol (Rolke et al. 2006), the participants were instructed to move the slider to a position greater than "0" if a sensation was experienced as being painful.

Standardized noxious stimulation

For standardized noxious stimulation, we used mechanical and thermal stimuli derived from the QST protocol (Rolke et al. 2006). We decided to use a fixed stimulus intensity on each participant by applying a fixed mechanical force or stimulating with a constant peak contact heat temperature rather than determining each participants' individual threshold. This allowed for a robust inter-group comparison as we kept the stimulus energy constant and eliminated the influence of fluctuations in stimulus energy on our EEG response. In addition, the recent studies that published normative data for CHEPS also administered a fixed peak stimulation

temperature (Granovsky et al. 2016; Jutzeler et al. 2016; Rosner et al. 2018). In order to ensure that our stimuli were perceived as painful by our healthy participants, we set the fixed stimulus intensity according to the QST reference values as described in the following paragraphs (Magerl et al. 2010). In order to avoid peripheral sensitization or stimulus wind up due to repetitive stimulation, both mechanical and contact heat stimulation were applied 12 times with a randomized or pseudo-randomized inter-stimulus interval. The number of trials in literature ranges from 7 trials (Granovsky et al. 2016; Anders et al. 2022) up to 20 trials (Rosner et al. 2018). We stimulated an area of 9 cm x 8 cm on the dorsal area of the dominant foot and applied the stimuli in a randomized pattern across the whole stimulation area. We asked the participants to rate each stimulus approximately 2 seconds after onset on the VAS. We verbally announced every single stimulation with the pinprick to the participant with a trigger word, approximately 1 second prior to the stimulus. We asked the participants to keep their eyes open during the test and to avoid blinking for 2 seconds directly after the trigger word and to be alert on the upcoming noxious stimulus.

Pinprick evoked potentials (PEP)

We used a pinprick (MRC Systems, Heidelberg, Germany) with a force of 512 mN to selectively activate both A- and C-fiber mechanosensitive nociceptors (Ziegler et al. 1999; Magerl et al. 2001; van den Broeke et al. 2015). The force of 512 mN was chosen as it is above the 95% confidence interval of the mechanical pain threshold (MPT) in the QST reference data for feet stimulation in the age range of 15 – 35 years and should thus be perceived as painful by a healthy participant (Magerl et al. 2010). The mean values are 73.02 mN (10.97 mN; 486.09 mN) for males and 69.39 mN (9.92 mN; 485.16 mN) for females, with the 95% confidence interval in brackets (Magerl et al. 2010).

We modified the pinprick to generate a 5 V TTL trigger pulse for our EEG recordings by drilling two opposite holes into the stationary holding tube right above the moving weight, as it is described in literature (Iannetti et al. 2013). We equipped the holes with a photodiode and a phototransistor in a way that the photoactive parts were facing each other. When we applied the pinprick onto the skin, the weight was moved upwards and disrupted the visual connection between the sensor/emitter pair. The 5 V TTL trigger pulse was then generated via an LM393 (Texas Instruments, Dallas, United States of America) and an ATmega32U4 (Microchip, Chandler, United States of America). We programmed the Integrated Circuit (IC) using the

Arduino IDE (Arduino, Somerville, United States of America). We randomized the inter-stimulus interval between 8 s and 12 s.

Contact heat evoked potentials (CHEPS)

We again stimulated the dorsal area of the dominant foot and applied thermal stimuli using a MEDOC PATHWAY Pain and Sensory Evaluation System (Medoc Limited, Ramat Yishai, Israel) which we connected with its 5 V TTL trigger-output to our EEG. The thermal probe for recording CHEPS delivers short heat bursts by increasing its temperature at a fixed rate of 70°C/s and selectively activates A- and C fiber nociceptors if an adequate peak temperature is chosen (Madsen et al. 2012; Rosner et al. 2018). The contact area of the CHEPS thermode is circular, with a diameter of 27 mm. We pseudo-randomized inter-stimulus interval between 8 s and 12 s. We set our baseline temperature to 32°C and our peak temperature to 54°C. The peak temperature of 54°C was chosen as it is above the 95% confidence interval of the heat pain threshold (HPT) in the QST reference data for feet stimulation in the age range of 15 – 35 years and should thus be perceived as painful by a healthy participant (Magerl et al. 2010). The mean values are 45.12 °C (40.42 °C; 49.81 °C) for males and 43.69 °C (38.20 °C; 49.19 °C) for females, with the 95% confidence interval in brackets (Magerl et al. 2010).

Conditioned pain modulation (CPM) with PEP as readout

As a CS, we used a cold water bath, which we kept at 8 °C using ice packs. We applied the conditioning stimulus by having the participants submerge their non-dominant foot into the cold water. We asked the participants to rate the initial painfulness on the VAS right after inserting their foot into the cold water bath.

We then applied the TS on the same stimulation area as before (dorsal area of the dominant foot). We used our modified pinprick as outlined in the methods section for “PEP” with 12 stimuli in the same area. The only difference to the PEP was a reduced inter-stimulus interval which we randomized between 3 – 5 s and a summarized VAS rating after a twelfth stimulus (i.e., not every single stimulus was rated on the VAS). We recorded PEPs three times: (1) as a baseline recording before applying the CS, (2) ten seconds after the participants submerged their foot into the cold water bath, while having the foot submerged during the whole application of the TS and (3) sixty seconds after the participants took their foot out of the cold

water bath. Other settings and sequences were carried out as described in the methods section for “PEP”.

EEG recording and pre-processing

The study took place with each participant sitting, in a quiet room. We asked the participant to place their dominant leg on a height-matched rack to allow for comfortable sitting during the whole study period. The investigators equipped the participants with a 64-channel (g.Tec g.SCARABEO, Guger Technologies, Schiedlberg, Austria) EEG cap (g.Tec g.GAMMAcap²). We decided to use active EEG electrodes to guarantee for an exceptionally low output impedance (below 1 Ω) and to minimize artifacts from movement of the electrode cables.

After recording the raw EEG in g.Tec’s proprietary .hdf5 format and storing it offline, we imported it into the MATLAB toolbox EEGLAB (Delorme and Makeig 2004). We down-sampled our EEGs to 256 Hz for the purpose of data reduction by utilizing EEGLAB’s function *pop_resample*. This function automatically applies the necessary low-pass filter. We applied a zero-phase bandpass filter by utilizing EEGLAB’s *eegfiltnew* function between 1 Hz and 100 Hz and reduced line noise at 50 Hz with the EEGLAB CleanLine plugin. By visually inspecting our datasets, we removed corrupted channels (e.g., due to electrode popping) and interpolated them via spherical spline interpolation (Ferree 2006). On average, we rejected and interpolated 4 channels, while our area of interest (the Cz electrode) was never affected. By subsequently utilizing Artifact Subspace Reconstruction (ASR) with a tolerance parameter of 20, we applied an automated artifact rejection routine to our datasets to eliminate artifacts such as eye blinks and jaw clenching (Chang et al. 2018). As a last step, we epoched our data from -1 s before the onset of each stimulus to +2 s after the onset of each stimulus.

We calculated the Event-Related Spectral Perturbation (ERSP) and the Inter-Trial Coherence (ITC) using EEGLAB's *newtimef*-function with a divisive baseline from -1 s to 0 s, a resolution in time of 400 points from -1 s to +2 s and a frequency resolution of 200 points between the frequencies of 3 Hz and 100 Hz (Grandchamp and Delorme 2011; Herrmann et al. 2014). The function *newtimef*, which incorporates both a wavelet transform and a short-term Fourier transform, ran with 3 cycles at the lowest frequency (3 Hz) and 20 cycles at the highest frequency (100 Hz). Our electrode of interest for EEG analysis was the Cz (Iannetti et al. 2013; Granovsky et al. 2016; Anders et al. 2020). For comprehensibility, we show the ERSP between 3 Hz and 45 Hz. In the ERSP, to be considered a response to a stimulus, we set a threshold for

the changes in EEG power of [-2 dB; 2 dB] and outlined areas that exceeded that threshold with a grey area in our figures.

Fitness testing

We obtained the participants' heart rates following the Astrand Rhyming Step test as a derivate of the submaximal heart rate of the participants. We additionally evaluated the heart rate at the beginning and at the end of the EEG measurement as an estimation of the resting heart rate in order to classify the performance level and cardiovascular capacity of the participants. The Astrand Rhyming Step test is a valid and reliable submaximal variation of the Harvard test (Marley and Linnerud 1976).

For the fitness test, the participants must alternately climb a step with a gender-adjusted height for five minutes at a predetermined frequency of 90 steps per minute. We ensured the observance of the beat with an acoustic signal (metronome). The height for women was 33 cm, while for men this was 40 cm. Fifteen seconds after the measurement, we measured the participants' heart rates manually at the wrist.

Statistics

Due to our small sample size (Mishra et al. 2019) and because of its suitability for the analysis of EEG data (Maris and Oostenveld 2007), we adhered to a non-parametrical statistical approach throughout our analysis. In order to statistically evaluate possible differences between the groups (ERSP and VAS) we calculated the area under the receiver operating characteristics (AUROC), together with 1000-fold bootstrapped 95% confidence intervals using the MES toolbox for MATLAB (Hentschke and Stüttgen 2011). An effect or difference can be considered significant if the 95% confidence interval for the AUROC does not include 0.5 (Hentschke and Stüttgen 2011). An AUROC = 0.5 indicates a completely random relationship, while an AUROC = 1 or AUROC = 0 indicates a perfect separation of the values between the groups, i.e., a perfect classifier (Jordan et al. 2010). According to the traditional point system, we reported effects presented as AUROC values as being excellent in the range of between 1 and 0.9, as good in the range of between 0.9 and 0.8, as fair in the range of between 0.8 and 0.7, as poor in the range of between 0.7 and 0.6 and as fail when they are below 0.6 (Tape 2001). For dependent data, we compared the relative change between two conditions versus a fixed value of 1 using the *auroc* function of the MES toolbox. For comprehensibility, we

extracted the maximum ERSP and AUROC values out of the mentioned regions to extract the most objective ERSP response that was not dependent on the chosen window size.

To account for multiple comparisons, instead of a common approach of an alpha level adjustment, we applied a cluster-based approach, as it has been used in literature both for 2-dimensional (Akeju et al. 2014; Kreuzer et al. 2020) and 3-dimensional (Lutz et al. 2022; Reiser et al. 2022) EEG data. We only reported results as being significant if they occurred in clusters of at least 4 x 4 adjacent significant pixels; this translates to a frequency range of 1.5 Hz and a time range of 15 ms.

We compared demographics between both groups (questionnaire scores and age) as well as the VAS scores using the Wilcoxon-Mann Whitney test, and binary questionnaire responses (Yes/No) using the Chi-squared test. Furthermore, we compared the VAS scores for the three different conditions during CPM testing (before, during and after the cold water bath) using the Friedman's test. For posthoc testing, we utilized the Matlab function *multcompare*. For all median values, we show the 25% and the 75% interquartile ranges in square brackets.

Data and code availability statements

The datasets generated during and/or analysed during the current study are not publicly available due to the need for a formal data sharing agreement but are available from the corresponding author on reasonable request.

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11 Deutsche Zusammenfassung

Die vorliegende Thesis untersucht die Möglichkeit, inwieweit das Elektroenzephalogramm (EEG) in der Lage ist, Schmerz und Nozizeption zu messen, zu quantifizieren, und darzustellen. Schmerz ist eine stark subjektive Empfindung und nicht zwingend das Ergebnis von Nozizeption. Die Charakterisierung und Behandlung von Schmerz ist anspruchsvoll, da es große Unterschiede in der beschriebenen Qualität und Quantität von Schmerz zwischen einzelnen Individuen gibt. Aus diesem Grund wurde in den letzten Jahrzehnten immer wieder versucht, Nozizeption und vor allem Schmerz zu quantifizieren, um eine Vergleichbarkeit der Werte zwischen Proband*innen oder Patient*innen zu ermöglichen. Die derzeit valideste Methodik ist eine einfache verbale oder visuelle subjektive Schmerzskala, bei der der/die Proband*in oder Patient*in seine individuelle Empfindung mittels einer Zahl einschätzt. Dies funktioniert für akuten oder chronischen klinischen Schmerz, der beispielsweise als Reaktion auf eine Verletzung oder ein Trauma auftritt, oder für neuropathische Schmerzen. Eine Erweiterung der einfachen subjektiven Schmerzskala ist die Quantitativ Sensorische Testung (QST). Diese bedient sich standardisierter nozizeptiver und schmerzhafter Reize, die von den Proband*innen oder Patient*innen subjektiv bewertet werden. Durch gute Datenlagen gesunder Vergleichskohorten lassen sich die Reaktionen der Proband*innen/Patient*innen hinsichtlich des Normbereichs bewerten, welcher bedingt durch die beschriebenen interindividuellen Variationen allerdings recht groß ist. Dies führt dazu, dass teilweise nur Extremfälle als pathologisch erkannt werden. Verständlicherweise wird in der Wissenschaft nach Alternativen gesucht, welche im Idealfall als robuster und reproduzierbarer Biomarker für Schmerz oder als Ergänzung zur etablierten Schmerzskala fungieren könnten.

Bereits lange ist bekannt, dass bestimmte somatosensorische Reize eine messbare Antwort im EEG evozieren. Diese Reize können dabei von verschiedener Qualität sein, sei es auditorisch, visuell, oder auch nozizeptiv bzw. schmerzhaft. Sie unterliegen alle einer gewissen Anforderung an Dauer und Intensität: gemessen werden können nur sogenannte „time-locked“-Reize, also kurze Reize, deren Beginn millisekundengenau mit der Aufnahme des EEGs synchronisiert ist. Die dabei entstehende Signatur ist bei den verschiedenen Reizqualitäten zumindest vergleichbar. Dies wirft bei schmerzhaften Reizen die Frage auf, wie schmerzspezifisch die Antwort ist und welche Faktoren noch einen Einfluss haben. Eine weitere Voraussetzung ist, dass die Reize wiederholt appliziert werden. Nur so ist die evozierte Antwort im EEG sichtbar, da sie sich von der cortikalen Grundaktivität abhebt.

Das Ziel der vorliegenden Thesis war es zu überprüfen, unter welchen Bedingungen sich Schmerz und Nozizeption im EEG darstellen lassen, und inwieweit die im EEG gemessenen evozierten Signaturen spezifisch für Schmerz sind. Dazu wurden drei Studien mit jeweils eigenen Hypothesen durchgeführt. Aus der Gesamtheit der Ergebnisse konnte so eine Schlussfolgerung für die Frage gezogen werden, ob

das EEG in Kombination mit standardisierten nozizeptiven bzw. schmerzhaften Reizen ein robuster, reproduzierbarer und holistischer Biomarker für Schmerz ist.

In der ersten Studie wurde die Methodik der Messung von Schmerz bzw. Nozizeption im EEG etabliert. Dazu wurden 21 gesunde Proband*innen ab 18 Jahren aller Geschlechter rekrutiert, die in einer weiteren Studie als gesunde Kontrollgruppe fungierten. Weiterhin wurden verschiedene Parameter sowohl für die Aufnahme der EEGs als auch für die standardisierte schmerzhafte Reizung verwendet. Zusammengefasst durchliefen alle Proband*innen die Stimulation mittels sogenannter kontakthitzeevozierter Potenziale. Beim oben angesprochenen somatosensorischen Reiz handelte es sich in diesem Fall also um kurze, schmerzhafte Kontakthitzestöße, bei denen eine Maximaltemperatur von 54°C eingestellt wurde. Auf Basis der QST-Normdaten ist davon auszugehen, dass ein gesunder Mensch, egal welchen Alters oder Geschlechts, den Reiz ab einer Kontakthitzetemperatur von 50 °C als schmerzhaft empfindet. Ergebnisse aus in-vitro Studien haben gezeigt, dass sich hitzesensitive Rezeptoren wie der TRPV1 bereits ab einer Temperatur von 45 °C aktivieren lassen. Insgesamt wurden 7 dieser Reize auf die Unterseite des dominanten Unterarmes appliziert, wobei zwischen zwei Reizen ein Intervall von 40 Sekunden ohne Stimulation lag. Die Probanden bewerteten jeden einzelnen Reiz auf einer verbalen Schmerzskala zwischen 0 (kein Schmerz) und 100 (individuell maximal vorstellbarer Schmerz). Die Thermode wurde über die gesamte Stimulation an der gleichen Stelle belassen. Während der gesamten Messung wurde ein EEG aufgenommen und jeder einzelne Reiz millisekundengenau im EEG markiert. Primäres Ziel der Studie war es herauszufinden, ob die von uns gewählten Parameter zu einer sichtbaren evozierten Antwort im EEG führten. Als sekundäres Ziel haben wir verschiedene Parameter der EEG-Aufnahme sowie EEG-Analyse ausprobiert und untersucht. In der Etablierungsstudie gelang es uns, mit den gewählten Parametern die evozierte Antwort im EEG auf standardisierte, schmerzhafte Kontakthitzereize im Zeit-Amplituden-Spektrum darzustellen und diese mit den subjektiven Schmerzscores der Probanden auf die Reize in Bezug zu setzen. Die methodischen Erkenntnisse aus der Studie führten zu hilfreichen Erfahrungen, die in den weiteren Studien im Design berücksichtigt werden konnten. Dies bezieht sich insbesondere auf die Art der Stimulation als auch die Aufnahme und Auswertung der EEGs. Zusammengefasst lässt sich sagen, dass 54 °C Kontakthitzereize in den meisten Fällen zu einer reproduzierbaren evozierten Antwort im EEG führen.

In der zweiten, anwendungsbezogenen Studie, die das Akronym IMPACE trägt, wurden 17 Patient*innen im Rahmen eines chirurgischen Routineeingriffes an dem Universitätsklinikum Frankfurt rekrutiert. Ziel der Studie war es zu überprüfen, ob das EEG eine geeignete nicht-invasive Methode zum intraoperativen Monitoring von Schmerz, Nozizeption und Analgesie darstellt. Dazu wurde

untersucht, ob die evozierten EEG-Signaturen, welche in der Etablierungsstudie erarbeitet wurden auch noch nach der Gabe klinischer Dosen des Narkotikums Propofol sowie des Analgetikums Remifantil (sowohl nach alleiniger Gabe, „mono“, als auch in Kombination) reproduzierbar darstellbar sind. Als Schmerzreiz dienten in dieser Studie anstatt der Kontakthitzereize aus der Etablierungsstudie schmerzhafte Konstantstrom-Reize, welche von den Patient*innen im wachen Zustand auf einer subjektiven Schmerzskala durchschnittlich mit einem Wert von 60 aus 100 bewertet wurden. Die Patient*innen wurden im Rahmen der klinischen Routine rekrutiert und waren alle für eine Trauma-Operation mit einem niedrig zu erwartendem Risiko eingeplant. Das Narkoseschema wurde dabei via sogenannter Target Controlled Infusion (TCI) gesteuert, bei der die Effektorgankonzentrationen der Medikamente Propofol und Remifentanil anhand des Geschlechtes, der Körpergröße und des Gewichts modelliert werden. Die Zielkonzentrationen orientierten sich dabei am klinischen Standard des Universitätsklinikums Frankfurt und wurden für die klinische Beobachtung nicht angepasst. Das Studiendesign unterscheidet sich dabei deutlich von dem einer kontrollierten klinischen Studie, da das Hauptziel einer klinischen Anästhesie der zeitnahe Verlust von Bewusstsein, Schutzreflexen und Schmerzempfinden ist. Insgesamt wurden den Patient*innen dadurch deutlich schneller deutlich höhere Dosen an Narkotika verabreicht.

Es stellte sich heraus, dass die alleinige Gabe von Remifentanil zu einer nicht-signifikanten Reduktion insbesondere der sogenannten evozierten N2-Komponente als Antwort auf den schmerzhaften Elektrostimulus führte. Die subjektiven Schmerzscores nahmen im Vergleich ebenfalls nicht-signifikant ab. Die alleinige Gabe von Propofol führte wie erwartet zum Bewusstseinsverlust, sodass keine subjektiven Schmerzscores mehr erhoben werden konnten, als auch zum vollständigen Verschwinden der evozierten Antwort im EEG. Nach der Kombination von Remifentanil und Propofol im Rahmen einer stabilen Vollnarkose konnte ebenfalls keine EEG-basierte Antwort mehr abgeleitet werden. Auch der stark schmerzhafte tetanische Reiz (1500 Elektroschocks innerhalb von 30 Sekunden mit einer Stärke von 50 mA) führte nicht zu einer reproduzierbaren und robusten Veränderung im EEG, wie sie bei einem Biomarker notwendig wäre. Es lässt sich feststellen, dass Propofol, welches keinerlei relevanten analgetischen Eigenschaften besitzt, die Verwendung des EEGs als Biomarker für Schmerz nach standardisierter schmerzhafter tonischer Stimulation verhindert. Die Ableitung jeglicher evozierter Antworten im klinischen Patienten sowohl im Zeit-Amplituden-Spektrum als auch im Zeit-Frequenz-Spektrum schlägt durch Applikation des Narkotikums fehl. Auf Basis unserer Daten lässt sich der Grund für diesen Effekt nur spekulativ beantworten: die hier vorgestellten EEG-Antworten werden cortikal abgeleitet, und ein Bestandteil der Schmerzantwort ist die Weiterleitung von Schmerzinformationen vom Thalamus zum sensorischen Cortex über Aktionspotenziale. Werden diese Aktionspotenziale durch Substanzen, welche inhibierende Neurone aktivieren, gedämpft oder ausgeschaltet, so kann auch kein cortikales Potenzial mehr abgeleitet werden. Es wurde gezeigt, dass

Propofol in die Kommunikation zwischen Thalamus und Cortex („thalamocortical loop“) eingreift, wodurch beispielsweise auch die charakteristische frontale Alpha-Schwingung während einer Vollnarkose entsteht. Der genaue Wirkmechanismus von Propofol auf diese Kommunikation ist jedoch noch nicht bekannt. Selbst bei fehlender oder gestörter Kommunikation zwischen Thalamus und sensorischem Cortex finden aber weiterhin eine Vielzahl an Schmerzprozessen statt, welche vom EEG nicht erfasst werden. Zu nennen sind hier die Schmerzverarbeitung an Verschaltungsstellen vor dem Thalamus bzw. Cortex wie dem Rückenmark, oder auch Schmerzreflexe. Aus unserer Studie ergibt sich deshalb, dass das EEG einen Teilprozess der Schmerzverarbeitung abbildet. Diese Ableitung kann durch Substanzen wie Propofol, welches in diesen Teilprozess eingreifen, verhindert werden. Das EEG erfüllt also nicht den Anspruch an einen vollumfänglichen und reproduzierbaren Biomarker für Schmerz.

In der dritten Studie mit dem Akronym SPINE haben wir in einer Literaturrecherche Elite-Ausdauer-Leistungssportler als eine Proband*innen-Gruppe identifiziert bei der man annimmt, dass sich die Verarbeitung und Bewertung von Schmerz von der einer normalsportlichen Grundgesamtheit unterscheidet. In der vorhandenen Literatur wird darauf hingewiesen, dass Leistungssportler im Laufe ihrer Karriere in Bezug auf Schmerz deutlich resilienter werden. Es verschieben sich in mehreren Publikationen die Grenzwerte nach hinten, ab denen ein Reiz als schmerzhaft beschrieben wird. Es sollte analysiert werden, ob diese Unterschiede auch in der von uns rekrutierten Probandenkohorte messbar sind und sich im EEG darstellen lassen. Dazu haben wir 26 Elite-Ausdauer-Leistungssportler*innen rekrutiert, die auf kompetitivem Niveau einer der Ausdauer-Beinsportarten Rudern, Triathlon, Speedskating oder Laufen mit mindestens 15 Trainingswochenstunden nachgehen, sowie eine alters- und geschlechtergleiche normalsportliche Vergleichskontrollgruppe mit ebenfalls 26 Proband*innen, die lebenslang nie mehr als 9 Wochenstunden sportliches Training durchgeführt haben. Als standardisierte schmerzhafte Stimulation wandten wir zusätzlich zu den bereits vorgestellten Kontakthitze- und Elektroreizen, schmerzhafte mechanische Reize mittels Pinprick-Stimulator an. Dieser Stimulator wird auch in der QST-Testbatterie verwendet. Zusätzlich haben wir die Fähigkeit der endogenen, körpereigenen Schmerzunterdrückung mittels Conditoned Pain Modulation (CPM) zwischen den Gruppen verglichen. Beim CPM applizierten wir einen schmerzhaften mechanischen Testreiz mittels Pinprick und überprüften, ob ein schmerzhafter Konditionierungsstimulus (8 °C kaltes Wasserbad) an einer anderen Körperstelle zu einer Reduktion der Schmerzbewertung des Testreizes führt. Während der gesamten Studie wurde zusätzlich ein EEG mit den in der Etablierungsstudie erarbeiteten Parametern aufgenommen und ausgewertet.

Zusammengefasst lässt sich sagen, dass sich die subjektive Schmerzwahrnehmung der Leistungssportler nur im CPM, aber nicht im Ruhezustand nach standardisierter Schmerzstimulation von der der normalsportlichen Kontrollgruppe unterschied. Beim CPM wurde der Teststimulus nur von

der Kontrollgruppe, nicht jedoch von den Leistungssportlern während der Applikation des Konditionierungsstimulus als weniger schmerzhaft beschrieben. Im EEG zeigten sich jedoch signifikante Unterschiede: in allen Testparadigmen zeigten die Elite-Ausdauer-Leistungssportler im Vergleich zur normalsportlichen Kontrollgruppe eine deutlich stärkere evozierte Antwort im somatosensorischen Cortex auf die Reize. Dies interpretierten wir als ein erstes Anzeichen einer zentralen Sensitivierung, welche nicht in unseren subjektiven Schmerzscores sichtbar war. Die Gründe dafür waren höchst wahrscheinlich unsere geringe Gruppengröße (n=26), sowie die hohe Variabilität und geringe Robustheit subjektiver Quantifizierungsmethoden für Schmerz. Analog zu den Ergebnissen aus anderen Studien schlussfolgerten wir weiterhin, dass die Intensität der evozierten Antwort im EEG nicht nur von schmerzspezifischen Faktoren bestimmt wird. Einer dieser Faktoren ist die Salienz, also die Wahrnehmung des Reizes außerhalb des normalen Bewusstseins. Eine erhöhte Aktivierung im EEG in der Gruppe der Leistungssportler kann ein Indiz dafür sein, dass die Salienz eines Schmerzreizes bei Leistungssportlern signifikant erhöht ist. Im Bezug auf die endogene Modulation von Schmerz wäre ein logischer Rückschluss aus unseren Daten, dass unsere Kohorte an Leistungssportlern eine geringere Kapazität zur endogenen Schmerzmodulation besitzt. Da dies sich nicht mit den aktuellen Erkenntnissen aus der Literatur vereinbaren lässt, ist eine methodische Limitierung unserer Studie wahrscheinlich: der Teststimulus (Pinprick) ist noch nicht für die Nutzung innerhalb einer CPM-Testung validiert. Weiterhin wurde der Konditionierungsstimulus von den Athleten als signifikant weniger schmerzhaft beschrieben, sodass es wahrscheinlich ist, dass auch hier das endogene Schmerzmodulations-System geringer aktiviert wurde.

Auf Basis unserer Ergebnisse lässt sich schlussfolgern, dass das EEG keinen vollumfänglichen und robusten Biomarker für alle schmerzassoziierten Prozesse darstellt. Im klinischen Kontext soll mit weiteren Studien überprüft werden, ob beispielsweise andere Stimulationstechniken, oder sogar klinisch persistenter Schmerz im EEG reproduzierbar abbildbar und quantifizierbar ist. Die von uns verwendeten kurzen, tonischen Schmerzreize werden im EEG unter anderem auch durch die Salienz bestimmt. Trotzdem gibt es für die hier vorgestellten Methoden insbesondere in der pharmakologischen Forschung, sowie in Tiermodellen oder bei der Untersuchung nonverbaler Gruppen wie neugeborener Kinder, nachgewiesene sinnvolle Anwendungszwecke. So kann das EEG in der hier vorgestellten Form mit einer klinischen Untersuchung kombiniert werden, da im Rahmen von kontrollierten klinischen Studien neuer Analgetika reproduzierbare Ergebnisse erzielt werden. Auch ist der Anwendungszweck zur frühen Erkennung einer zentralen Sensitivierung denkbar, da das EEG auch bei kleineren Stichprobengrößen robustere Ergebnisse liefert als eine reine subjective Schmerztestung. Weiterhin kann das EEG als indirekter Marker für die Funktion des nozizeptiven Systems eingesetzt und es können z.B. Erkrankungen wie Small Fiber Neuropathien erkannt werden.

12 Publications

Peer-reviewed journal papers

Dreismickenbecker E*†, Zinn S†, Romero-Richter M, Kohlhaas M, Fricker L, Klippstein M, Petzel-Witt S, Walter C, Kreuzer M, Toennes S, **Anders M**: The EEG-based effects of acute alcohol intake on the pain matrix. 2022. *Submitted to peer review/in review*

† indicates a shared first authorship

Anders M*, Dreismickenbecker E, Fleckenstein J, Walter C, Enax-Krumova EK, Fischer MJM, Kreuzer M†, Zinn S† (2022) EEG-based sensory testing reveals altered nociceptive processing in elite endurance athletes. Experimental brain research (Online ahead of print). doi:10.1007/s00221-022-06522-4

† indicates a shared senior/last authorship

Anders M, Anders B, Dreismickenbecker E, Hight D, Kreuzer M, Walter C, Zinn S* (2023) EEG responses to standardised noxious stimulation during clinical anaesthesia: a pilot study. BJA Open Volume 5 (March 01). doi:10.1016/j.bjao.2022.100118

Lutz R, Müller C, Dragovic S, Schneider F, Ribbe K, **Anders M**, Schmid S, García PS, Schneider G, Kreuzer M*, Kratzer S. The absence of dominant alpha-oscillatory EEG activity during emergence from delta-dominant anesthesia predicts neurocognitive impairment- results from a prospective observational trial. J Clin Anesth. 2022 Nov;82:110949. doi: 10.1016/j.jclinane.2022.110949. Epub 2022 Aug 29. PMID: 36049381

Anders B*, **Anders M**, Kreuzer M, Zinn S, Fricker L, Maier C, Wolters M, Köhm M, Behrens F, Walter C. Sensory testing and topical capsaicin can characterize patients with rheumatoid arthritis. 2022. Clinical Rheumatology, DOI: 10.1007/s10067-022-06185-0

Anders M*, Anders B, Kreuzer M, Zinn S, Walter C. "Application of Referencing Techniques in EEG-Based Recordings of Contact Heat Evoked Potentials (CHEPS)". 2020. Frontiers in Human Neuroscience, DOI: 10.3389/fnhum.2020.559969

** indicates the corresponding author*

Other publications

Bauko B, **Anders M**, Neb H, Zacharowski K, Zinn S (2021). „COVID-19: Intensivpflichtige Patienten“. Arzneimitteltherapie, 39(03):70-80

Conference Abstracts

Anders M, Dreismickenbecker E, Fleckenstein F, Walter C, Enax-Krumova E, Fischer M, Kreuzer M, Zinn S (2022). „EEG-based sensory testing reveals altered pain processing in elite endurance athletes". 12th Congress of the European Pain Federation, Pain in Europe XII, Dublin/Ireland, 27 - 30 April 2022

Anders M, Zinn S, Anders B, Dreismickenbecker E, Kreuzer M, Walter C (2022). „Propofol Eliminates Pain-Related Evoked Potentials in the EEG". IASP World Congress on Pain, Toronto/Canada, 19 - 23 September 2022

Dreismickenbecker E, Fleckenstein F, Walter C, Enax-Krumova E, Fischer M, Kreuzer M, Zinn S, **Anders M** (2022). „Pain Processing of Elite Athletes Varies Between Sport-Specific Loads". IASP World Congress on Pain, Toronto/Canada, 19 - 23 September 2022

Heyer Y, **Anders M** (2022). "Pupillometry for pain tracking of induced heat stimuli". BMT 2022 - Joint Annual Conference of the Austrian, German and Swiss Societies for Biomedical Engineering, 28 – 30 September 2022

Supervised bachelor's and master's theses

Number of supervised bachelor students: 1

Topic: Messung der Schmerzwahrnehmung mittels EEG von Ausdauer-Leistungssportlern im Vergleich zu einer Kontrollgruppe

University/completion: Goethe-University Frankfurt/April 2021

Number of supervised master students: 3, and 1 incompleted thesis

Topic: The influence of high-performance sport on somatosensory EEG signatures and pain modulation in elite athletes

University/completion: Goethe-University Frankfurt/October 2021

Topic: Entwicklung eines Schmerztrackers mittels EEG und Pupillometrie auf Basis eines Rasberry Pi

University/completion: Hochschule Koblenz/June 2021

Topic: Technische Spezifikation des Anästhesie- und Sedierungsmonitors Treaton MGA-06 und Einfluss von physiologischen Reizen und Änderungen in der Narkosetiefe auf den Anästhesieindex

University/completion: Hochschule Furtwangen University/May 2021

Topic: The EEG-based effects of acute alcohol intake on the pain matrix

University/completion: Goethe-University Frankfurt/not completed

13 Declaration on the collaborative work in this thesis

Except where stated otherwise by reference or acknowledgment, the work presented was generated by myself under the supervision of my advisors during my doctoral studies. The material listed below was obtained in the context of collaborative research:

Methodology establishment study: Application of Referencing Techniques in EEG-Based Recordings of Contact Heat Evoked Potentials (CHEPS) [2]

Collaboration partner:	Björn Anders, at the time of the study PhD student at Fraunhofer ITMP
His contribution:	Study design, data collection, data analysis, data interpretation
Collaboration partner:	Sebastian Zinn, anesthesiologist at Goethe University Hospital
His contribution:	Assistance during data analysis
Collaboration partner:	Matthias Kreuzer, scientist at TU Munich
His contribution:	Assistance in the statistical analysis of the results
Collaboration partner:	Carmen Walter, Fraunhofer ITMP
Her contribution:	Direct supervisor at Fraunhofer ITMP, organizational assistance, study design
My contribution:	**Study design, data analysis, data visualization, paper first and final draft, thesis chapters, figures, tables**

All text-based results, figures and tables are a result of the abovementioned collaboration. These Figures and Tables were analyzed and designed completely by myself, the collaborative part only includes the study design and the data collection process. The EEG part of the study was designed by myself and Björn Anders.

Project IMPACE: EEG responses to standardised noxious stimulation during clinical anaesthesia: a pilot study [3]

Collaboration partner:	Björn Anders, at the time of the study PhD student at Fraunhofer ITMP
His contribution:	Assistance during the application for ethics approvement
Collaboration partner:	Elias Dreismickenbecker, at the time of the study: Masters' student at Fraunhofer ITMP/Goethe University Frankfurt, supervised by myself
His contribution:	Assistance during data analysis
Collaboration partner:	Darren Hight, Inselspital Bern University Hospital
His contribution:	Proofreading of the first draft of the manuscript
Collaboration partner:	Matthias Kreuzer, scientist at TU Munich
His contribution:	Statistical analysis of the results, figures, data analysis
Collaboration partner:	Carmen Walter, Fraunhofer ITMP
Her contribution:	Direct supervisor at Fraunhofer ITMP, organizational assistance
Collaboration partner:	Sebastian Zinn, anesthesiologist at Goethe University Hospital
His contribution:	Study design, Anesthesiological procedures, subject recruitment, guidance during data analysis in anesthesiological topics, paper final draft, figures, tables, review process
My contribution:	**Study design, data collection, data analysis, data visualization, paper first draft, thesis chapters, figures, tables**

All results are a result of the abovementioned collaboration. These Figures and Tables were analyzed and designed completely by myself and Sebastian Zinn, the collaborative part further includes the study design, the data collection process, where Sebastian Zinn carried out the anesthesiological procedure, as well as guidance from Sebastian Zinn for the parts that analyzed and discussed the anesthesiological procedures. I also wrote the first draft of the paper, whereas Sebastian Zinn finalized the manuscript and the reviews during the review process. All co-authors revised the manuscripts (but not this thesis) and gave additional guidance during data analysis.

Project SPINE: EEG-based sensory testing reveals altered nociceptive processing in elite endurance athletes [4]

Collaboration partner:	Elias Dreismickenbecker, at the time of the study: Masters' student at Fraunhofer ITMP/Goethe University Frankfurt, supervised by myself
His contribution:	Assistance during the data collection
Collaboration partner:	Johannes Fleckenstein, Goethe University Frankfurt
His contribution:	Assistance in the writing of the first draft of the manuscript, payment of the participants, sports-related testing and analysis
Collaboration partner:	Carmen Walter, Fraunhofer ITMP
Her contribution:	Direct supervisor at Fraunhofer ITMP, organizational assistance
Collaboration partner:	Elena Enax-Krumova, BG Universitätsklinik Bergmannsheil gGmbH Bochum
Her contribution:	Assistance in the writing of the first and final draft of the manuscript
Collaboration partner:	Michael Fischer, Medizinische Universität Wien
His contribution:	Assistance in the writing of the first draft of the manuscript
Collaboration partner:	Matthias Kreuzer, scientist at TU Munich
His contribution:	Assistance in the statistical analysis of the results, figures, statistics
Collaboration partner:	Sebastian Zinn, anesthesiologist at Goethe University Hospital
His contribution:	Assistance in the study design, data collection, data interpretation
My contribution:	**Data collection, data analysis, data visualization, paper first and final draft, thesis chapters, figures, tables**

All results are a result of the abovementioned collaboration. These Figures and Tables were analyzed and designed completely by myself, the collaborative part only includes the abovementioned people and responsibilities. I was also responsible for the first and final draft of the paper, as well as the review process. All co-authors revised the manuscripts (but not this thesis) and gave additional guidance during data analysis.