UNIVERSITY OF AMSTERDAM

Cognitive Neurobiology & Clinical Neurophysiology (MSc)

Research Project

An Exploration of Alpha Rhythms in a CLIS-ALS Patient

Searching for Temporal Windows for Optimal BCI Communication

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Abstract

Application of BCI devices for CLIS-ALS patients has so far failed to establish even basic communication. One explanation for this ineffectiveness is that the level of cognitive capacity in these patients may vary throughout the day. Previous work in this patient group identified a prominent power spectral peak at sub-alpha frequencies. The functional implications of this ostensibly downshifted alpha rhythm are unknown. The first aim of our project was to assess the neurophysiological properties of this enigmatic sub-alpha rhythm. To do this, we quantified the amount of event-related desynchronisation (ERD) to trains of auditory stimuli. We found no evidence of any significant ERD of the sub-alpha (4 – 6Hz) rhythm. Our second aim was to explore distinct brain states which may be associated with varying levels of cognitive function. We found that our patient possessed two distinct brain states associated with a prominent power spectral peak at either sub-alpha (4 – 6Hz) or high-alpha (10.5-12.5Hz) frequencies. Next, we explored state-specific responses to auditory stimulation and tested for between-state differences. Our results were inconclusive and so we could not infer whether these brain states conferred different levels of cognitive function to our patient. Looking forward, we suggest that an improved and expanded experimental setup may reveal important functional distinctions between these two brain states, with important ramifications for the development of improved BCI-based communication strategies.

Introduction

Application of BCI-Based Communication in CLIS-ALS Patients

Amylotrophic lateral sclerosis (ALS) is a rare, neurodegenerative disease which results in a progressive and debilitating loss of motor function. It is caused by a degeneration of motor neurons in several motor-related regions of the central nervous system [Wijesekera, L. C., & Leigh, P. N. (2009)]. Patients in the later stages of the disease may enter a 'completely locked-in state' (CLIS) where they lose the ability to execute any meaningful motor actions. Patients in this phase are no longer capable of controlling the eye-tracker-based spelling devices commonly used in partially paralysed patients. Consequently, communication with these patients becomes impossible through conventional means.

Many attempts have now been made to establish communication with CLIS patients using brain-computer interface (BCI) technology. The goal of this enterprise is to bypass the patients' dysfunctional motor system by recording neurophysiological signals directly from the brain to infer intended actions. Application of BCI technology has shown some success with severely paralysed ALS patients [Birbaumer et al, 1999; Kubler et al, 2005; Nijboer et al, 2008]. However, it has proved more challenging to establish communication with patients who are completely locked-in. One study collected data from seven CLIS-ALS patients and showed that even basic communication had not been achieved in any of the patients [Kübler & Birbaumer, 2008]. Another meta-analysis found that the classification accuracy of BCIs used in CLIS-ALS patients was not significantly different from chance [Marchetti et al, 2015]. The reason for this lack of success is unclear but could involve specific aspects of the CLIS-ALS disease pathology, the inadequacy of current BCI technologies or both.

Neurophysiological and Cognitive Changes Associated with the Completely Locked-in State

It is imperative that we understand what cognitive and neurophysiological changes, relevant to the application of BCIs, occur during the transition to the completely locked-in state. Neuropsychological evaluation of the cognitive function of CLIS patients is not possible and so the exact degree and type of cognitive function that CLIS patients possess remains elusive. Two strategies have been deployed to better understand the mental capabilities of CLIS patients. The first has been to characterise the relationship between disease progression and cognitive function in ALS patients and then extrapolate findings to predict the capacities of patients who lose all motor function. The second is to investigate whether neurophysiological markers, indicative of cognitive capacities, are intact or diminished in the CLIS. While these approaches do not directly measure mental function, they do allow for analysis of patients while they are in the CLIS and so no extrapolation is required.

Studies have shown that ALS is associated with cognitive dysfunction although the relationship between the disease progression and the level of

impairment is still uncertain. One study found that 35.6% of patients demonstrated clinically significant deficits in multiple cognitive tasks [Massman et al, 1996]. The authors also found that the degree of motor impairment was a risk factor for cognitive dysfunction which could indicate that cognition is increasingly affected as the disease progresses. Another study found that half of the patient sample displayed cognitive impairment but, importantly, they found no correlation between the degree of impairment and the severity/duration of motor symptoms [Ringholz et al, 2005]. This contradicts the idea that cognitive function declines linearly as the disease progresses or suggests that any correlation is weak. The nature and degree of cognitive impairment appears to manifest in a highly heterogenous manner across the patient population which makes it difficult to make general claims about cognition in the CLIS.

Neurophysiological studies have found that CLIS patients exhibit a partial retention of ERPs (event related potentials) to a variety of cognitive tasks [Hinterberger et al., 2005; Kotchoubey et al., 2005]. Although this does not prove that paralysis leaves cognitive functioning intact it does suggest some processing modules are spared by the disease. One study monitored the changes to event-related cortical responses across a nine-month period, enabling the authors to observe the degeneration from a partially paralysed state to a CLIS. They demonstrated that cortical responsiveness to auditory and proprioceptive stimuli were unaffected whereas the response to vibrotactile stimuli was lost as the patient lost all motor function [Ramos et al, 2011]. This suggests that cognitive function, involving specific sensory modalities, may persist in the CLIS. As they only monitored a single patient, the findings may not hold true for all patients. While it is not possible to make any definitive claims about the cognitive landscape that accompanies the CLIS, there are certainly signs that elements of cognition may persist.

A recent study has made an interesting contribution to the debate surrounding the cognitive capacity of CLIS patients. The authors found that the resting-state, alpha peak frequency (APF) in two CLIS-ALS patients was significantly down-shifted compared to non-CLIS patients and healthy controls. The APF has been shown to be a reliable neurophysiological indicator of cognitive function in healthy subjects [Grandy et al, 2013]. The lowering of the APF may reflect underlying cognitive decline in the patients. However, the downshifted APF was recorded at a frequency well below the typical alpha range which raises questions about the functional significance of this putative alpha rhythm. Additionally, the patients were not recorded prior to complete paralysis and so it is not known at what stage of the disease the downshift occurred. Better characterisation of the functional properties of alpha rhythms in CLIS patients is needed to make any further claims about the implications of this striking observation.

The retention of some neurophysiological indicators of cognition and the heterogenous nature of disease progression suggest that some aspects of cognitive processing may persist in some, if not all, CLIS-patients. Whether this is sufficient to enable effective communication through a BCI device is still an open question.

Theories Explaining the Lack of Success of BCI-Based Communication with CLIS-ALS Patients

The ineffectiveness of BCI-based communication with CLIS-ALS patients has led some researchers to question the viability of this project. It has been proposed that the loss of motor function leads to a loss of goal-directed behaviour [Kübler & Birbaumer, 2008]. From this perspective, the complete loss of the ability to interact with the 'outside world' causes a rapid acceleration in the rate of cognitive decline in patients. This idea is aligned with other theories which emphasise the importance of sensory-motor interactions in consciousness and cognitive function [James, 1890; O'Regan & Noë, 2001]. Attempts to design BCIs which do not require goal-directed behaviour have been unsuccessful so far [Chaudhary et al, 2017; Spuler, 2018]. If this theory is correct then it seems very unlikely that communication with CLIS patients will be possible, regardless of what technological progress is made.

An alternative explanation is that the level of cognitive function in CLIS-ALS patients may vary considerably throughout the day [Grosse-Wentrup, 2019]. This theory is displayed in figure 1. Support for this idea came from preliminary research on EEG data taken from a CLIS-ALS patient. The analysis showed a consistent variability in both the power and frequency of alpha rhythm across the day. As alpha-variability is considered a hallmark of transitions between resting and task-focused, brain states [Klimesch, 1999] the high alpha-variability observed in these patients could indicate a retention of some cognitive processing.

What does this mean for the development of BCI-based communication with CLIS patients? Previous attempts may have failed because

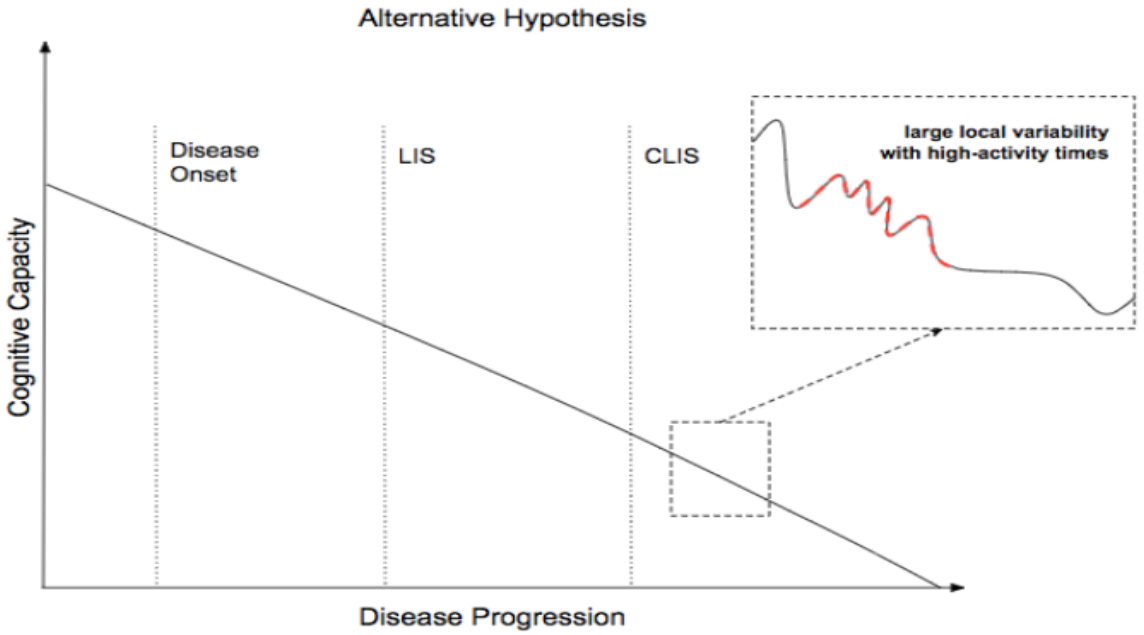

Figure 1: Conceptual diagram describing an alternative theory for cognitive decline in CLIS-ALS patients [diagram from M. Hackl, unpublished]

patients drift in and out of states of high/low cognitive capacity across the day. Successful BCI-based communication may require a system capable of identifying temporal windows which are optimal for BCI communication. Deploying BCI technology during these windows would give a better chance of successful communication.

Project Goals

Previous analysis from this lab had identified several potential brain rhythms in a CLIS-ALS patient [Grosse-Wentrup, unpublished]. One such rhythm was identified by a prominent power spectral peak in the range of 4.5 to 6Hz. This oscillation was of particular interest because it corresponds with the downshifted alpha rhythm highlighted by previous work on CLIS-ALS patients [Hohmann et al, 2018]. The functional implications of these sub-alpha rhythms are unknown. Therefore, our first goal was to investigate the functional properties of this sub-alpha rhythm. To do this, we tested for the presence of event-related desynchronisation (ERD) to trains of auditory stimuli at this frequency range. We hypothesised that if the sub-alpha rhythm was truly a downshifted, alpha rhythm then it would exhibit desynchronisation after stimulus presentation.

There were several reasons why we chose ERD as our neurophysiological marker. Typical alpha rhythms (7 – 13Hz) display ERD in a variety of cognitive tasks, including auditory stimulation [Pfurtscheller, Lopes da Silva, 1999; Klimesch, 1999, Krause et al, 1994]. ERD was an ideal neurophysiological phenomenon for our setup for several reasons. ERD, especially in the lower-alpha range, is topographically widespread and therefore more likely to be identified with the Dreem headset [Klimesch et al, 2007]. ERD is also considered to be a signature of general, attentional processes [Klimesch et al, 2007] and is therefore a suitable metric for assessing general levels of cognitive capacity. Additionally, our use of a stimulus train, as opposed to a single stimulus, was done to take advantage of the fact that ERD duration is known to correlate with task duration [Kaufman et al., 1990, 1992; Michel et al., 1994]. Importantly, cortical responses to auditory stimulation have been shown to persist in a CLIS-ALS patient [Ramos et al, 2011] suggesting that this would be the preferred sensory modality to elicit ERD.

Our next aim was to look for potentially distinct brain states and establish whether these states were associated with distinct neurophysiological properties. Identifying state-specific responses to trains of auditory stimulation could indicate a state-specific retention of cognitive function. Based on preliminary work, we were able to identify two distinct brain states and categorise our epochs as belonging to either state. With this subdivided data, we set out to compare both ERPs and ERD in each brain state and test for any between-state differences.

Results

Analysis of Epoched Data

Our dataset was compiled by combing the data taken from 10 recording sessions of a single CLIS-ALS patient. The recordings were conducted using a low-cost, Dreem headset which enabled us to record across the entire day. An external device was used to deliver trains of five auditory stimuli with inter-stimuli interval of 3 seconds and each stimulus train was initiated at random times. Epoched data was extracted relative to the onset of each stimulus train and included the data for five good EEG channels. For full details of the setup, please see the methods section.

Previous analysis of the entire dataset had identified four potential brain rhythms of interest (BR1: 0.8 - 1 Hz on CH7, BR2: 2.7 - 3.3 Hz on CH3, BR3: 4.5 - 6 Hz on CH1 and CH5, BR4: 10.5 – 11 Hz on CH4) [Grosse-Wentrup, unpublished]. To verify whether these brain rhythms were robust enough to manifest in our epoched dataset, we performed power spectral analysis on all resting-state segments. As shown in Figure 2, BR3 (4.5 to 6 Hz) was clearly present in the baseline data for CH1 and CH5. These two channels were recording a similar signal and so the power spectra for these two channels shared similar characteristics. However, none of the other previously identified rhythms were visible in our analysis of the epoched data. We assumed that these ostensible brain rhythms were insufficiently robust to manifest themselves in the shorter, epoched dataset. Nevertheless, we were encouraged by the prominence of the sub-alpha (4 – 6Hz) peak in the power spectra of CH1 and CH5 and next sought to investigate how this brain rhythm responded to auditory stimulation.

Event-Related Desynchronisation of the Sub-Alpha Rhythm

As figure 3 shows, we used a variety of approaches to explore how the sub-alpha rhythm changed in response to auditory stimulation. The ERP plots for the two channels of interest (figure 3, top) revealed no clear depression locked to the stimulus onset. Examination of the average ERP plots suggests the possible presence of a delayed downward deflection, appearing two seconds after the stimulus. However, no clear trend was present across individual epochs and this delayed depression was only visible in a very small number of epochs. This suggested the downward deflection was not a consistent neural response to the stimuli but the result of a few outlying epochs. Furthermore, our recording electrodes were positioned above frontal brain regions and therefore unlikely to pick up changes in the ERP anyway. Although they were not particularly informative by themselves, our ERP plots were useful for allowing us to compare our abstract frequency-domain data with the raw EEG signal used to derive it. This allowed us to asses whether aspects of our frequency-domain data could be caused by noise in the raw EEG signal, as opposed to true brain signal.

To test whether the sub-alpha rhythm was exhibiting ERD we generated power spectra for baseline (-15 to 0s) and response (0 to 15s) segments. Our long response segments contained the data associated with each stimulus train (5 individual stimuli). We focused our analysis on CH1 and CH5 and frequencies in the range of 4 to

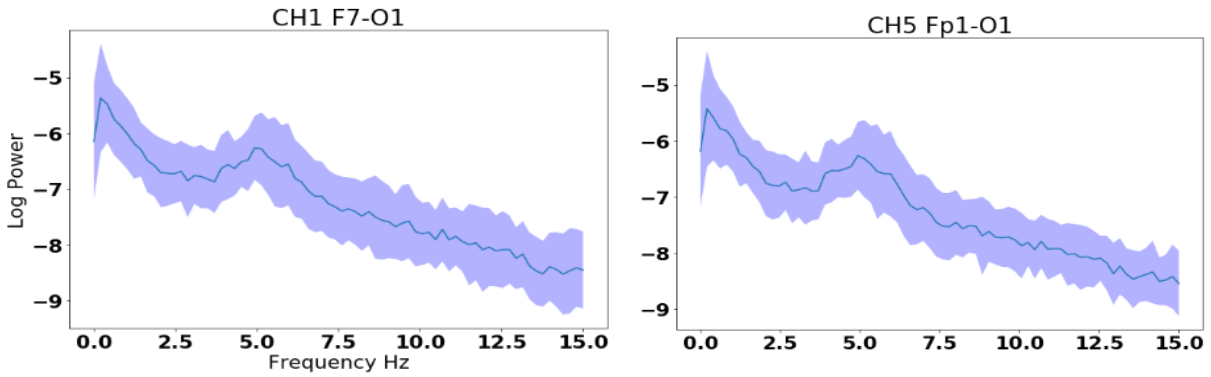

Figure 2: Sub-Alpha Peaks in the Power Spectra: Power spectra were generated by performing a fast fourier transform with a Hanning window on baseline/resting segments(-15 to 0s). Log power values were averaged across epochs (n = 265) and standard deviation is displayed. The two channels exhibiting a prominent peak around 4 – 6Hz are shown.

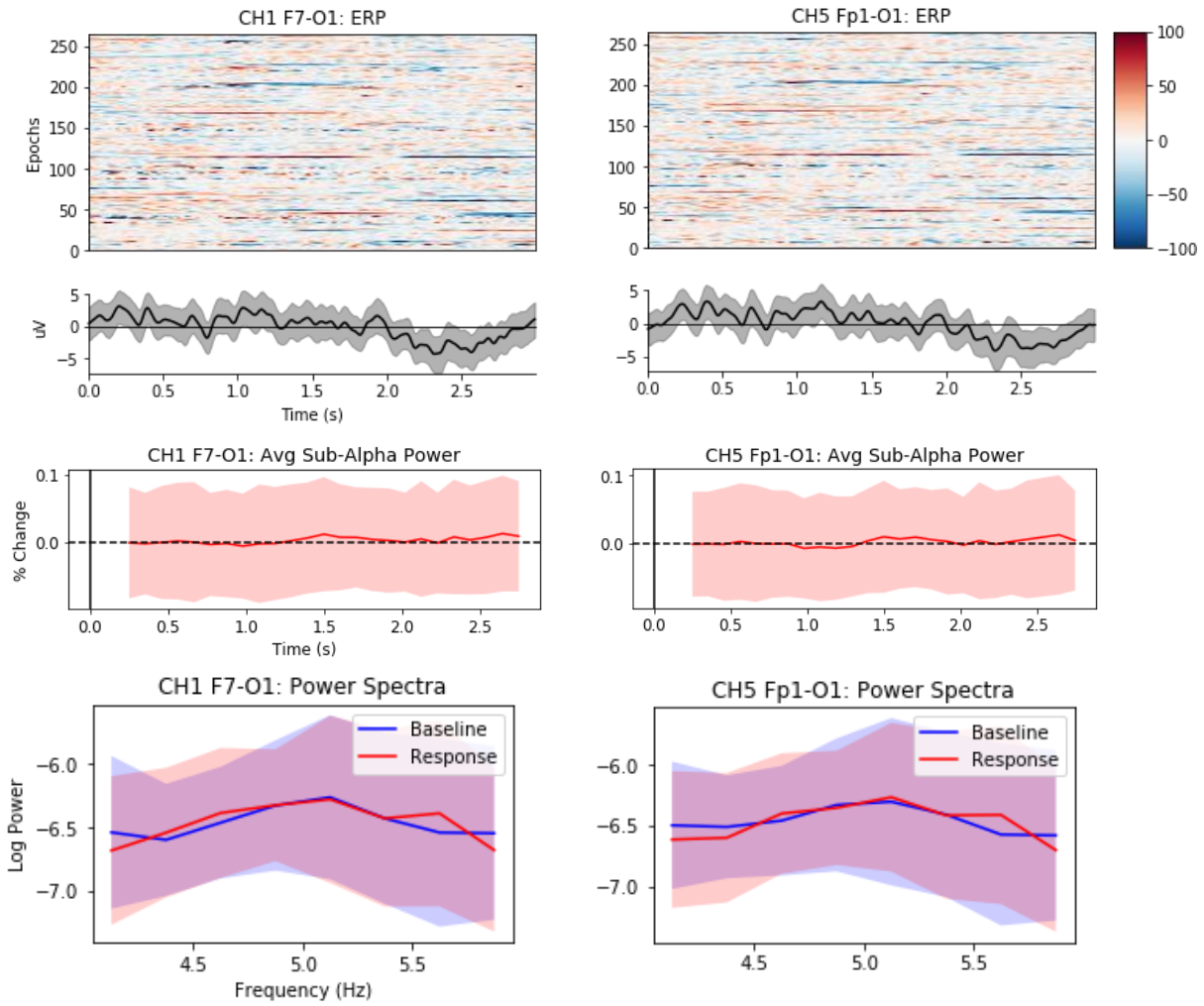

Figure 3: Event-Related Responses of the Sub-Alpha (4-6Hz) Rhythm: (Top) Event-related potentials capture the response period (0 to 3s) following presentation of each auditory stimulus calculated. Data was filtered between 0.1 and 15Hz and displayed across both individual epochs and as an average. (Middle) Plot shows how the average power of the sub-alpha rhythm (4-6Hz) varied during the response period following each auditory stimulus. Standard deviation shown as a colour fill. The sub-alpha power rhythm did not significantly deviate from baseline at any timepoint n = 265). (Bottom) Comparison of power spectra of long baseline (-15 to 0s) and response (0 to 15s) periods – each long epoch incorporating data from five stimuli. No significant differences were present between baseline and response at any sub-alpha frequency.

6Hz. Figure 3 (bottom) shows the power spectra for the baseline and response segments superimposed on each other. The most notable feature of this graph is the large size of the variance for both power spectra. The size of the differences in the average power values were miniscule compared to the variance and so we could not visually identify any signs of ERD at these frequencies. To confirm this statistically, we performed non-parametric statistical analysis at each frequency of interest (details in methods section). This allowed us to test for significant differences between the power spectral density of baseline and response segments. The p values generated for each sub-alpha frequency did not approach significance and so we were unable to conclude that any significant, sub-alpha desynchronisation had taken place.

To get an idea about how the sub-alpha rhythm was evolving over time we also performed a time-frequency analysis. We applied a fourier transform decomposition of the signal with a sliding time window to retain temporal information. As we were specifically interested in the sub-alpha rhythm, we performed this analysis on a single frequency band (4-6Hz). Our results are displayed as a percentage change relative to baseline in figure 3 (middle). No clear trends were visible across the response window. To verify this statistically, we tested each timepoint to assess whether the average sub-alpha power dropped significantly below baseline levels. The results of this permutation-based analysis

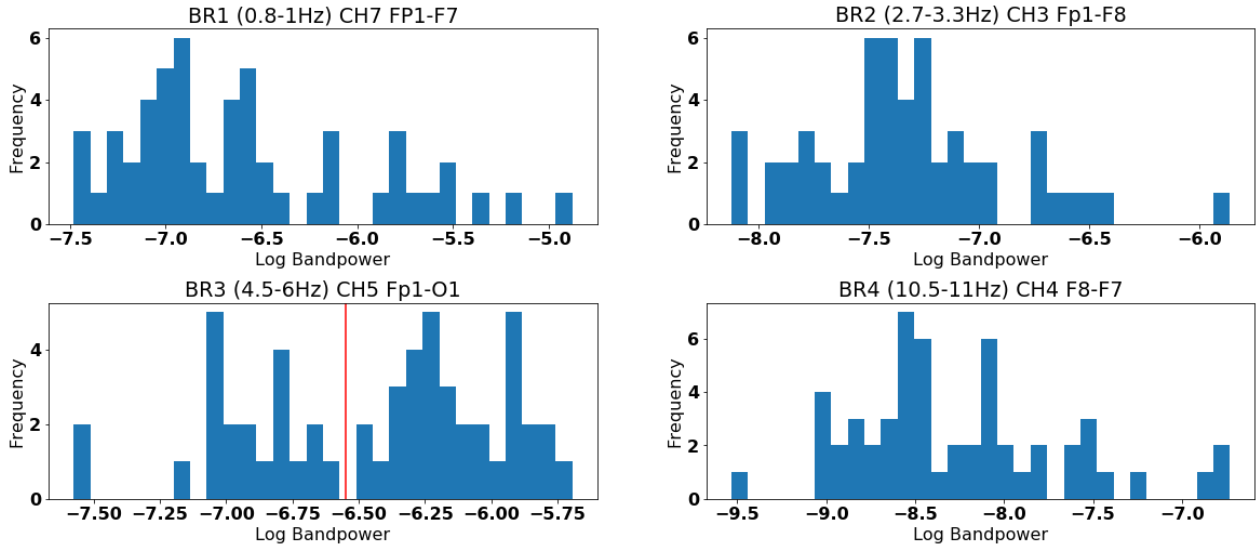

Figure 4: Power Distribution of Putative Brain Rhythms: For each of the four brain rhythms, identified from previous analysis of the patient data, we generated histograms showcasing the spread of average power values across long baseline segments (-15 to 0s) of our epoched data. Each histogram was generated from a fast fourier analysis of a unique frequency band in a different EEG channel. Brain Rhythm 3 (BR3) produced a clear bimodal distribution. Setting a threshold value (red line) allowed us to subdivide epochs into two distinct brain states for future analysis. Number of stimulus trains = 53.

indicated that no significant sub-alpha ERD was occurring across the response window. This fell in line with the findings of our power spectral analysis. Consequently, we accepted the null hypothesis – that any variation in sub-alpha synchronisation following stimulus presentation was due to chance.

Defining Brain States Based on the Sub-Alpha Rhythm

Previous work on this dataset suggested that the power values of the sub-alpha, brain rhythm (4.5 to Hz) exhibited a bimodal distribution. This raised the possibility that the high and low sub-alpha, power values corresponded to two distinct brain states. To confirm whether this feature was also present in our epoched dataset, we replicated the histograms of power values for each brain rhythm. As shown in figure 4, we found that the spread of power values for the sub-alpha rhythm (4.5 – 6Hz) roughly formed a bimodal distribution. This contrasted with the normal distributions exhibited by the other brain rhythms. By themselves, these distributions incorporate too few epochs to reliably reveal the underlying distribution. Instead these graphs are included to highlight that the robust distribution patterns seen in previous analysis of the continuous dataset were also present in our restricted epoched dataset.

The division point between the two sections of the bimodal distribution matched to the value seen in the analysis of the whole dataset. This suggested that these sub-alpha-defined states were a robust component of the data. To investigate functional distinctions between these brain states, we set a threshold power level (denoted by red line) in order to divide epochs into two groups corresponding to the two brain states. We defined ‘high’ epochs as those having above-threshold, sub-alpha power values in CH5 and ‘low’ epochs as those below threshold. To avoid any experimental bias due to the two brain states containing different numbers of epochs, we randomly sub-selected epochs from the state with a greater number of epochs.

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Power Spectral Features of Brain States

Once our trials were divided, we wanted to get an idea about the properties of our potential brain states. Our definition of ‘brain state’ was based on a single power spectral property. It was therefore important that we found some supporting evidence that these states were based on meaningful neurophysiological distinctions rather than irrelevant data features.

We performed a power spectral analysis on the baseline (-15 to 0s before train onset) and response (0 to 15s after train onset) segments of our epochs. This was done separately for epochs belonging to each brain state. Our results,

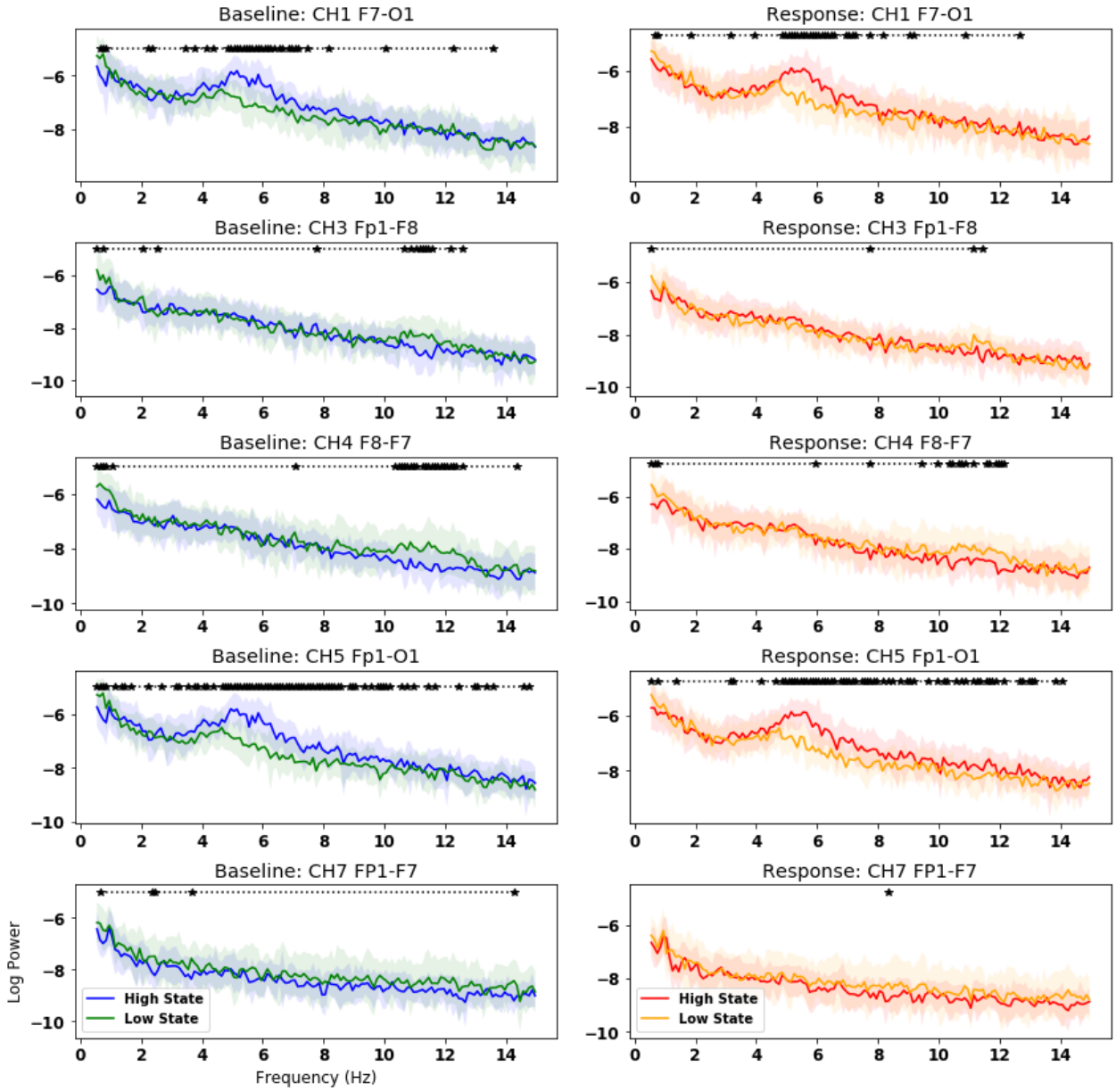

Figure 5: Power Spectral Differences Between Brain States*: Following subdivision of epochs into 'high' and 'low' brain states (n = 100), power spectral datasets were calculated for the four experimental conditions (baseline-low, baseline-high, response-low, response-high) using fft with a Hanning window. Averages were taken across corresponding epochs and standard deviation shown as a colour fill. Non-parametric statistical tests were used to investigate differences between brain states at each frequency and for baseline (left) and response (right) segments. Asterisks indicate the frequencies where there was a significant difference between 'high' and 'low' states.*

displayed in figure 5, show a comparison between brain state spectra for the two trial segments and the five channels. Each frequency value was tested for significant differences between 'high' and 'low' states. Our epochs were divided based on the 4.5 – 6Hz rhythm found in CH5. Unsurprisingly, significant differences between brain states were found at this frequency band in both baseline and response segments of the CH5 power spectra. However, significant differences, albeit smaller in size, appeared across a wide range of frequencies. This raised the possibility that our epoch-division was based on differences in the amplitude of the signal rather than specifically the sub-alpha, oscillatory power. Examination of the power spectra from the other channels gave us some reassurance that our division was based on some biologically meaningful properties. For example, power spectral analysis of CH1, a channel measuring a similar signal to CH5, displayed between-state differences which were mainly restricted to the 5 – 7Hz frequency range. This suggested to us that

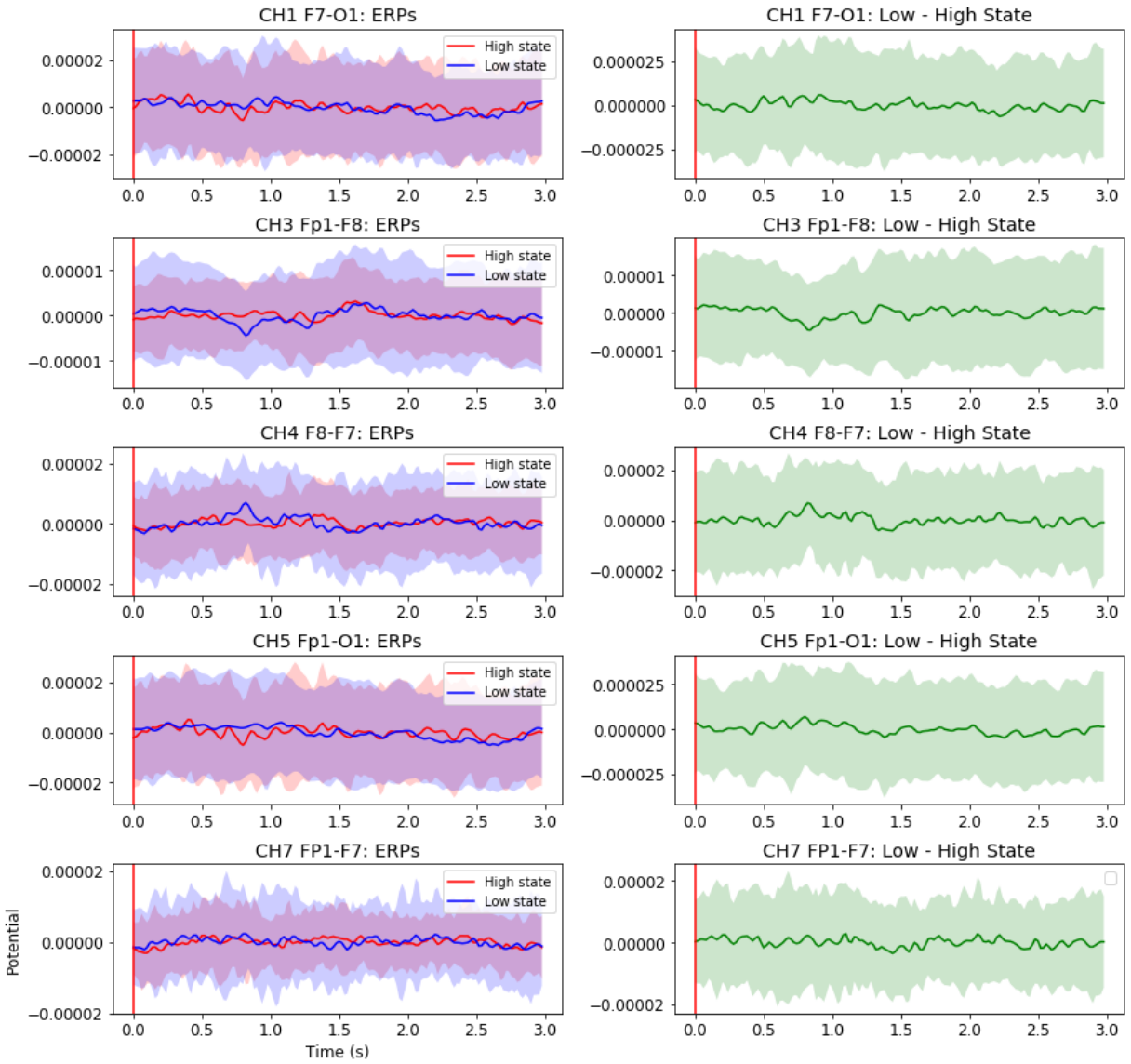

Figure 6: State-Specific Event-Related Potentials: (Left) Event-related potentials (ERP) for response windows belonging to each brain state were bandpass filtered below 15Hz and displayed as an average across epochs (n=100) with the standard deviation shown as a colour fill. (Right) The difference ERP was calculated as the average low state – average high state at each timepoint. Permutation statistics found no significant differences between high and low states.

our brain states were indeed associated with different intensities of sub-alpha oscillations.

Some other interesting properties of our brain states revealed themselves at this stage. Our low-state data exhibited a prominent peak in the 10.5 – 12.5Hz range. This feature was strongest in CH4 but also present in CH3. Fascinatingly, this peak was completely absent in the high-state epochs. In other words, in epochs where the sub-alpha frequency was low there was a peak at a high-alpha frequency. Whereas, in epochs with a strong, sub-alpha rhythm there was no peak at high-alpha frequencies. Our epochs appeared to be displaying either a peak at sub-alpha frequencies or high-alpha frequencies, but not both. Additionally, these distinct, brain rhythms were occurring in different brain regions; the sub alpha rhythm was found in the vicinity of CH1 and CH5 whereas the high-alpha rhythm occurred in CH3 and CH4. The presence of these two distinct brain states was striking. Given that the low-state epochs possessed an alpha rhythm residing within the typical range, it seemed more likely to be associated with a higher degree of cognitive function. Further testing would hopefully give us an answer to this suspicion.

There also appeared to be some between-state differences at the 1Hz frequency. As this effect was seen across most of the channels, we deduced that this rhythm was most likely due to contamination of the signal by cardiovascular noise rather than neural oscillations. The possibility that

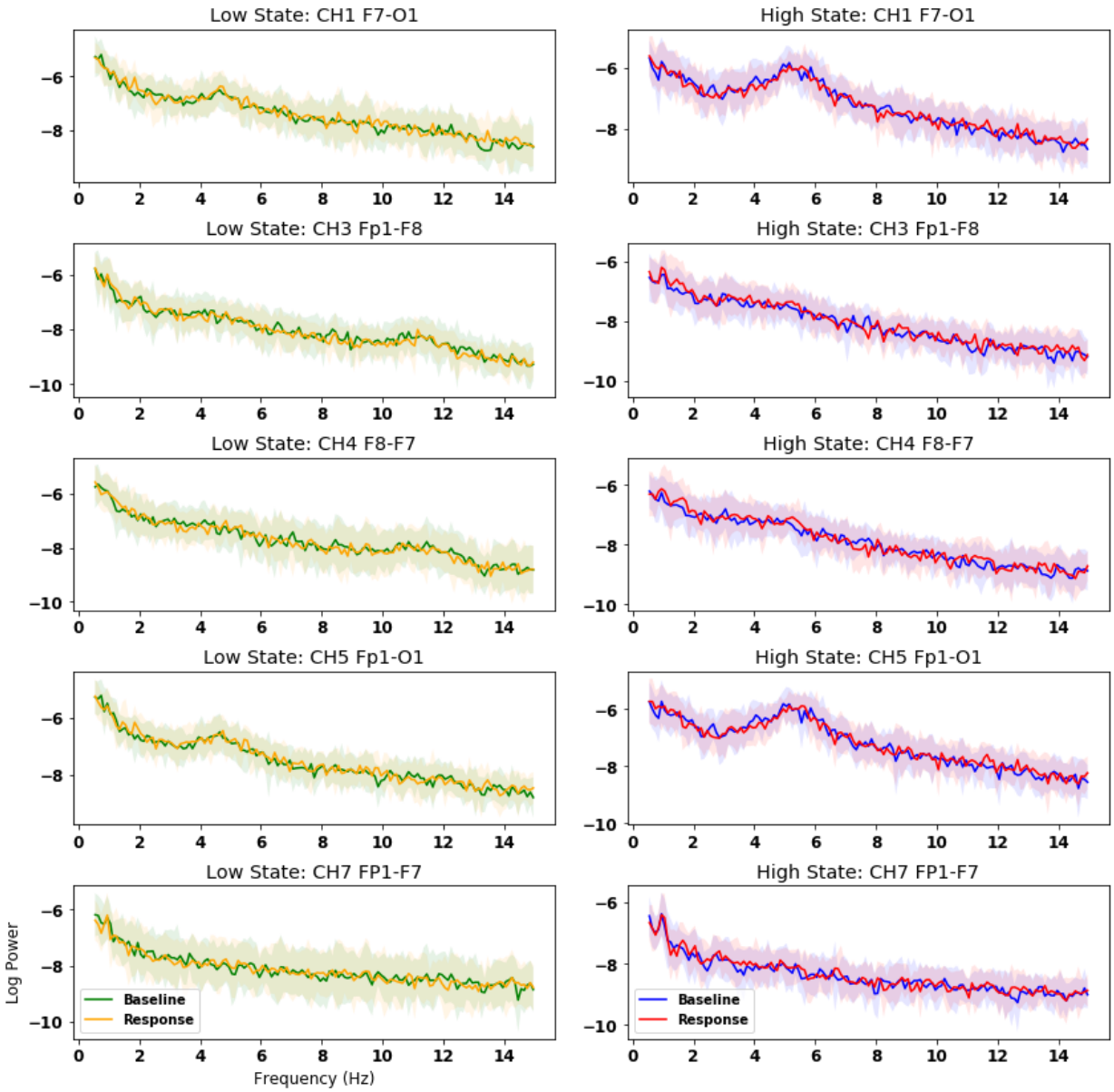

***Figure 7: State-Specific Oscillatory Responses to Auditory Stim**: Power spectra data for the four conditions (baseline-low, baseline-high, response-low, response-high) was rearranged to allow visualisation of any event-related desynchronisation in each brain state. Averages of the log power values were taken across epochs in each condition (n = 100) and the standard deviation is shown as a colour fill. For each frequency, non-parametric tests evaluated the significance of any potential differences between baseline (-15 to 0s) and response (0 to 15s) segments in each brain state – no differences were found.*

our brain states also exhibited distinct cardiovascular properties was interesting but not of great relevance to our project.

Comparison of Event-Related Potentials in 'High' and 'Low' Brain States

Our next goal was to assess whether our brain states were associated with distinct neurophysiological responses to auditory stimuli. We first examined the ERPs for the two brain states in each channel. If these two brain states corresponded to different levels of cognitive capacity, we supposed that this would manifest as a state-specific deflection in the ERP. Figure 6 (left) displays the superimposed ERPs for each brain state while figure 6 (right) shows the difference between ERPs of the two states.

We noticed some potential deviations around 0.5 to 1 seconds after stimulus onset. CH3 and CH4, the two channels associated with the 10.5 – 12.5Hz rhythm, showed mild deflections in their ERPs in low-state epochs. Interestingly, the deflections were in opposite directions, downwards in CH3 and upwards in CH4. This suggests that these two channels were measuring the source of

the deflection from opposite sides of the electrical dipole. However, statistical analysis indicated that the ERPs of our two brain states did not differ significantly at any channels/timepoints. This result was unsurprising given the size of the variance relative to the weak differences. Therefore, we could not show that these potential deflections related to real neurophysiological distinctions.

State-Specific Oscillatory Responses to Auditory Stim

We next set out to explore event-related changes in the frequency domain. We returned to our power spectral dataset for our four experimental conditions (high base, high resp, low base, low resp). At this stage, we unsure which brain state and which brain rhythm would display ERD, if any. For each brain state, we theorised that we might see ERD taking place at the alpha peak frequency associated with each brain state. Specifically, we anticipated that in CH1 and CH5 we might see some desynchronisation occurring in the sub-alpha range (4-6Hz), while for CH3 and CH4 we believed instead that ERD could manifest in the high-alpha frequency band (10.5 – 12.5Hz). The functional role of the sub-alpha rhythm was unclear so we had less confidence that this rhythm would undergo ERD in high epochs. As the high-alpha rhythm fell into the typical alpha range, we had greater confidence that it would display state-specific ERD and that this would occur in the low-state epochs, where the high-alpha rhythm dominates. Our primary, working hypothesis was that we would see ERD occurring selectively in CH3 and CH4 during low-state epochs.

To investigate this, we re-arranged and plotted our power spectral data in order to explore deviations between the baseline and response segments. To avoid missing any miscellaneous ERD and to give us a complete picture, we included a broader range of frequencies. As shown in figure 7, we found no clear indications that the oscillatory power was changing in response to stimuli. Whereas comparisons between brain states revealed a wealth of oscillatory distinctions, within-trial comparisons suggested that brain rhythms were relatively unperturbed by stimulus presentation. Furthermore, our statistical analysis did not reveal any frequencies where the power of the response period dropped significantly below the baseline segment. From this analysis, we did not find any evidence for ERD occurring in either brain state.

State-Specific ERD with Time Dimension

One potential flaw with the analysis of discrete time windows was that we were diluting any potential ERD. If the desynchronisation was occurring weakly or in temporal-restricted windows, then eliminating the time dimension would reduce our ability to register these effects. To remedy this, our next approach was to construct time-frequency representations of the response period for each brain state. From this we could assess whether any response timepoints were associated with significant desynchronisation relative to baseline. Additionally, we could then calculate a time-frequency representation of the difference between the baseline-corrected values for each brain state. As with our previous approach, we suspected that high-state epochs might show ERD in the sub-alpha range, if at all, and the low-state epochs would show this at a high-alpha frequency. In alignment with this, we also expected to see significant differences in the time-freq plots of each state at these two frequency bands.

Our results are shown in figure 8. Each plot incorporates baseline-corrected power values at each time-frequency pixel in a grey scale. Superimposed on each plot are the significant pixels, emphasised with a color scale. CH1 appeared to show some ERD at 4Hz and 10.75Hz in a brief window after the stimulus (0.5 to 1s). Counter to our intuitions, this only occurred in low-state epochs and not at all in high-state trials. We were surprised to see signs of sub-alpha ERD occurring selectively in the low state because this state possesses a significantly suppressed sub-alpha rhythm. While the sub ERD at 4Hz was not statistically significant, the difference between the low and high states did reach significance. The peak at 10.75Hz in the low state was unexpected in CH1 because the high-alpha rhythm was not visibly present in this channel. Although this feature was also selectively found in the low state, where the high-alpha rhythm dominates, it did not reach significance.

CH5 exhibited some similar features to CH1, as would be expected. A general trend for greater ERD in low-state epochs was also apparent in CH5. From examination of the low-state plot alone, it was difficult to pick out any well-defined time-freq clusters and none reached significance. However, between 0.5 and 1s after stimulus presentation,

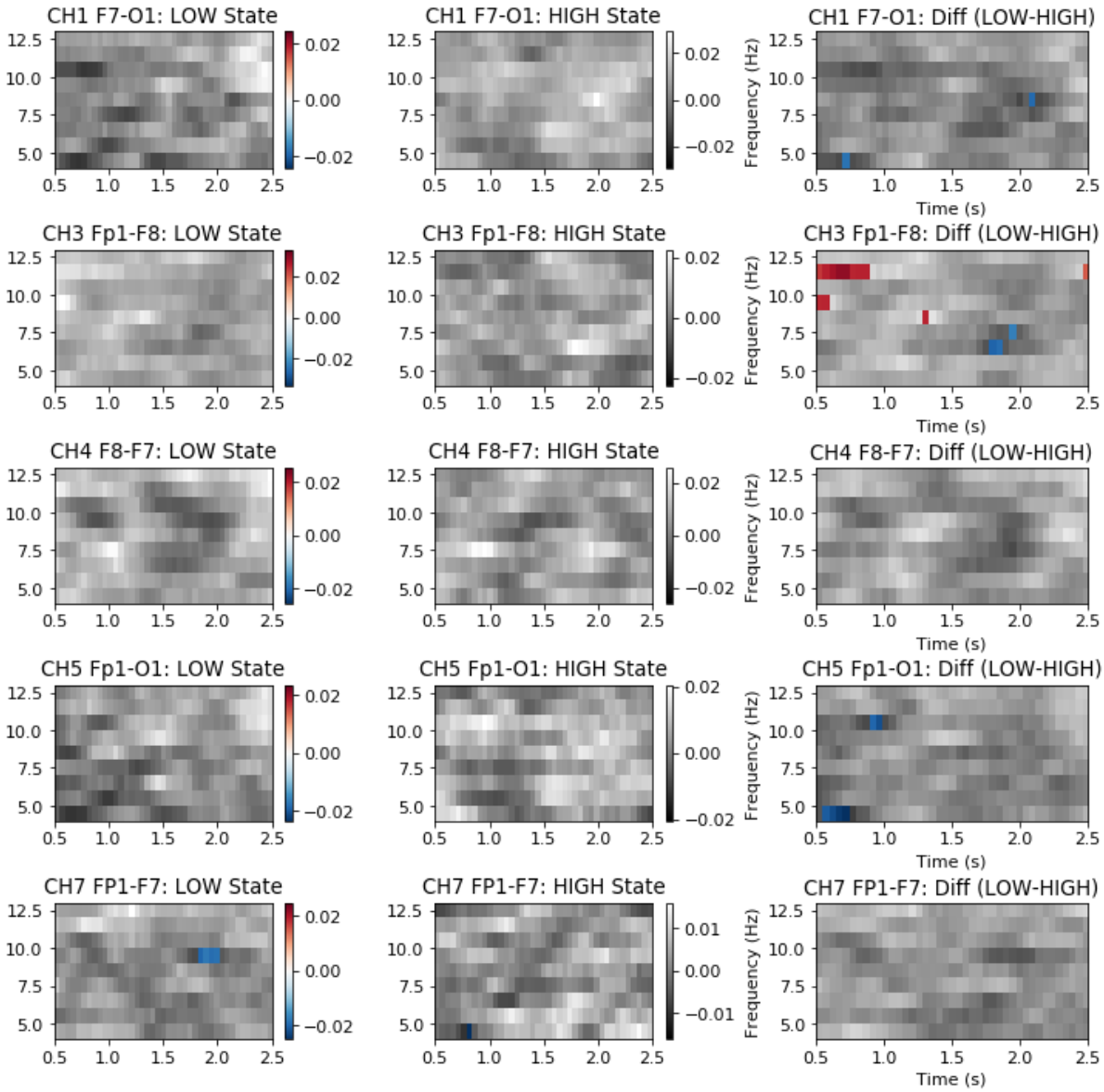

Figure 8: State-Specific ERD Across Time: Time-freq data was calculated with a fft and sliding time window of 1s length and further processed through baseline-correction (% change). Time axis indicates the 0.5 to 2.5 seconds window following auditory stimuli. Grey scale indicates relative change from baseline (dark = desynchronisation, light = synchronisation) for low-state epochs (left), high-state epochs (middle) and the difference between the two states (right). Colour overlay emphasises time-freq pixels which differed significantly from baseline (left and middle) or significant differences between low and high (right). Number of epochs = 100.

the low state showed significantly more ERD than the high state at 4Hz and 10.75Hz.

For CH3, no clear ERD was evident based on the time-freq plots of either brain state. However, these two brain states differed significantly between 0.5 and 1 second after stimulus presentation. The direction of this difference was the reverse of our expectations; the data suggested that in the high state there was significantly more ERD at 11.9Hz than in the low state. As the high-alpha rhythm is suppressed in the high state it was surprising to find greater desynchronisation. Given that there was no sign of ERD in the high state alone, we were not convinced that this significant between-state difference reflected a true neurophysiological distinction.

We were not able to identify any significant responses for CH4. However, there was a general trend for desynchronisation at higher-alpha frequencies selectively in the low state. This aligned with our expectations that CH4 would show ERD selectively in low-state epochs. However, as none of these effects were significant, we were unable to draw any conclusions from this. Finally, examining the data for CH7 revealed a significant

ERD event at 9.6Hz, taking place almost 2s after the stimulus. We had no prior expectations regarding this channel as it displayed neither of the two dominant alpha rhythms and so the implications of this peak were uncertain.

We found no evidence to support our primary hypothesis that we would see high-alpha ERD occurring selectively in CH3 and CH4 in low-state epochs. The picture was muddier for CH1 and CH5. Both CH1 and CH5 showed some tentative signs of significantly greater ERD (0.5 to 1s) occurring in the low state. Interestingly, this potential ERD was at both sub-alpha and high-alpha frequencies. The implications of this were unclear because the sub-alpha is suppressed in this state and the high-alpha rhythm was not visibly present in either of these channels. Additionally, neither channel showed any significant ERD relative to baseline. Taken together, we did not find any strong evidence to support the idea that these brain states possessed distinct neurophysiological properties.

Discussion

Absence of Sub-Alpha ERD

Our first aim was to explore the neurophysiological responses of the enigmatic sub-alpha rhythm (4-6Hz) in our patient. We investigated whether this sub-alpha behaved like a typical alpha rhythm by quantifying the ERD at this frequency after stimulus presentation. As figure 3 shows, we found no sign of ERD at this frequency. The lack of ERD to auditory stimulation may be reflective of the fact that this sub-alpha rhythm is not functionally equivalent to a typical alpha rhythm.

No Clear State-Specific Responses to Auditory Stimulation

After identifying the presence of two distinct brain states, our next aim was to assess whether these states were associated with distinct neurophysiological responses. To do this, we again measured ERD to auditory stimuli but over a wider range of frequencies. Power spectral analysis of discrete time windows suggested that no significant ERD was occurring in either brain state (figure 7). Unlike the between-state comparison, the difference in the oscillatory characteristics between baseline and response segments appeared to be negligible. By itself, this result suggested that neither brain state was associated with an intact neurophysiological response to auditory stimuli. The picture was more nuanced when we examined oscillatory changes across the time dimension of the response window. However, we were unable to find any convincing evidence to suggest that the brain states were associated with distinct cognitive capacities.

We were also not able to make any solid conclusions about the roles of the two distinct alpha rhythms. As the high-alpha rhythm sits within the normal range, we believed it may be reflective of improved cognitive function. We thought that this heightened function would reveal itself through greater ERD occurring in the low state. However, the two channels possessing prominent high-alpha rhythms did not show any significant ERD or preference for the low state. Also, the few signs of greater ERD occurring in low state compared to the high state manifested in channels with no visible high-alpha rhythm. Similarly, we couldn't make any strong conclusions about the relevance of the sub-alpha rhythms in each brain state. Hints of sub-alpha ERD seemed to show a preference for the low state, where the sub-alpha rhythm is suppressed. This could suggest that the sub-alpha rhythm reflects underlying cognitive dysfunction. This, in turn, would explain why sub-alpha ERD was not present in the high state.

In summary, it was certainly possible that we had witnessed some temporally restricted ERD. The window 0.5 to 1s, and also around 2s, after stimulus appeared to show some hints of desynchronisation at sub-alpha and high-alpha frequencies. The fact that potential ERD events were focused in these bands suggested our sub-alpha and high-alpha rhythms may have produced some subtle effects. These subtle effects may have been masked by noise in the data. On a qualitative note, our time-freq plots appeared to contain considerable noise and the potential ERD events were not strongly distinguishable from the background noise. We corrected for multiple comparisons using the Benajmin Hochberg method. This approach had the benefit of reducing our false negative but may inadvertently have increased our false positive rate. Consequently, we felt we had no strong evidence to claim that one brain state was associated with greater cognitive capacities than the other.

Presence of Two Distinct Alpha Rhythms

The observation that each brain state was clearly associated with a distinct alpha-peak frequency was striking. In each epoch, our patient was displaying either a sub-alpha (4 – 6Hz) rhythm or a high-alpha (10.5 – 12.5Hz) rhythm. We were unable to show that these brain rhythms possessed distinct, functional properties. As a result, the implications of these distinct alpha rhythms on the assessment of our patient's level of cognitive capacity is unknown.

One potentially important feature of these rhythms is that they appeared in different channels suggesting they made be topographically distinct. The sub-alpha (4 – 6hz) rhythm was present in channels linked to the '01' occipital electrode. Alpha rhythms in posterior brain regions have been proposed to underlie many important visual and cognitive functions [Clayton et al, 2017]. The significance of the dramatic lowering of alpha peak frequency (APF) is still an open question. Subtle slowing of the APF has been documented in cases of mild cognitive impairment [Garcés et al, 2013]. Whether a dramatic lowering of the APF indicates a dramatic drop in cognitive function is unknown. The authors also found a correlation between APF in posterior regions and hippocampal volume. It is intriguing to speculate that a downshift in posterior APF is reflective of the degeneration of certain brain regions and could be the topic of future research efforts.

The high-alpha (10.5 – 12.5 Hz) rhythm appeared to emanate from the 'F8' frontal electrode. As this rhythm resides within the typical alpha range it could indicate the persistence of normal function. It has been suggested that alpha rhythms in frontal regions are involved in top-down, inhibitory control of perceptual processes [Misselhorn et al, 2019] and affective states [Sikka et al, 2019]. Although the possibility that these high-alpha rhythms may reflect some persisting cortical function is intriguing we weren't able to verify that through our project.

Considerations, Limitations and Future

What reasons could explain the lack of clear ERD in our patient? It may be that the cognitive decline in our patient had reached a stage where the processing modules responsible for the ERD phenomenon are no longer functional. Cortical responsiveness to auditory stimulation has been observed in another CLIS-ALS patient [Ramos et al, 2011]. However, the heterogeneity of the cognitive decline associated with ALS implies that our patient may not share this quality. Furthermore, cortical responsiveness does not necessarily prove that higher-level cognitive or attentional processes are intact. The absence of ERD in both brain states could be taken as evidence that underlying processing modules are impaired in our patient, with no substantial variance in their functionality occurring through the day.

I believe that, while our results do not provide evidence in support of our patient possessing varying levels of cognitive function, they do not provide strong evidence against this theory either. Our analysis of oscillatory changes occurring over time revealed some hints of state-specific ERD although these were not conclusive. Our ability to detect these differences may have been hindered by some of the technical issues we experienced. We encountered some difficulties with the identification of stimulus timepoints which limited the number of epochs we could reliably extract and use for analysis. Additionally, our quantity of available epochs was further restricted by the presence of stimulus-artefacts in some sessions. Consequently, we may not have had adequate statistical power to find any real, subtle effects in the data. This note is especially relevant to us as EEG recordings are prone to contamination with considerable noise form a variety of sources. The resulting low signal to noise ratio requires a high number of trials to 'cancel-out' the noise and emphasise the desired signal. Inclusion of more epochs from future recording sessions could help to enhance the statistical power of our analysis. A previous researcher had also suggested that there may be some temporal drift between the stimulus channel and the EEG channels. In some sessions, we found that stimulus-related artefacts appeared in the EEG recordings with a constant 0.5s latency. This encouraged us, that at least in these sessions, the discrepancy between the stimulus channel and EEG channel could be rectified by a simple time shift. A built-in mechanism for synchronising stimulus timepoints with EEG recordings would have been ideal for improving data quality but would have come with added costs and inconvenience to our patient.

Secondly, the topographical limitations of the Dreem headset may have prevented us from registering any ERD. Previous work on ERD from auditory stimulation found that while the low-alpha (8 – 10Hz) ERD was topographically widespread across the cortex, the high-alpha (10 – 12Hz) ERD was localised to temporal and parietal regions [Krause et al, 1994]. We had identified two alpha peaks in our data; a sub-alpha (4.5 – 6Hz) rhythm

as well as high-alpha (10.5 – 12Hz) rhythm. The high alpha rhythm could be expected to follow the same trend as previous work and desynchronise selectively over temporal and parietal regions. No previous work has been performed on the ERD characteristics of sub-alpha rhythm and so the topographical pattern of ERD at these frequencies is unknown. Our recording electrodes were positioned above frontal, prefrontal and occipital regions. Consequently, it is plausible that potential ERD in our data was not sufficiently widespread for us to be able to register it. Future analysis of CLIS-ALS patients using a complete array of EEG electrodes would allow us to record ERD taking place in topographically isolated regions.

Summary

While our results don't provide evidence in favour of our CLIS-ALS retaining some level of cognitive function, they also do not provide evidence against. The presence of two distinct brain states associated with a dramatically distinct APF was a very promising finding. This is especially true for the high-alpha rhythm which resides within the normal alpha range for healthy subjects. The implications of our patient exhibiting these neurophysiological traits is uncertain and raises many more questions to answer. I believe further work, incorporating a great number of epochs and a full array of EEG channels would be needed to elucidate the functional roles of these distinct alpha rhythms. Furthermore, providing stimuli in a variety of different sensory modalities would give us a more complete understanding of the cognitive capacities of each brain state.

Methods

Experimental Setup

The recordings were performed using a low cost, Dreem headset. The comfort and portability of this type of device enabled long-term recordings of EEG data in the patient's environment. The main limitation of this device was that it featured only five electrodes and so our analysis was topographically restricted. By referencing between these electrodes, we were able to work with seven EEG channels.

Additionally, the Dreem headset did not feature any hardware or software to incorporate the auditory-stimulation aspect of the experiment. Therefore, auditory stimulation was applied using an external device. Each auditory stimulus train consisted of five stimuli interspersed at three second intervals. These stimulus trains were delivered to the patient at random intervals throughout the day. Using separate devices for the recording and stimulation produced the issue of synchronising the timing of the auditory stimulation with the recorded EEG signal. To get around this, the plethysmograph photodiode (pleth diode) on the headset, which was designed to monitor cardiovascular signal, was manipulated so that it was recording a signal emitted from the external stimulation device. Through this adaptation, we were able to monitor the timing of the auditory stimuli and align this information with the EEG signal.

Epoch Extraction

Initially, we constructed epochs by identifying the onset of stimulus trains and then extracting data 15+ seconds before (baseline) and 15+ seconds after (response) this timepoint. We experienced a few issues with our ability to accurately extract epochs around presentation of the auditory stimuli. This was primarily the result of the improvised, stimulus-timing setup. One problem was that the pleth signal was prone to saturation. We were unable to establish the precise timing of some potential stimulus events because of this issue. However, for other stimuli, we observed that the pleth signal was effective at indicating the timing of the events. To define the time points where the auditory stimuli occurred, we noted the timepoints where the pleth signal exceeded a threshold value. This threshold value was based on the variance of signal and was automatically altered to compensate for the difference in variance between recording sessions. Next, we applied visualisation tools from the MNE toolbox to manually reject 'false' stimuli and any select only the first stimulus of each stimulus train.

Preprocessing

For analysis which did not require a time dimension we performed analysis on the whole baseline (-15 to 0) and response (0 to 15) segments. Through this method, each response period incorporated five individual stimuli. When a time dimension was retained (ERPs and time-freq analysis), we further segmented the baseline and response segments into 3 second windows. This

allowed us to focus on the responses to individual stimuli and, by doing so, increase our number of epochs and create more reliable averages.

Prior to analysis, we excluded two EEG channels which featured a very high level of noise and were deemed to be faulty. Visualisation tools were used to comb through the combined data and manually exclude epochs which contained obvious electrical artefacts or instances of signal saturation, which would likely contaminate any subsequent frequency-domain analysis.

Stimulus-Related Artefacts

One problem we encountered was that our EEG signal appeared to be contaminated by the stimulus itself. Specifically, we observed deflections in the EEG channels which accurately matched the timing of the auditory stimulation and resembled the pleth signal. This occurred prominently in one of our recording sessions and weakly in three other recording sessions. Somehow, it seemed that the signal emanating from the LED on the external auditory device was not only affecting the intended target of the pleth diode but also 'infecting' the EEG channels. Given the close proximity of the pleth diode to some of the electrodes and the makeshift nature of the setup, this contamination is understandable.

To remedy this, we attempted to use independent component analysis (ICA). We hoped that the application of machine learning algorithms would allow us to isolate and remove these artefacts from the EEG data. While these algorithms were able to register the artefacts, they were not able to separate them from the 'good' signal. One reason for this is that the maximum number of independent components is limited by the number of EEG channels. As we had only five channels, we were only able to generate five independent components which was not sufficient for the algorithm to be able to selectively isolate the stimulus-induced artefacts. Another factor was likely the small size of the epoched data which may have reduced the effectiveness of the algorithm. Using the intact, raw data may have provided a greater quantity of data but the relative prominence of the stimulus-induced artefacts was weaker than the epoched data. As a result, neither ICA-based approach was able to successfully remove the artefacts without also removing important signal. Although not ideal, our solution to this problem was to manually exclude epochs which contained the artefacts. This had the effect of reducing our total amount of available epochs for analysis.

Data Analysis

Data used for ERPs was filtered between 0.1 and 15Hz. The average ERP was calculated by taking a mean average across epochs. To generate power spectral plots for discrete time windows, we used a fast fourier transform (fft) method with Hanning windows and then log transformed all power values. To assess the temporal evolution of brain rhythms we applied our fft algorithm with a sliding window. The length of this window was either 0.5 or 1 second depending on the analysis applied to.

We divided our trials into either 'high' or 'low' classes. To do this, we examined the sub-alpha (4.5 – 6Hz) power histograms generated from CH5 and set a threshold power level which best split the bimodal distribution. Trials which exceeded the threshold value were labelled as 'high' whereas falling below the threshold were labelled as 'low'. Finally, the number of trials in the high and low conditions was equalised by randomly excluding excess trials from the condition with the greatest number. This step was critical as it mitigated against the biasing effect of trial number in the calculation of power values.

Non-Parametric Statistics

We used non-parametric, permutation statistics to test for differences between-state (high vs low) or within-trial (baseline vs response). To do this we used a standard monte-carlo randomisation procedure with 1000 permutations, as described in detail [Maris & Oostenveld, 2007]. When testing for between-state differences we generated test statistics by comparing randomising epochs belonging to each brain state and calculating the average difference. For within-trial comparisons of power spectra, we applied the same principal. For within-trial comparisons of time-freq changes we applied a slightly altered method. For each frequency, we first averaged the baseline across the time dimension and then compared each time-freq pixel in the response period to the time-averaged baseline value.

For instances, where we were specifically looking for a unidirectional change, we applied a one tailed test. This was the case for our analysis of whether the sub-alpha oscillation underwent ERD in response to auditory stimulation. For comparison

between brain states, we were unsure which state would be associated with heightened cognitive capacity. Therefore, we tested for differences in ERD occurring in both directions using a two tailed approach.

For comparison of power spectral values within a limited frequency range, our number of tests was low and so a simple Bonferroni correction was used to adjust the p values. However, for comparisons across many frequencies or time-frequency pixels, we instead used a Benjamin Hochberg correction. This was done to lower the inflated false negative rate which would be expected from use of the conservative Bonferroni method.

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