Resting State EEG Variability and Implications for Interpreting Clinical Effect Sizes

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***Abstract*— Resting state electroencephalography (rsEEG) is widely used to investigate intrinsic brain activity, with the potential for detecting neurophysiological abnormalities in clinical conditions from neurodegenerative disease to developmental disorders. When interpreting quantitative rsEEG changes, a key question is: how much deviation from a healthy normal brain state indicates a clinically significant change? Here, we build on the existing rsEEG variability literature by quantifying how this baseline rsEEG range can be attributed to common but underinvestigated sources of variability: experiment day, time of day, and pre-recording exercise level. We found that even within individuals, frequency band powers and entropy measures can vary by 7% (sample entropy and relative alpha power) to 28% (absolute delta power). Absolute and relative delta power increased significantly after running, while relative theta power decreased significantly. Relative beta and gamma power were significantly higher in the afternoon compared to morning trials. Sample entropy and alpha power were relatively consistent. The coefficients of variability we found are similar to some clinical rsEEG effect sizes identified in prior literature, bringing into question the clinical significance of these effect sizes. Furthermore, time of day and activity level accounted for more rsEEG variability than experiment day, indicating the potential to reduce variability by controlling for these factors in repeated-measures studies.**

***Index Terms*— Frequency band power, resting state electroencephalography (rsEEG), sample entropy, within-participants variability.**

I. Introduction

ELECTROENCEPHALOGRAM (EEG) recordings taken during resting state are increasingly applied in neuroscience research to study intrinsic brain activity. Resting state EEG (rsEEG) metrics have demonstrated sensitivity to the altered neurophysiology in brain diseases and injuries, and have accordingly been suggested for clinical application [1], [2], [3]. For example, rsEEG band power analyses have shown slowing of brain activity (shift towards lower frequency bands) in psychiatric [4], [5] and neurodegenerative conditions [6], [7], as well as mild traumatic brain injury [8], [9]. Meanwhile, rsEEG complexity generally decreases in neurodegenerative, developmental, and psychiatric disorders, with numerous reports of decreased entropy in Alzheimer's Disease (AD), Autism Spectrum Disorder (ASD), and mood disorders [10], [11].

Interpreting the clinical significance of quantitative rsEEG changes depends on the variability in the rsEEG representation of brain states. Prior to clinical application, we need to address the question: how much deviation from a healthy normal brain state indicates a clinically significant change? It is generally well-established that inter-participant variations can be substantial, yet intra-participant rsEEG patterns remain relatively stable [12], especially when the measurement system is controlled [13]. High test-retest reliability scores within participants have been found between repeated trials performed a few minutes apart [14], a few days apart [15], and several weeks or even months apart [16], [17]. The theta/beta band power ratio for attention deficit/hyperactivity disorder (AD/HD) diagnosis has demonstrated reliability over several days and consistency with the time progression of established measures of attentional control [18]. The alpha band, which constitutes the dominant EEG rhythm during eyes-closed resting state, has also shown low variability and has thus been the focus of many rsEEG analyses [19].

While the day-to-day variability of rsEEG metrics is relatively well-established, it is known that a number of biochemical, physiological, and behavioral factors can modify EEG patterns [20], and as rsEEG is moving towards more ambulatory applications [21], further quantification of potential variability is required for clinical translation. Circadian rhythms in alertness and wakefulness have been correlated with EEG metrics, and these neuroelectric changes are hypothesized to be mediated by time-dependent neurotransmitters such as melatonin [22]. Shifting from morning to evening trials has demonstrated increases in alpha power, beta power, and entropy [23]. The physical stress level of a study participant may also be reflected in rsEEG recordings. In healthy adults, moderate aerobic exercise (e.g., running on a treadmill)

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This work involved human subjects or animals in its research. Approval of all ethical and experimental procedures and protocols was granted by the University of British Columbia Research Ethics Board under Application No. H20-02313, and performed in line with the Declaration of Helsinki.

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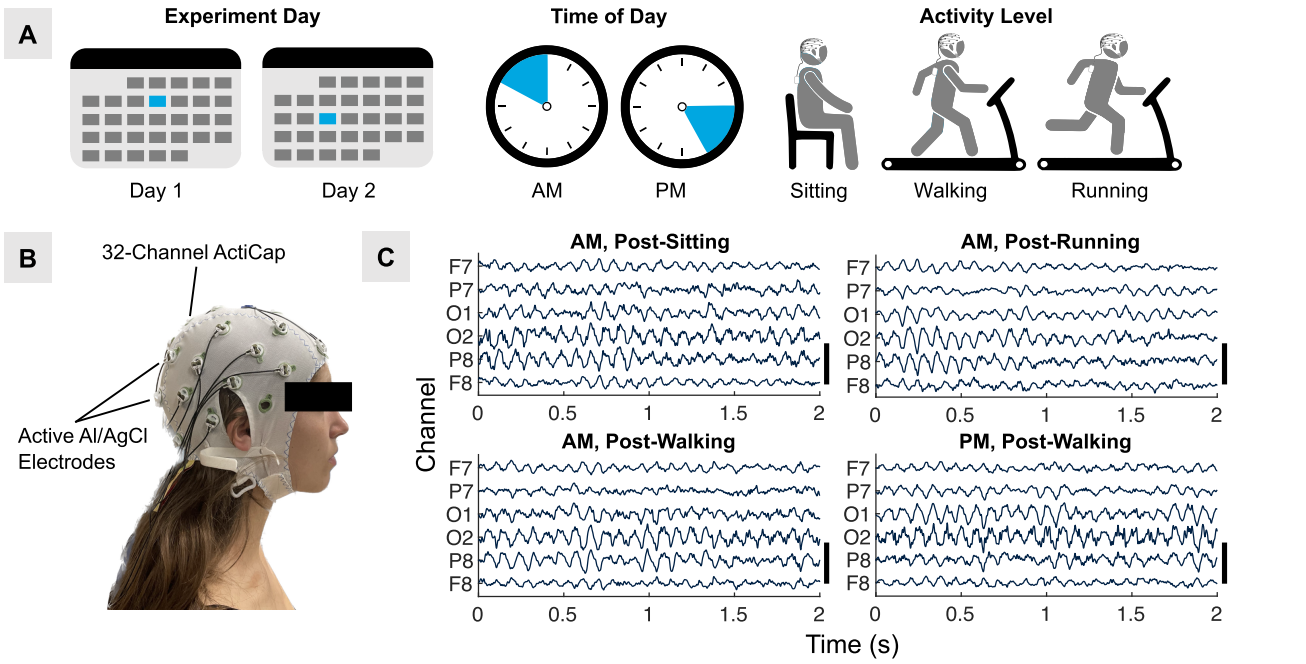

Fig. 1. Overview of methods. The experimental design (A) consisted of 2 experimental days, 2 times of day, and 3 pre-resting state activity levels, for a total of 12 trials per participant. We used the Brain Products LiveAmp with active electrodes and the 32-channel ActiCap (B). Clean EEG signals (C) were obtained through bandpass filtering, ICA, and manual artifact pruning. The scale bar represents 100 μV.

significantly reduced the relative theta power in subsequent resting state recordings [24]. A similar effect has also been observed in children with AD/HD, with exercise decreasing the theta/beta ratio [25]. Our study presents a novel analysis of the specific contributions of these common variables to an individual's overall rsEEG variability.

In this study, we examined the variability in rsEEG power spectrum metrics and sample entropy over repeated trials on different days, at different times of day, and after varying levels of pre-recording physical activity. We hypothesized that the rsEEG changes due to these factors contribute significantly to intra-individual variability and that quantifying these contributions can inform clinical interpretations of effect sizes and potential strategies to control for variability.

II. Methods

A. Participants

12 healthy young adults (5 female, 7 male) aged 18 to 26 years (mean age of 22.3 $\pm$ 2.60 years) participated in the study. Participants were instructed to carry out daily activities as normal for the 24 hours before the experiment and were advised against unusually strenuous exercise or sleeping less than normal. Consuming alcohol or recreational drugs was not permitted in the 24 hours before each experiment session. The human participant protocol for the study was approved by the University of British Columbia Research Ethics Board (H20-02313), and informed consent was obtained from all participants.

B. Experimental Setup

Experiment sessions included measurements across different days, times of day, and pre-rsEEG activity levels (Figure 1A). On each experiment day, participants participated in a morning session (between 10:00am and 12:00pm) and an afternoon session (between 3:00pm and 5:00pm). Each session consisted of 5-minute eyes-closed rsEEG measurements after different activities (3 minutes of sitting, treadmill walking, and treadmill running, in that order). Overall, we took 12 rsEEG recordings from each participant (2 days, 2 times, and 3 activities).

To simulate normal exertion levels for the non-stationary activities, participants each chose comfortable speeds between 2.5 mph to 3.5 mph for walking and between 4.5 to 5.5 mph for running. After EEG setup and verbal instructions, participants were provided a set of earplugs with a 29 dB attenuation rating (3M, Saint Paul, USA) to block ambient noise. During rsEEG measurements, participants were instructed to sit quietly, relax, keep their eyes closed, and avoid dwelling on any particular thought.

C. EEG Instrumentation

EEG was recorded through 32 Ag/AgCl active electrodes arranged using the International 10-20 system on the actiCAP slim cap and amplified using the Brain Products LiveAmp amplifier and SuperVisc conductive gel (Brain Products, Gliching, Germany) (Figure 1B). Electrode-scalp impedances were kept below 25 kΩ. EEG data were recorded at a sampling rate of 500 Hz and sent via Bluetooth to a PC running the Brain Products Recorder software for live monitoring. The FCz electrode was used as the initial recording reference, and the ground electrode was placed between Fp1 and Fp2. Resting state recordings were started after visual confirmation of participant compliance with instructions, cessation of eye blink artifacts in Fp1 and Fp2, and decreased motion in the accelerometer channels. 5 minutes of eyes-closed rsEEG were

then collected, with an additional 10 seconds of buffer before and after to account for edge artifacts.

D. EEG Pre-Processing

EEG recordings were imported into EEGLAB [26] running on MATLAB r2022a (Mathworks Inc., Natick, MA). The data were first re-referenced to the common average reference, then 1-100Hz bandpass filtered using a 4th-order forward-backward Butterworth filter. Large and transient multi-channel artifacts were removed manually, resulting in an average loss of 5.68s per 300s recording (1.9%). Independent component analysis (ICA) was performed using the extended infomax algorithm [27]. The physiological signature (brain, muscle, eye, heart, line noise, channel noise, or other) of each independent component (IC) was determined using the ICLabel EEGLAB plugin for automatic IC classification. Components classified as artifacts with greater than 85% confidence were flagged for removal. These classifications and component power spectra were used to manually select independent components with significant artifacts to remove from the data. The cleaned signal was then reconstructed from the remaining ICs (Figure 1C).

E. Quantitative EEG Metrics

Whole, processed rsEEG trials were separated into 2-second epochs to create time series of relative frequency band powers for each trial. Epochs had a 50% overlap and were windowed using a Hamming window. The power spectral density of each epoch was computed using the Fast Fourier Transform. According to the convention in the literature, power spectra were reported using a logarithmic (log) scale (dB) [26], and subsequent variability analyses were performed on non-transformed values (μV^2). Because log-scale analyses could be valuable for comparison with studies using log-scale, we include them in the Supplementary Materials (Supplementary Figures 1-3). Frequency bands were defined as delta (1-4 Hz), theta (4-8 Hz), alpha (8-13 Hz), beta (13-22 Hz), and gamma (22-40 Hz) [28]. Frequency band powers were computed from the area under the power spectral density, and relative band powers were determined by dividing each frequency band power by the total spectra power.

For each epoch, we also computed sample entropy with standard parameter values of $m = 2$ and $r = 0.2$. Sample entropy is estimated as the conditional probability

$$\mathrm{SE}(m, r) = -\log\left[A^m(r)/B^m(r)\right], \tag{1}$$

where $B^m(r)$ is the probability of two vectors of length m in the time series exhibiting a similar pattern, $A^m(r)$ is the probability of two vectors of length $m+1$ exhibiting a similar pattern, and r is the tolerance level for this similarity [29]. Sample entropy quantifies the likelihood of pattern repetition in a time series as a proxy for complexity. Power spectra and sample entropy values for whole trials were determined by averaging across the 2-second epochs and across EEG channels.

F. Statistical Analysis

We used the coefficient of variability (CV), defined as the standard deviation normalized by the mean, to quantify the overall variability of rsEEG metrics for each participant. CVs were expressed as percentages to facilitate comparisons with literature effect sizes. A repeated-measures analysis of variance (ANOVA) was performed to determine the within-participants effects of experiment day, time of day, and activity level on rsEEG relative band power and sample entropy. Response data (band powers and entropy) were log-transformed to pass the Kolmogorov-Sinai test for normality prior to performing the ANOVA. A separate analysis was performed for each rsEEG metric. *Post hoc* tests corrected for multiple comparisons using Tukey's HSD were used to determine significant contrasts between levels of the fixed effects. All statistical testing was performed in R.

G. Intra-Trial Variability

Since we noticed non-stationary behavior in the time series of the power and entropy parameters, we also examined and quantified this behavior. Time series were first smoothed using a moving average filter with a window size of 25 points (MATLAB *smoothdata* function) to reveal global trends. Settling patterns were considered present if the mean of the initial 5 seconds of recording was either 2 standard deviations above (downward settling) or below (upward settling) the steady-state value (mean of the second half of the recording). This heuristic method provided settling pattern determinations consistent with visual examinations of settling behavior. Settling time was defined as the time point at which the settling pattern crossed the time series mean. Correlations between time series of different metrics were calculated using the Pearson correlation coefficient.

III. Results

A. Overall Intra-Participant Variability

The shapes of the rsEEG power spectra across trials within participants were consistent (Figure 2A-L). While the spectra for each participant demonstrated peaks in both the alpha and beta frequency bands, the height, width, and center frequencies of these peaks varied between participants (Figure 2M). The rsEEG metrics tested in this study showed varying levels of intra-participant variability (Figure 3). Of the relative band powers, the least variable metric was relative alpha power (0.49 ± 0.03, CV = 7%) (Figure 3C, Figure 4A) and the most variable metric was relative delta power (0.17 ± 0.02, CV = 13%) (Figure 3A). Of the absolute band powers, the least variable metric was absolute beta power (4.46 ± 0.59 μV^2, CV = 12%) (Figure 3J) and the most variable metric was absolute delta power (5.69 ± 1.45 μV^2, CV = 28%) (Figure 3G). Total absolute power over all frequency bands (41.87 ± 4.86 μV^2, CV = 13%) (Figure 3L) was less variable than the absolute power in any individual frequency band, agreeing with the similarities in power spectra size and shape within participants. Sample entropy had low intra-participant variability (0.59 ± 0.04, CV = 7%) (Figure 3F).

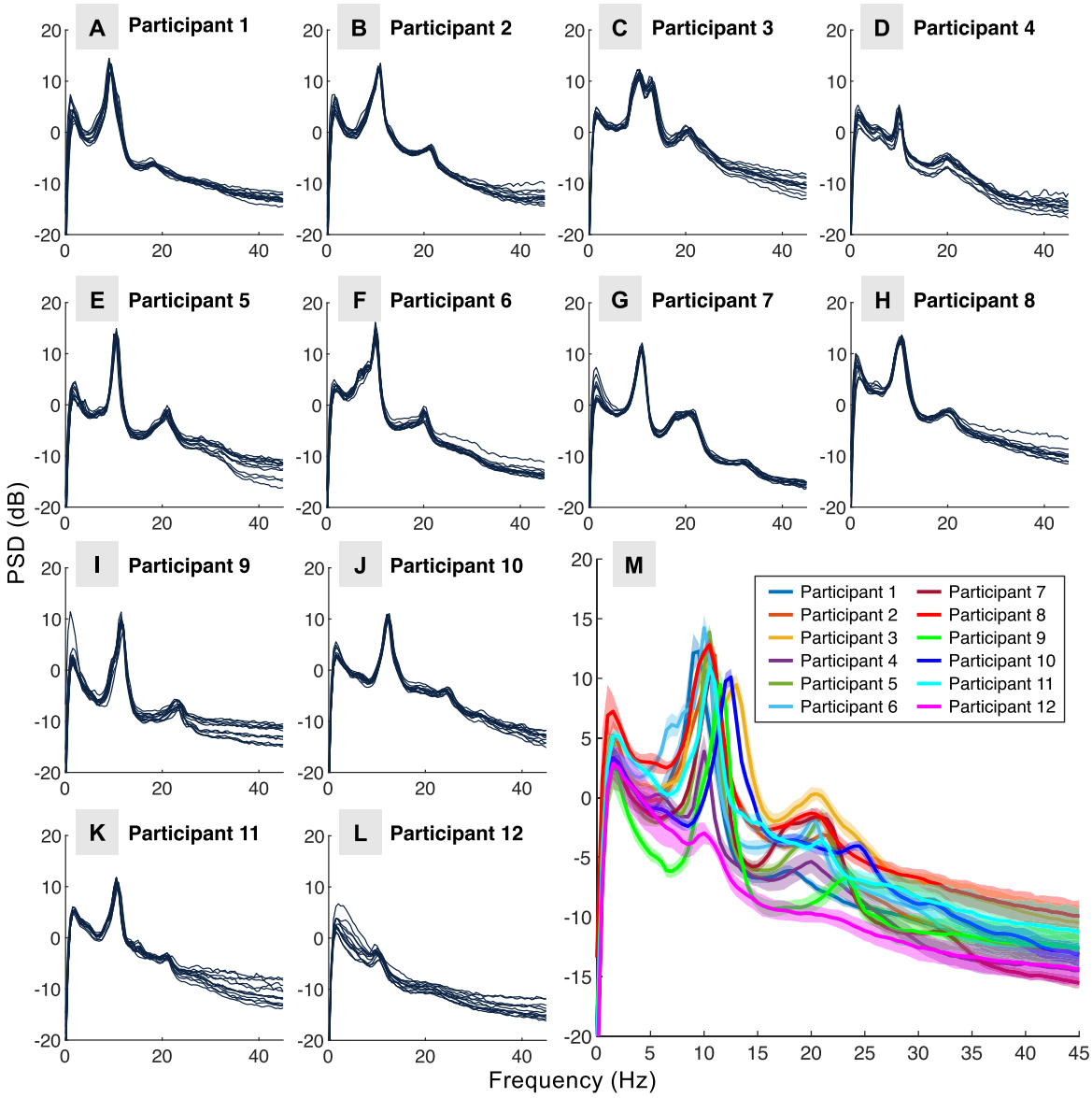

Fig. 2. Power spectral densities. Power spectra for single rsEEG trials (navy blue, A-L) were consistent in shape within participants, while average power spectra over repeated trials (M) varied in shape between participants. Standard deviations are shaded.

B. Effects of Experimental Factors on rsEEG Variability

The experimental factors we examined explained up to about 20% of the variance in rsEEG metrics (Figure 4B). Time of day and activity level accounted for more of the variance than experiment day. The repeated-measures ANOVA revealed that time of day had a significant effect on relative beta power ($p = 0.001$) and relative gamma power ($p = 0.001$). Across activity levels pre-resting state, main effects analysis showed a significant difference in relative delta power ($p = 0.003$), relative theta power ($p < 0.001$), absolute delta power ($p < 0.001$), and total power ($p = 0.002$). Neither time of day nor activity level significantly affected sample entropy (Figure 5F). Experiment day did not have a significant effect on any rsEEG metrics (Figure 5A).

Post hoc comparisons for relative beta and relative gamma power showed an increase in PM sessions for both metrics ($p < 0.001$ for both) (Figure 5B). Relative delta power was higher after running compared to after walking ($p = 0.006$) (Figure 5C) and after sitting ($p = 0.01$) (Figure 5D). Absolute delta power was higher after running compared to both the walking and sitting conditions ($p < 0.001$ for both) (Figure 5C,D). Total power was higher after running compared to the sitting condition ($p = 0.002$) (Figure 5D) and after running compared to after walking ($p = 0.04$) (Figure 5C). Theta power responded in an opposite manner to delta and total power, experiencing a decrease after running compared to both the walking and sitting conditions ($p = 0.004$ and $p < 0.001$, respectively) (Figure 5C,D). *Post hoc* testing revealed no significant differences between the walking and sitting conditions (Figure 5E). We found no significant interaction effects on any of the rsEEG metrics.

C. Intra-Trial rsEEG Variability

We observed that quantitative rsEEG measurements vary within individuals not only between sessions (time scale of

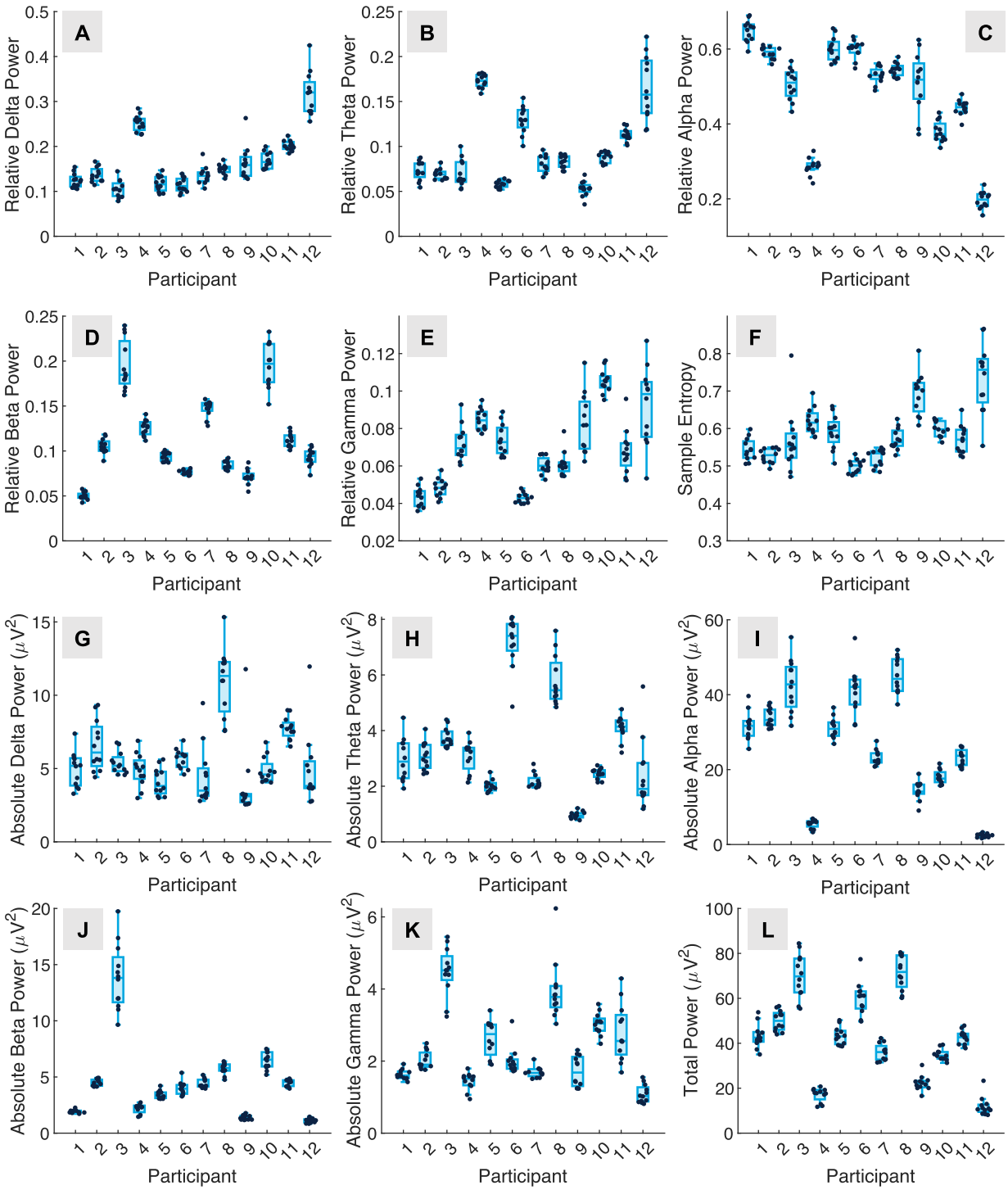

Fig. 3. Within-participants variability in rsEEG metrics. The spread of the distribution of measurements for each participant (boxes represent individual distributions) depended greatly on the participant and the rsEEG metric.

hours to days) but also within trials (time scale of seconds to minutes). Of the 144 sample entropy time series collected over all experiment sessions, 87 time series exhibited a decreasing settling pattern (Figure 6A). The relative band power time series exhibited fewer settling patterns overall, and these were mostly found in the alpha (47 of 144) and gamma (69 of 144) frequency bands. Gamma power settled with similar patterns as sample entropy (Pearson's correlation $\rho_{\text{avg}} = 0.80$), while alpha power was negatively correlated with sample entropy ($\rho_{\text{avg}} = -0.59$) (Figure 6B). A mixed-effects logistic regression revealed no significant effects of experiment day, time of day, or activity level on the presence of a settling pattern. However, the number of settling patterns depended significantly on the participant ($p < 0.001$). For sample entropy trials with settling patterns, the average settling time over all trials was 85.0 seconds. The difference between the initial

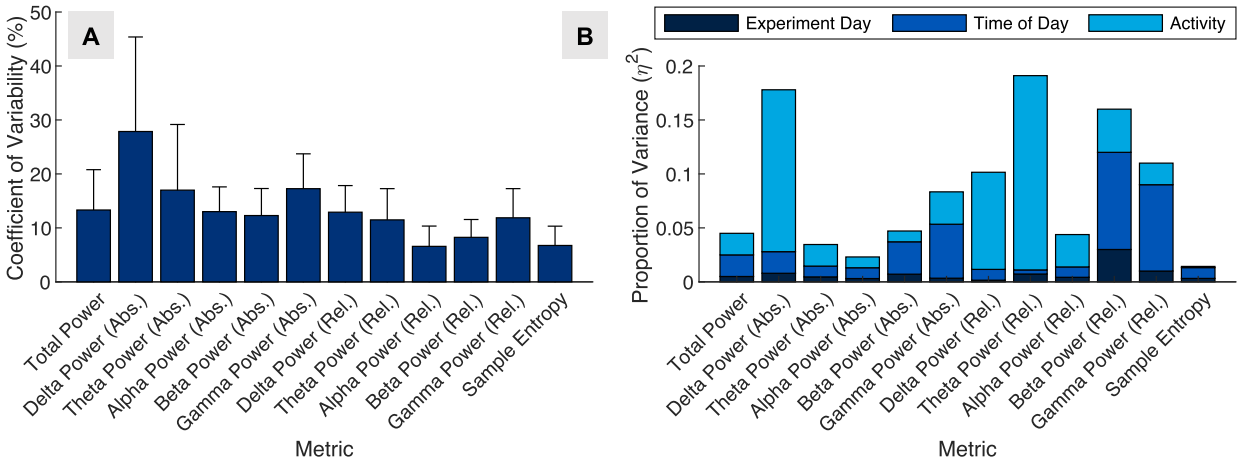

Fig. 4. Quantification of rsEEG variability. Within-participants coefficients of variability, averaged over all participants, were greatest for absolute delta, theta, and gamma power, and lowest for relative alpha power and sample entropy (A). Absolute delta power, relative theta power, and relative beta power had the greatest ANOVA effect sizes (B). Activity level and time of day were the main contributors to this variance.

response and the steady state value was 0.206 (+27.0%) for sample entropy, −0.280 (−113%) for relative alpha power, and 0.073 (+54.6%) for relative gamma power. Repeated-measures ANOVA showed that the settling time and settling magnitude did not depend significantly on the experimental factors.

D. Brain Region Effects on rsEEG Variability

Lastly, we explored how the intra-participant variability in rsEEG metrics depends on brain region using CV scalp maps (Figure 7). We observed that variability can be highly localized to certain brain regions, with peripheral brain regions generally exhibiting higher variability than central brain regions.

IV. Discussion

As rsEEG becomes more common as a research and clinical tool, understanding the variability in rsEEG measurements due to healthy normal variations and experimental factors will be crucial for making meaningful interpretations. We found that even within individuals, rsEEG metrics can vary by over 25% from trial to trial, which has implications for how clinically significant effect sizes for these metrics are determined and interpreted. We demonstrated that this variability may be mediated by often overlooked factors such as time of day and physical activity. Research studies or clinical tests using rsEEG may need to further control protocols to account for this sensitivity to normal changes in experimental conditions.

A. Overall EEG Variability: Implications for Interpreting Clinical Effect Sizes

The variability scores expressed in Figure 4 quantify the normal spread of within-participants rsEEG measurements. For a given participant, a rsEEG recording taken at one time point may lie anywhere within this spread, and can only be meaningfully assessed to be normal or abnormal after this variability has been quantified. Here, we quantified the normal variability for 12 participants, and the average of these variabilities for each EEG metric can be compared to literature effect sizes to estimate clinical significance.

For example, in a review of rsEEG band power metrics in psychiatric disorders, Newson and Thiagarajan summarized that AD/HD patients exhibited an 11% increase in relative theta power and a 25% increase in absolute theta power over healthy controls [30]. We observed an 11% average intra-participant coefficient of variability (CV) in relative theta power and a 17% CV for absolute theta power. The effect sizes in the AD/HD studies fall within or close to the normal trial-to-trial variability we found, suggesting that the observed magnitude of effects may be within or near the level expected for healthy normal variation. The same review found that studies on absolute gamma power in schizophrenia and relative gamma power in ASD demonstrated average differences of 45% and 50% between patient and control groups [30]. These effect sizes are much larger than the 17% absolute gamma variability and 12% relative gamma variability we found and are more likely to represent true pathophysiology. Similarly, Geraedts et al. found relative beta power changes of 19% (non-dementia Parkinson's vs. controls) to 52% (dementia Parkinson's vs. controls) in a systematic review of rsEEG [31]. Comparing these figures with our observed relative beta variability of 8%, disease progression may indeed be associated with increasing effect sizes, and the normal variability we quantified may aid in interpreting this progression.

Notably, despite the variability in band power metrics, rsEEG power spectra shapes were surprisingly consistent within participants. Quantifying the frequency distribution may be a promising method of analyzing repeated rsEEG recordings with lower intra-participant variability. This finding agrees with Levin et al., who used the Fitting Oscillations and One-Over-F (FOOOF) algorithm to parameterize power spectrum profiles and demonstrate their within-participants stability [32]. While the quantification of power spectral shape is still an emerging analysis method, it has the potential to help minimize the intra-participant variability in clinical EEG.

Sample entropy was also highly consistent within participants, suggesting that for each individual, there may be underlying dynamics that determine the complexity of their rsEEG patterns regardless of the external noise introduced by

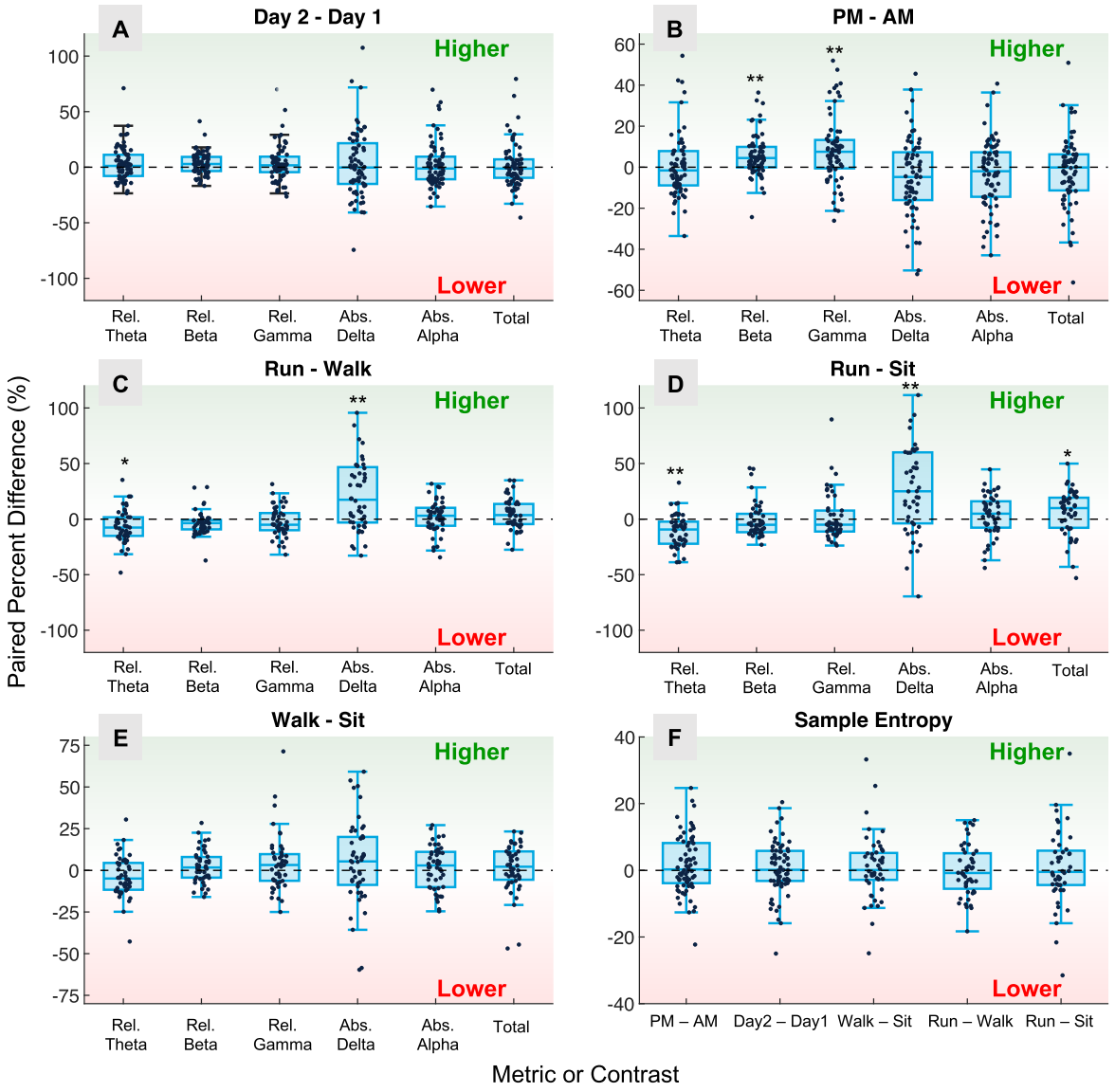

Fig. 5. Pairwise differences in rsEEG. Experiment day had no effect on rsEEG (A). Relative beta and gamma powers were significantly higher in the afternoon compared to the morning (B). Absolute delta power was significantly increased after running, compared to both walking (C) and sitting (D), whereas relative theta power was significantly decreased. There were no significant differences between walking and sitting (E) or for sample entropy (F). *: $p < 0.01$ and **: $p < 0.001$.

experimental variables. However, because all rsEEG recordings were performed in an eyes-closed state, the consistency in sample entropy may also reflect the inherent order of the dominant alpha rhythm. Further exploration of sample entropy in different experimental conditions is required before application to clinical settings.

The assumption that rsEEG is a stationary signal is crucial for measuring and interpreting significant changes in brain activity. However, the within-trial settling patterns we observed suggest that variability may also exist on smaller time scales. The lack of a significant effect of experiment day, time of day, or activity level on the presence of these settling patterns suggests that they may be an inherent feature of EEG recordings. For example, the gradual decrease in sample entropy and relative gamma power and gradual increase in relative alpha power could be the neural response to the transition into calm resting states. We determined the typical time to reach a "steady state" to be about one minute. This delay should be accounted for in future resting state studies to ensure that participants have fully reached the resting neural state before collecting data.

B. Sources of EEG Variability: Implications for Study Design

1) Experiment Day: The lack of a significant effect of experiment day on any rsEEG metrics was consistent with the existing literature on rsEEG reliability. The consistency of individual rsEEG from day to day was supported by the similarity of rsEEG power spectra across trials, regardless of experimental condition. These results demonstrate that rsEEG records the unique electrophysiological signature of each individual's cortical activity. While time of day and physical activity may cause small perturbations in this signature, the underlying mechanics are consistent.

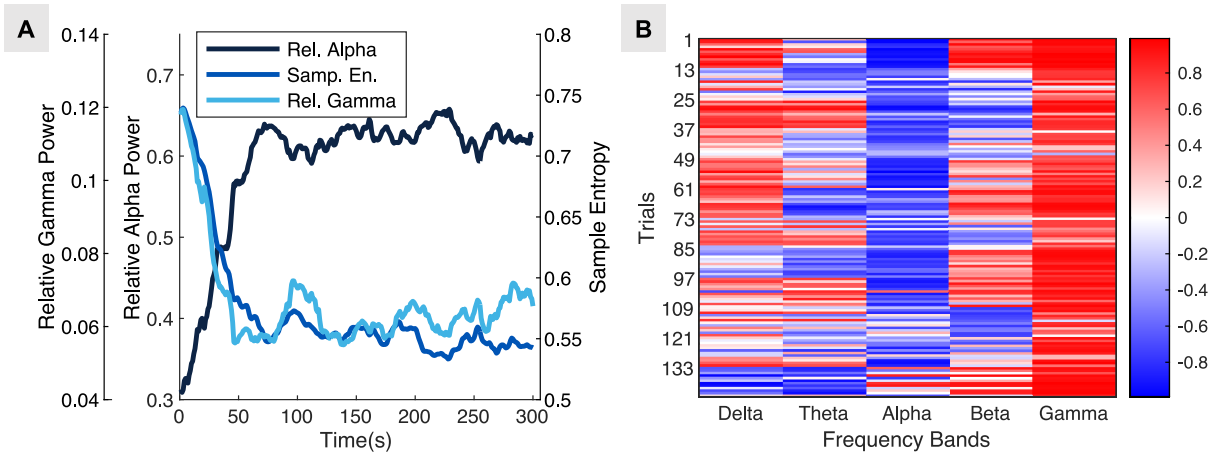

Fig. 6. Correlations between band power and entropy time series. Relative gamma power correlates positively with sample entropy and relative alpha power correlates negatively with sample entropy (representative time series, A). These correlations are consistent across almost all rsEEG trials (B). The average alpha band correlation was $\rho_{\text{avg}} = -0.59$ and the average gamma band correlation was $\rho_{\text{avg}} = 0.80$.

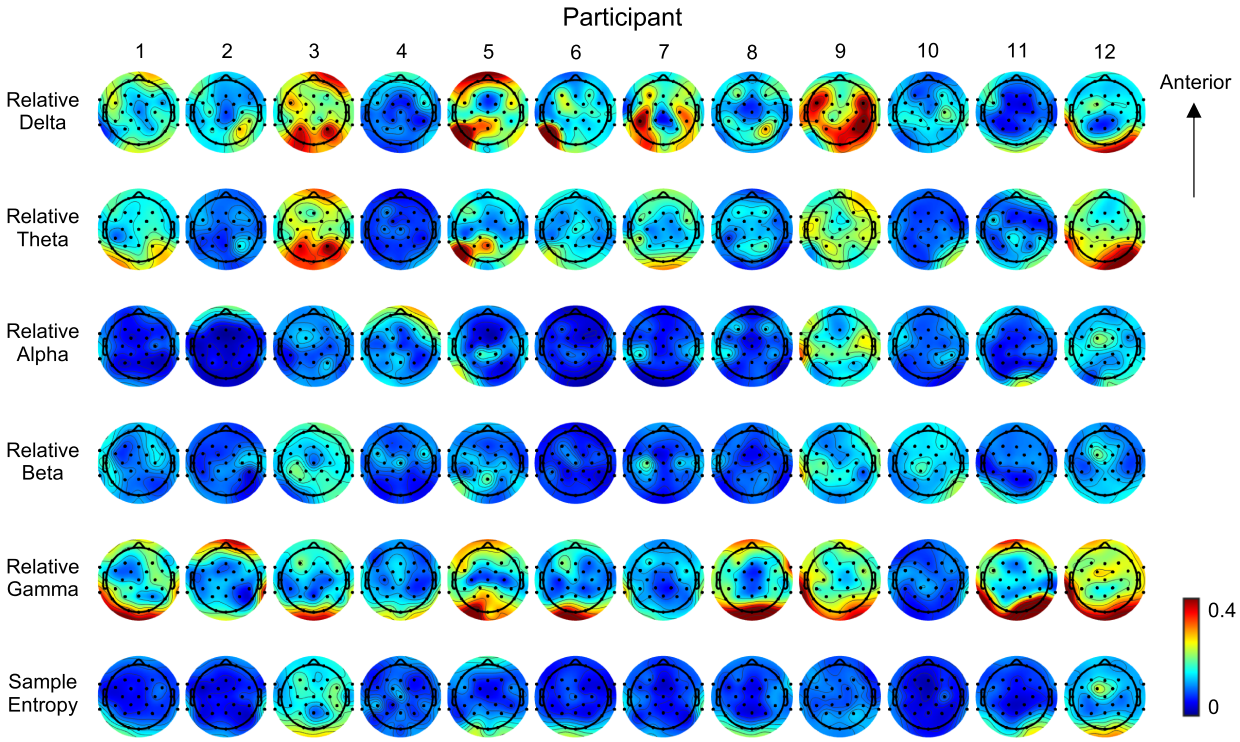

Fig. 7. Relative band power and sample entropy CV scalp maps. CV distributions depend on the participant and rsEEG metric studied.

2) Time of Day: We anticipated that time of day would introduce variability into metrics related to alertness and wakefulness. Our results showed increased beta and gamma power in the afternoon compared to the morning. These metrics are typically associated with higher-level cognitive functions such as problem-solving and memory retrieval. Participants could be more cognitively active in the afternoon, or more restless when going through the second experimental session of the day. These changes may also indicate an overall shift in individual power spectra away from lower frequencies, which is characteristic of a peak in the wakefulness circadian rhythm commonly observed in the late afternoon [33]. However, making conclusions about higher cognitive function from resting state data can be complicated by the contribution of ocular and muscle artifact to beta and gamma power [34], [35]. While muscle and ocular artifact removal was performed using ICLabel, further artifact identification and mitigation may be explored to determine potential sources of variability in the beta and gamma bands (e.g., empirical mode decomposition as described in [36]).

These time-of-day effects suggest that whenever possible, repeated rsEEG measurements should be conducted at the same time of day. Because the time-of-day effects may be mediated by wakefulness, the sleep duration and wake time before repeated rsEEG measurements should be kept consistent. Alternatively, baseline changes in rsEEG between

time points could be taken to account for potential drift. Dedicated electromyogram (EMG) electrodes placed close to EEG electrodes most susceptible to muscle artifacts (e.g., temporal locations) may also benefit the removal of this noise.

3) Activity Level: The decreased relative theta power post-running compared to the post-walking and post-sitting conditions is consistent with the existing literature on exercise and EEG. Combined with the link between decreased theta power and improved performance on cognitive tasks, the theta changes we observed may demonstrate a transient effect of exercise on cognitive alertness. However, we also observed large increases in relative and absolute delta power, which are traditionally markers of decreased cortical activation. While these changes could reflect the onset of fatigue after exercise, an alternative explanation may involve sweat artifacts. In our data, these large-amplitude, low-frequency oscillations were present for most participants for the running condition but were visually undetectable after the rsEEG data were processed using standard EEG artifact removal methods.

While running or other forms of aerobic exercise are not common in traditional clinical or research settings, understanding the rsEEG response in a stressed physical state can inform study designs for more specific applications. For example, patients with psychiatric conditions may become nervous or agitated during rsEEG collection, causing sympathetic responses such as sweating or increased heart rate [37]. RsEEG is also regarded as a potential method of assessing athlete brain condition on the sidelines during contact sports [38], [39] where the effects of physical activity are highly relevant. Baseline recordings for participants after different activity intensities should be taken, when possible, to determine individual rsEEG variability. Other physiological measurements, such as heart rate, breathing rate, and skin conductance, may be used in tandem with EEG measurements to characterize physical state.

Increasing the pre-recording activity level from sitting to walking did not cause statistically significant changes in any rsEEG metrics, suggesting that they are robust to a low level of physical activity. This result allows for a tolerance of movement in clinical settings without affecting rsEEG readings, and also has implications for the use of rsEEG in ambulatory settings. At-home rsEEG can be critical for monitoring diseases that have acute episodes, such as epilepsy or bipolar disorder [40]. The knowledge that mild, everyday activity does not interfere with rsEEG readings aids the interpretation of the data. However, because our study was carried out entirely within a designated experiment room, these results are valid only for the isolated effects of walking, without the potential accompanying changes in surroundings. Previous studies have shown significant EEG effects of walking through different settings [41]. Neuroelectric responses to movement may also be different between the healthy young adults in our study and older or diseased populations.

C. Limitations and Future Directions

Our study participation was limited to healthy young adults. However, there may be differences in rsEEG variability between population subsets based on factors such as sex and age. We did not find any sex differences in variability, but our sample size was inadequate for making any general conclusions. Improving the demographic precision of intra-participant variability measurements will increase their relevance to clinical research, where participants are often sampled from specific patient groups. While our sample size provided adequate statistical power, G*Power analysis revealed that a larger sample size could improve statistical power for rsEEG metrics with smaller effect sizes (Supplementary Tables 1-2). The study design could also be improved by expanding the range of experimental conditions to better understand potential sources of rsEEG variability. For example, activities may be performed outside controlled lab settings, where lighting and temperature can present novel stimuli for rsEEG differences. Changes in alertness and emotional state, measured using tools like the Stanford Sleepiness Scale and Self-Assessment Manikin, may elucidate the effects of continuously changing mental states on rsEEG measurements. Finally, characterization of rsEEG variability can be expanded beyond traditional quantitative EEG metrics. For example, machine learning classifiers have been applied to determine pathophysiology in rsEEG [42], so variability in classification performance across varying conditions may be clinically important.

Our examination of CV scalp maps revealed that variability may be concentrated in localized brain regions. The tendency for certain electrodes (e.g., peripheral ones) to exhibit greater variability may be related to the dominant EEG rhythms in those brain regions or their proximity to sources of noise. Our data suggest that central electrode locations may be optimal for achieving more consistent rsEEG readings. Statistical tests segregated by brain region should be incorporated into future analysis pipelines to quantify region-specific variability.

V. Conclusion

Within-participant rsEEG variability can meet or exceed literature effect sizes for neurological conditions. Routine variations in experimental conditions, such as physical activity and time of day, can contribute significantly to this variability. Future studies should be designed to keep these experimental conditions consistent or collect reference recordings to control these effects. Given the effect sizes we found, the baseline variability within participants should be assessed prior to interpreting the clinical significance of observed rsEEG changes. Our quantification of normal rsEEG variability calls for a critical examination of established research methods and provides a reference baseline for interpreting clinical results.

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