UNIVERSITY OF CALIFORNIA,
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Computational EEG markers of seizures in neonates with Hypoxic-Ischemic Encephalopathy

THESIS

submitted in partial satisfaction of the requirements
for the degree of

MASTER OF SCIENCE

in Biomedical Engineering

by

Alicia MacDonald

Thesis Committee:
Associate Professor Beth Lopour, Chair
Associate Professor Christine King
Associate Professor Jennifer Gelinas

2026

DEDICATION

To

my parents and friends

in recognition of their worth

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ACKNOWLEDGMENTS

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ABSTRACT OF THE THESIS

Computational EEG markers of seizures in neonates with Hypoxic-Ischemic Encephalopathy

by
Alicia MacDonald
Master of Science in Biomedical Engineering
University of California, Irvine, 2026
Associate Professor Beth Lopour, Chair

Hypoxic-ischemic encephalopathy (HIE) is a leading cause of death and long-term neurodevelopmental disability in neonates globally [1]. Independent of etiology, high seizure burden is a significant risk factor for mortality in neonates [2]. While prior EEG studies have shown that measures such as amplitude, power, entropy, and long-range temporal correlations can differentiate HIE severity grade [3-8], it is unknown whether these same measures can differentiate neonates who develop seizures in the first few days of life from those who do not. Understanding seizure risk in this population is critical for developing a treatment plan. Therefore, we investigated computational EEG markers of seizures in a retrospective cohort of 96 neonates with HIE who underwent therapeutic hypothermia (TH) at Children's Hospital of Orange County. Continuous scalp EEG was recorded throughout the cooling and rewarming period, and six categories of EEG metrics were computed across the 6-90 hour postnatal window: amplitude, spectral edge frequency (SEF), power spectral density (PSD), Shannon and permutation entropy, detrended fluctuation analysis (DFA) scaling exponent and intercept, and cross-correlation connectivity. Metrics were compared between neonates with (SZ+) and without (SZ-) seizures using the Wilcoxon rank-sum test, and across neonatal encephalopathy (NE) severity grades (mild, moderate, severe) using the Kruskal-Wallis test followed by pairwise comparisons, with false discovery rate (FDR) correction applied in both cases.

Broadband amplitude and PSD in the delta, theta, alpha, beta, and broadband frequency bands were lower in the SZ+ group across most of the 84-hour study window; Shannon entropy in the delta, theta and alpha bands was also lower in SZ+ neonates, but this difference emerged later. Permutation entropy in the delta band was higher in SZ+ neonates and also emerged later in the timeframe. The DFA scaling exponent in the delta, theta, and alpha bands was higher in SZ+ neonates at most time points, and cross-correlation connectivity was higher in the SZ+ group during the majority of the recording. SEF, and DFA intercept did not differentiate the groups consistently across the timeframe. Most of these same metrics, with the exception of mean connectivity, also differentiated the severe NE grade from the mild and moderate grades, with no consistent differences found between mild and moderate grades.

To our knowledge, this is the first study to show that seizure status in neonates with HIE can be quantitatively assessed from EEG activity during TH. These findings indicate that background EEG activity is altered in neonates who develop seizures, and this change can be detected early, during TH, using computational EEG metrics. This work identifies candidate biomarkers that could support earlier identification of seizure risk and inform clinical monitoring and intervention strategies for this vulnerable population.

INTRODUCTION

There are nearly 850,000 deaths annually due to asphyxia at birth (2000-03 data) [9]. Asphyxia occurs when there is a disruption in the exchange of oxygen and carbon dioxide in the respiratory system [10]. A major complication of perinatal asphyxia is hypoxic-ischemic encephalopathy (HIE), which impacts approximately 1.5 out of 1000 live births in developed countries and approximately 15 out of 1000 live births in developing countries [11]. HIE is defined as disruption to both the oxygen (hypoxic) and blood flow (ischemic) to the brain, leading to neural injury (encephalopathy).

Neonates with HIE are scored using the neonatal encephalopathy (NE) scale to classify severity and to determine treatment plans. The Iberoamerican Society of Neonatology (Siben) defines three levels of NE: mild, moderate, and severe [11]. This method incorporates information regarding the patient's level of consciousness, spontaneous activity, posture, tone, suck, Moro reflex, pupillary exam, respiratory rate, and the presence or absence of seizures. A Siben score of 4-7 corresponds to mild grade, a score of 8-11 corresponds to moderate grade, and a score of greater than 11 corresponds to severe grade [11].

Neonates diagnosed with HIE are primarily treated with 72 hours of therapeutic hypothermia (TH) and rewarming, which have been proven to reduce death and disability at 18-month follow-up [12]. Ideally, the neonates start TH within the first six hours of life (HOL) [12]. Neonates are cooled using either preferential head cooling or whole-body cooling, and temperature is monitored to maintain a target of 33.5˚C-34.5˚C [12].

HIE is the most common etiology underlying seizures in neonates (38%), followed by ischemic stroke (18%), and intracranial hemorrhage (11%) [2]. In neonates with HIE, seizures can be difficult to diagnose using clinical features alone [13-15]. Therefore, continuous

multichannel video-electroencephalogram (EEG) has become the gold standard for monitoring seizures in neonates [16]. In addition to accurate diagnosis, identifying neonates at risk of developing seizures prior to onset would enable closer monitoring and earlier intervention. However, perinatal clinical variables have not reliably predicted which neonates with HIE go on to develop seizures [17]. Given the increasing pool of evidence that high seizure burden is detrimental to a neonatal brain [18-20], a better understanding of which neonates are most at risk for seizures would be extremely valuable.

Previous literature shows that EEG power, amplitude, entropy, and long-range temporal correlations can robustly differentiate the severity grades of HIE/asphyxia (Table 1). Full information as to how the literature search was performed can be found in Appendix B.

Table 1: Summary of Previous Literature for EEG analysis in neonates with HIE or related conditions.
Abbreviations: EEG = electroencephalogram, HIE = hypoxic-ischemic encephalopathy, RDP = relative delta power, SEF = spectral edge frequency, HOL = hours of life, TH = therapeutic hypothermia, DP = delta power, TP = total power, BSITD-III = Bayley Scales of Infant and Toddler Development III, SE90-EEG = 90% spectral edge frequency, ApEn = approximate entropy, LOS = length of stay, DFA = detrended fluctuation analysis

Study reference	**Comparison groups**	**EEG metrics tested**	**Timing of EEG recording**	**EEG Recording during TH?**	**Relevant findings**
I. Korotchikova et al. Clinical Neurophysiology, vol. 174, pp. 98–103.e1, 2016 [3]	EEG/HIE grades which were assigned by visual assessment of background EEG	Amplitude, RDP, SEF, discontinuity	1 h of EEG data free of seizures and artifacts, recorded near 6 HOL	No, this cohort was monitored before therapeutic hypothermia was widely used in medical centers	RDP, amplitude, and discontinuity could differentiate EEG/HIE grades (89% accuracy)
S. Kota et al. Pediatric Neurology, vol. 122, pp. 7–14, 2021 [4]	HIE severity groups (normal, mild, moderate, severe) classified by modified Sarnat score	DP, 0.5–4 Hz; TP, 0.5–20 Hz	First 3 hours of EEG recording in the first day of life, seizure-free and free of large artifacts (started within 6 HOL)	EEG segment used for analysis was recorded prior to the initiation of cooling	DP and TP significantly higher in mild vs. moderate group ($p<0.05$); both decreased with worsening severity
M. Fiammante et al. Computer Methods and Programs in Biomedicine, vol. 274, art. 109166, 2026 [5]	EEG/HIE grades classified by visual assessment of background EEG	Delta power (0.5-4 Hz)	Two datasets. 1. Within 6 h of birth 2. Up to 100 h after birth	EEGs were recorded before or during TH	Could differentiate between mild and Moderate/severe with 98% accuracy
N. Alotaibi et al. Frontiers in Human Neuroscience, vol. 15, art. 795006, 2022 [6]	2-year cognitive composite score (BSITD-III)	Sample entropy, permutation entropy, spectral entropy	Within the first week of life	This is not clearly specified in the paper	Delta band entropy (permutation and sample) performed the best at predicting the 2-year cognitive score
J. Burnsed et al. Journal of Clinical Neurophysiology, vol. 28, pp. 10–14, 2011 [7]	Length of stay for neonates with HIE	Approximate entropy, spectral edge frequency (90%)	Recording during re-warming after TH (day 3-4 of life)	Recording during re-warming after TH	Both SE90-EEG and ApEn had statistically significant associations with LOS

V. Matic et al. Frontiers in Human Neuroscience, vol. 9, art. 189, 2015 [8]	EEG background severity classified by visual assessment	Long-range temporal correlations, measured by DFA scaling exponent	Started within 2-48 hours HOL; median total duration was 31 hours; four 2-hour epochs analyzed per neonate	No therapeutic hypothermia mentioned	Conventional DFA did not have the desired prediction power, so the authors explored multi-factorial DFA

Considering the success of prior studies and the strong relationship between EEG features and HIE severity, we hypothesized that EEG features would also be correlated to the presence of seizures and could be used to predict the development of epilepsy. Therefore, our objective was to identify EEG-based biomarkers that could differentiate neonates who developed seizures in the first few days of life (SZ+) from those who did not (SZ-), with the goal of enabling earlier intervention or alternative treatment strategies to prevent epileptogenesis.

We retrospectively studied neonates with HIE at Children's Hospital of Orange County (CHOC) that underwent TH. We collected EEG recordings starting in the early hours of the active cooling and spanning the duration of TH and into rewarming. We then computed EEG metrics over the full duration of the recording to test for differences between the SZ+ and SZ- groups and quantify the evolution of the metrics over time.

CHAPTER 1: METHODS

1.1 Inclusion and exclusion criteria

Neonates diagnosed with HIE at CHOC from January 1, 2013, to December 31, 2025, who were treated with TH and had concurrent EEG recordings were retrospectively identified for inclusion in this study. The SZ+ patients were defined as those diagnosed with electrographic and/or electroclinical seizure(s) during the first few days of life while the neonate is under clinical observation/EEG recording, while the SZ- patients were those having no seizures or being monitored due to clinical concern only. Clinical concern means that the staff noted some body movement that was concerning, but no seizure activity was confirmed electrographically. The seizure designation was assigned by a board-certified pediatric epileptologist.

1.2 Ethical considerations

Approval of this retrospective study was obtained from the CHOC institutional review board (IRB # 200564).

1.3 Clinical treatment protocol

At CHOC, TH is the standard of care for neonates with HIE. During the cooling and rewarming period, the neonates are monitored with scalp EEG to monitor for potential seizures or other brain health indicators. The neonates underwent total body cooling with a target temperature of 33.5˚C, measured via rectal probe. The neonates with HIE were given an NE grade (following the Siben method [11]) by a board-certified neonatologist or a neonatology fellow physician.

1.4 EEG pre-processing

The EEG electrodes were placed at FP1, FP2, T3, T4, C3, C4, O1, O2, and Cz and positioned using the 10-20 International Standard for electrode placement system, modified for

neonates. In addition, data from mastoid electrodes were collected (A1/A2 or M1/M2) and used for linked ears re-referencing. The sampling frequency was either 2000 Hz or 200 Hz. In the cases of 2000 Hz sampling frequency, the data were filtered to 0-100 Hz using a finite impulse response filter and then resampled to 200 Hz sampling frequency. The potential signal distortion and attenuation associated with downsampling (resampling) at higher frequencies did not affect our findings, as the highest frequency considered in this study was 55 Hz. For one patient, the O1 and O2 channels were mislabeled as POL01 and POL02, so these were renamed prior to the analysis. All pre-processing and analysis steps were completed using MATLAB R2025b (MathWorks, Natick, MA).

Following these pre-processing steps, the EEG data were re-referenced prior to calculating the computational metrics. Re-referencing strategies varied based on the metric and will be specified in the sections below.

1.5 Automated artifact detection

After pre-processing, 10-minute segments of EEG were passed through an automated artifact detection function to identify clean epochs for analysis. The automated artifact detector was based on methods first described in [21, 22] and is implemented as described in our prior work [23, 24]. First, it filtered the data from ~1.6 Hz to 40 Hz using a Butterworth filter design. Then, the mean and standard deviation of each channel were computed. Each channel's mean was subtracted such that each resulting channel had zero mean. Segments of the EEG data exceeding 7.5 standard deviations and greater than 200 microvolts were flagged as artifacts. A buffer zone of 0.9 seconds was added before and after each artifactual segment to ensure the entire artifact was removed.

In addition, impedance checks were detected. If a channel's voltage had no difference from one sample to the next (when rounded to four decimal places), and this was true for at least six channels at that time point, it was assumed that this was an impedance check and was also flagged as artifactual.

1.6 Calculation of EEG metrics

The EEG metrics used in this thesis were amplitude, power, spectral edge frequency (SEF), entropy, detrended fluctuation analysis (DFA), and cross correlation connectivity. We chose these because they have shown value for the analysis of seizures and epilepsy in prior studies [3-8, 23, 25]. For all the metrics, all EEG channels were analyzed. For the metrics that used a linked-ears re-referencing montage, the A1/A2 or M1/M2 channels were also used. After the computation of each metric, the median was taken across channels and epochs within a two-hour time bin for statistical analysis and visualization. The median was used instead of the mean because the data were non-normal and skewed, and the median is less sensitive to outliers. The data included both ictal and interictal periods, and for this analysis were considered holistically rather than differentiated into sleep/wake segments.

1.6.1 Amplitude

Given the prior success of using amplitude to differentiate between HIE/EEG grades [3], we chose to include it in our study. EEG signals were re-referenced using the linked ears method, and amplitude was then computed on clean 1-second epochs of broadband (1-55 Hz) data as the difference between the maximum and minimum values within each epoch (range).

1.6.2 Spectral edge frequency

SEF describes the spread of power across frequencies in a dataset, defined as the frequency below which 95% of the power resides [26], calculated over the 0-55 Hz range.

Although a prior study found that SEF did not differentiate between EEG/HIE grades [3], it remains a foundational descriptive measure of EEG and was included here. SEF was computed on 5-second epochs following linked-ears re-referencing.

1.6.3 Power spectral density

Following the successful differentiation of EEG/HIE grades or HIE severities in several prior works [3-5], power spectral density (PSD) was included here and studied for each standard frequency band. The EEG PSD was computed after linked ears re-referencing. PSD was computed for 5-second epochs in the delta (1-4 Hz), theta (4-8 Hz), alpha (8-13 Hz), beta (13-30 Hz) and broadband (1-55 Hz) ranges. The EEG epochs were windowed with a Hanning function and transformed to the frequency domain via FFT. The resulting one-sided power spectrum was converted to PSD by normalizing by the sampling rate and the window's power correction factor ($\sum w^2$, where w is the Hanning window), which corrects for the attenuation introduced by the windowing. PSD within each frequency band was determined by numerically integrating the PSD over that range (trapezoidal rule) and expressed in decibels.

1.6.4 Entropy

Entropy is related to the predictability of time-series data. Prior studies found success in predicting length of stay [7] and 2-year cognitive scores [6] for neonates with HIE when using different entropy measures (sample, permutation, spectral, approximate). Therefore, we chose two commonly used entropy measures (Shannon and permutation) to apply to this dataset [23]. Entropy was computed after linked ears re-referencing and was computed for the delta (1-4 Hz), theta (4-8 Hz), alpha (8-13 Hz), beta (13-30 Hz) frequency ranges in 30-second epochs.

Shannon entropy is derived from information theory and is only dependent on the probability distribution of the signal's amplitude values; it is therefore independent of the

temporal structure of the EEG. The Shannon entropy calculation is as follows (per channel), after discretizing the EEG data into n bins [27]:

$$H(X) = -\sum_{i=1}^{n} p(x_i) log_2 p(x_i) \quad (1)$$

where $p(x_i)$ is the probability of the data *x* lying in the i^{th} bin [28]. The number of bins was determined using the Freedman-Diaconis rule [29], resulting in bin sizes of 81, 86, 83, and 94 for the delta, theta, alpha and beta bands, respectively.

Permutation entropy is a measure of the EEG signal's complexity that takes the temporal order of the data into account using symbolic dynamics [30]. The permutation entropy calculation is as follows (per channel):

$$H(n) = -\sum_{j=1}^{n!} {p'}_j log_2 \left({p'}_j\right). \quad (2)$$

Here n is the embedding dimension, representing the number of sequential EEG data points in a pattern, and ${p'}_j$ is a representation of the relative frequencies of all possible patterns of that length. It was computed with two different sets of parameters. First, the embedding dimension *n=4* and delay *τ=1* were used following a previous study in infants with epileptic encephalopathy [23]. Secondly, we used the embedding dimension *n=3* and delay *τ=1* following a paper that analyzed neonatal EEG using permutation entropy [6].

1.6.5 Long-range temporal correlations

The strength of long-range temporal correlations in the EEG was calculated using a statistical estimation algorithm called detrended fluctuation analysis (DFA) [31, 32]. There is evidence that the temporal modulation of EEG amplitude occurs over periods lasting tens of seconds which could reflect the brain's ability to control its neuronal synchrony [33]. Previously, an extension of conventional DFA (multi-factorial DFA) was used to differentiate between EEG background severity of asphyxiated neonates [8]. Given that success, we tested whether DFA

could also differentiate between SZ+ and SZ- neonates with HIE. In this study, DFA was computed after bipolar re-referencing following methodology from previous studies analyzing neonatal EEG with DFA [8, 34]. It was computed for the delta (1-4 Hz), theta (4-8 Hz), alpha (8-13 Hz), and beta (13-30 Hz) frequency bands. Here, DFA was implemented following the code and methodology in Hardstone et al. [32], and we computed both the scaling exponent and the intercept. The intercept was calculated by extrapolation of the linear fit to determine the fluctuation value when the window size was one second (a value of zero on the logarithmic axis) [35]. The window size of one second was used for the intercept to ensure the intercept values were independent of the sampling frequency. The minimum and maximum window sizes were 10 and 60 seconds, respectively, the segment length was 15 minutes of continuous clean EEG [8], and the overlap of EEG windows was 50% [36].

1.6.6 Cross correlation connectivity

Cross correlation is an amplitude-based measure that can be used to evaluate the functional connectivity of an EEG network. For this analysis, we followed a method developed by Kramer et al. [37] and Chu et al. [38]. For a pair of electrodes ($x_i[t]$ and $x_j[t]$) with signal length *n,* the cross correlation at lag τ is defined as:

$$C_{ij}[\tau] = \frac{1}{\hat{\sigma}_i \hat{\sigma}_j n} \sum_{t=1}^{n-\tau} (x_i[t] - \bar{x_i})(x_j[t+\tau] - \bar{x_j}) \quad (3)$$

where $\bar{x_i}$ and $\bar{x_j}$ are the averages and $\hat{\sigma_i}$ and $\hat{\sigma_j}$ are the standard deviations of $x_i[t]$ and $x_j[t]$, respectively [37]. We chose cross correlation connectivity for our analysis because it has been applied to infant EEG and shown promise as a biomarker of epilepsy [23, 25]. Before computing cross-correlation connectivity, the data were re-referenced to the common average and broadband filtered (1-55 Hz) [24]. Within each 1-second epoch of EEG, the connectivity was determined to be significant if the maximum absolute value for the cross-correlation for that

channel pair met two criteria: (1) it occurred at a non-zero time lag, and (2) it was larger than a significance threshold obtained from a permutation resampling method. Then, the connectivity strength for each pair of channels was computed as the percentage of epochs in which the connection was significant. The global connectivity strength for each patient was quantified in two ways: (1) the number of connections with connectivity strength larger than 0.1 [25], and (2) the mean connectivity strength of the top 10% largest connectivity strength values.

1.7 Statistical testing

First, the normality of the data was assessed, and it was found that the data did not follow a Gaussian distribution and therefore non-parametric tests needed to be used. For the tests between the SZ+ and SZ- groups, we used a two-sided Wilcoxon rank-sum test followed by FDR correction using the Benjamini & Yekutieli (2001) method [39]. For the tests between the three NE grades (mild vs. moderate vs. severe), the statistical significance test was a Kruskal-Wallis test. Pairwise comparisons were then performed using Dunn-Sidak correction to determine which pairs of groups differed significantly, followed by FDR correction using the Benjamini & Yekutieli (2001) method [39].

1.8 Time window for analysis

We analyzed the EEG data starting at six hours from time of birth (we defined time of birth as time zero) and ending at 90 hours. We chose this timeframe because data from ~50% of the total patient population were available at its beginning and end.

CHAPTER 2: RESULTS

2.1 Subject demographics

There were 102 patients who met the inclusion criteria; however, six of the patients did not have complete timeline information (e.g., time of EEG start, time of cooling start, etc.) and were therefore excluded from this analysis, resulting in 96 patients. In addition, for the DFA analysis, clean 15-minute segments of EEG were required; therefore, two more patients were excluded from the analysis because they did not have any clean 15-minute segments available (n=94 for DFA). Demographic and EEG recording information is shown in Table 2 below.

Table 2: Summary of demographic and recording information for patient population.
Abbreviations: NE = neonatal encephalopathy, IQR = interquartile range, EEG = electroencephalogram.

Demographic information	**N (%)**
Seizures (SZ+ group, electrographic and/or electroclinical seizures)	35 (36.5%)
No seizures (SZ- group)	61 (63.5%)
Sex	
Female	40 (41.7%)
Male	56 (58.3%)
Race	
Black/African-American	2 (2.1%)
East Asian	4 (4.2%)
Multiple/Other/Unknown	44 (45.8%)
South Asian	1 (1%)
White/Caucasian	45 (46.9%)
Ethnicity	
Hispanic	56 (58.3%)

Non-Hispanic	35 (36.5%)
Unknown	5 (5.2%)
NE grade	
Mild	17 (17.7%); 14 SZ- and 3 SZ+
Moderate	55 (57.3%); 42 SZ- and 13 SZ+
Severe	23 (24.0%); 4 SZ- and 19 SZ+
Unknown (excluded from NE comparison)	1 (1.0%); 1 SZ-
Recording Characteristics	**Value**
EEG recording duration, median (IQR)	82 (77-92) hrs
Time from birth to EEG start, median (IQR)	7.9 (6.3-9.1) hrs
Patients contributing data to first time bin (6-8 hrs)	51
Patients contributing data to last time bin (88-90 hrs)	48

2.2 Multiple computational EEG metrics exhibit significant differences between SZ- and SZ+ groups during TH

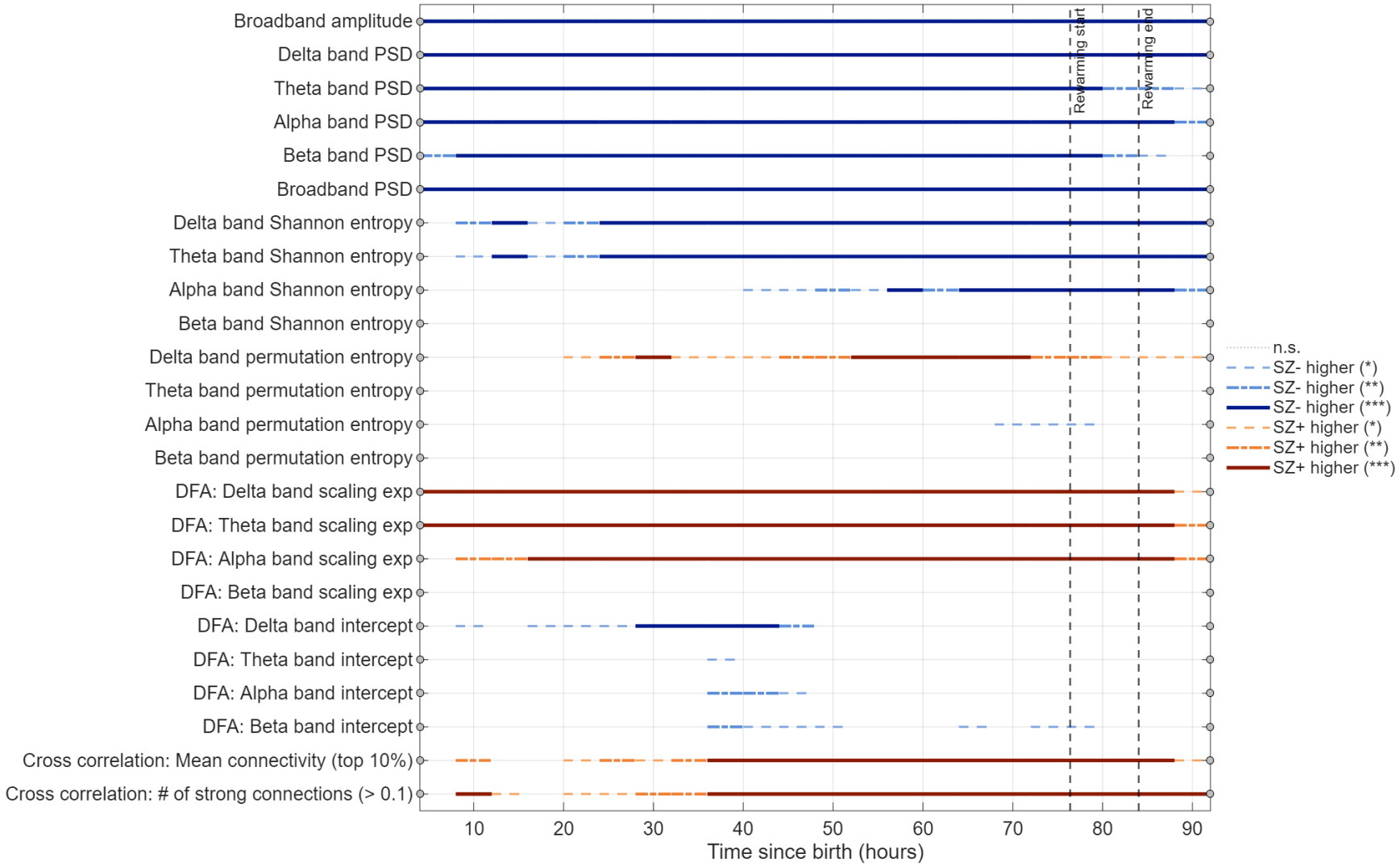

Figure 1: Summary of significant differences between the SZ+ and SZ- groups over 6-90 hrs postnatal.

Within each two-hour time bin, the median of each metric across all channels (Fp1, Fp2, T3, T4, C3, C4, O1, O2 and Cz) and epochs was calculated. These two-hour epochs were then pooled into four-hour time bins for visualization. The statistical significance test was the two-sided Wilcoxon rank sum test, followed by FDR correction using the Benjamini & Yekutieli (2001) method [39]. Line color indicates which group had higher values; within each color, the shade and line style together indicate significance strength. No significant difference between groups is represented by no line; the lightest color with the dashed line represents a significance level of $p < 0.05$ (*); the middle color shade with the dot-dash line represents a significance level of $p < 0.01$ (**); the darkest color shade with the solid line represents a significance level of $p < 0.001$ (***). The blue lines represent results in which the SZ- group had higher values, while the orange lines represent results in which the SZ+ group had higher values. The vertical lines labeled "Rewarming start" and "Rewarming end" indicate where the majority of patients transition from the cooling phase to the rewarming phase, and then from the rewarming phase to the post-rewarming phase (normothermia). Abbreviations: SZ+ = neonates exhibiting electrographic or electroclinical seizures, SZ- = neonates not exhibiting seizures, PSD = power spectral density, DFA = detrended fluctuation analysis, n.s. = no significance, FDR = false discovery rate.

Broadband amplitude and PSD in the delta, theta, alpha, beta and broadband ranges differed significantly between SZ- and SZ+ groups over the majority of the timeframe, with the SZ- group showing higher values (Figure 1). For PSD in the theta and alpha bands significance declined during the rewarming and post-rewarming phases and disappeared entirely for PSD in the beta band by the end of the recording period. Delta, theta, and alpha band DFA scaling exponent also differed significantly across the timeframe, with higher values in the SZ+ group (Figure 1). Significance decreased during rewarming but did not disappear. Delta band DFA intercept differed significantly in the middle of the timeframe, with higher values in the SZ- group. Delta, theta and alpha band Shannon entropy differed significantly in the mid-to-later part of the timeframe, with higher values in the SZ- group (Figure 1). Delta band permutation entropy differed significantly in the mid-to-later part of the timeframe, with higher values for the SZ+ group (Figure 1). Cross correlation connectivity, both the mean connectivity and number of strong connections, also differed significantly across the majority of the timeframe, with higher values in the SZ+ group (Figure 1, Figure 5, Figure 22). The other computational metrics (SEF, Shannon entropy in the beta band, permutation entropy in the theta, alpha and beta bands, DFA scaling exponent in the beta band, DFA intercept in the theta, alpha and beta bands) showed no significant differences between the two groups or only short, sporadic periods of significance.

For some metrics, the SZ+ and SZ- groups had different trends over time. The SZ- group appeared to have less variation than the SZ+ group in the PSD (Figure 2A-D), Shannon entropy (Figure 3A-D), and DFA scaling exponent metrics (Figure 4A-D). The median value for the delta (Figure 2A), theta (Figure 2B) and broadband (Figure 15) PSD also appeared to increase over the 6-90 hr timeframe for the SZ+ group. The median and range of SEF values appeared to decrease over the timeframe for the SZ+ group (Figure 6).

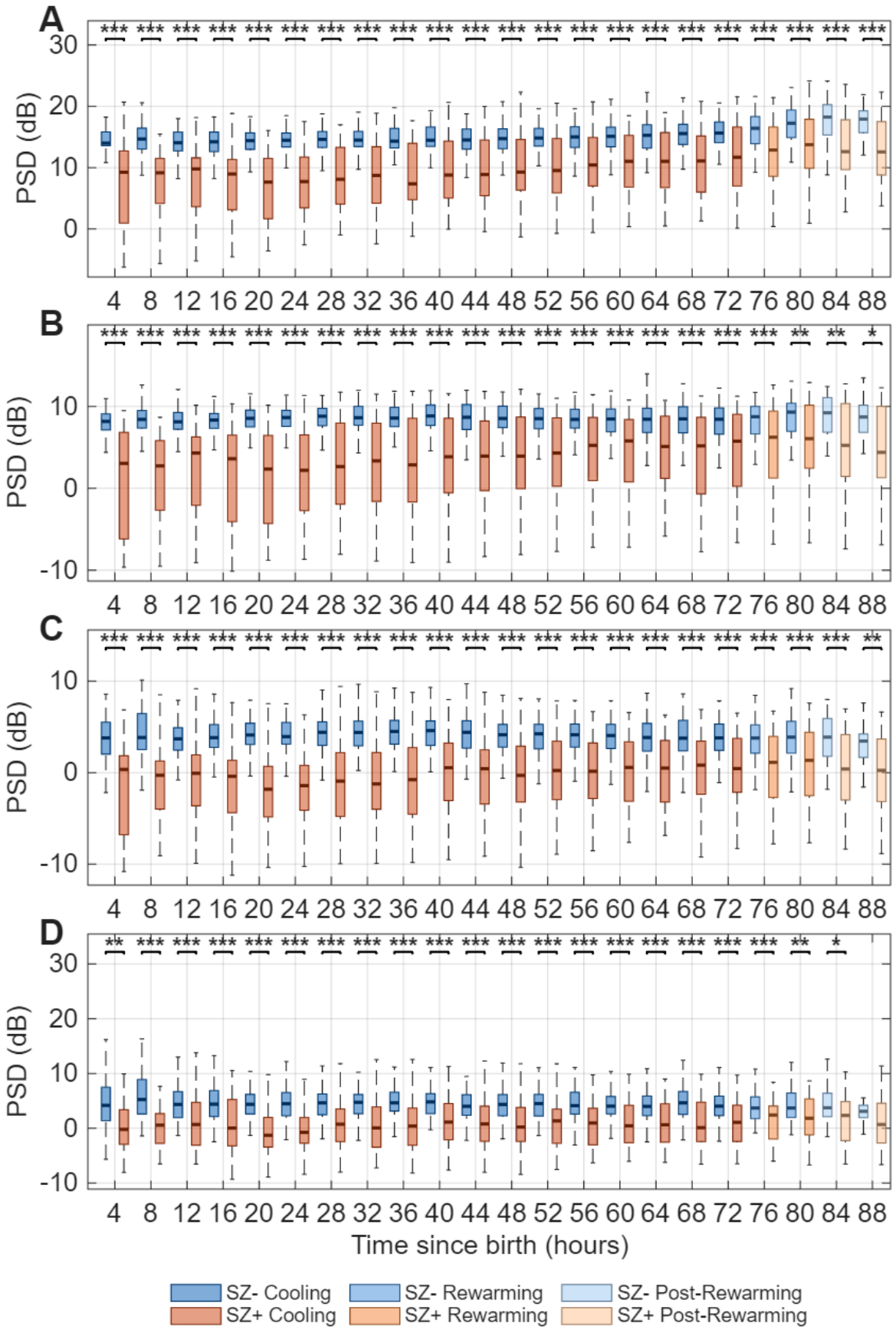
A
B
C
D
PSD (dB)
Time since birth (hours)
SZ- Cooling
SZ+ Cooling
SZ- Rewarming
SZ+ Rewarming
SZ- Post-Rewarming
SZ+ Post-Rewarming

Figure 2: Frequency band specific PSD results for SZ+ and SZ- groups over 6-90 hrs postnatal.
A, B, C, and D show the results for PSD in the delta, theta, alpha and beta frequency bands, respectively. Within each two-hour time bin, the median of each metric across all channels (Fp1, Fp2, T3, T4, C3, C4, O1, O2 and Cz) and epochs was calculated. These two-hour epochs were then pooled into four-hour time bins for visualization. The middle line in the boxes represents the median value, while the upper and lower limits of the boxes represent the IQR. The statistical significance test was the two-sided Wilcoxon rank sum test followed by FDR correction using the Benjamini & Yekutieli (2001) method [39]. The brackets and stars at the top of the plots represent a statistically significant result for that 2-hour time bin. One star (*) represents $p < 0.05$, two stars (**) represent $p < 0.01$ and three stars (***) represent $p < 0.001$. The color of the box plots changes to lighter shades when a majority of the epochs move from the cooling phase to the rewarming phase and then from the rewarming phase to the post-rewarming phase (normothermia). Abbreviations: SZ+ = neonates exhibiting electrographic or electroclinical seizures, SZ- = neonates not exhibiting seizures, IQR = interquartile range, FDR = false discovery rate, dB = decibels.

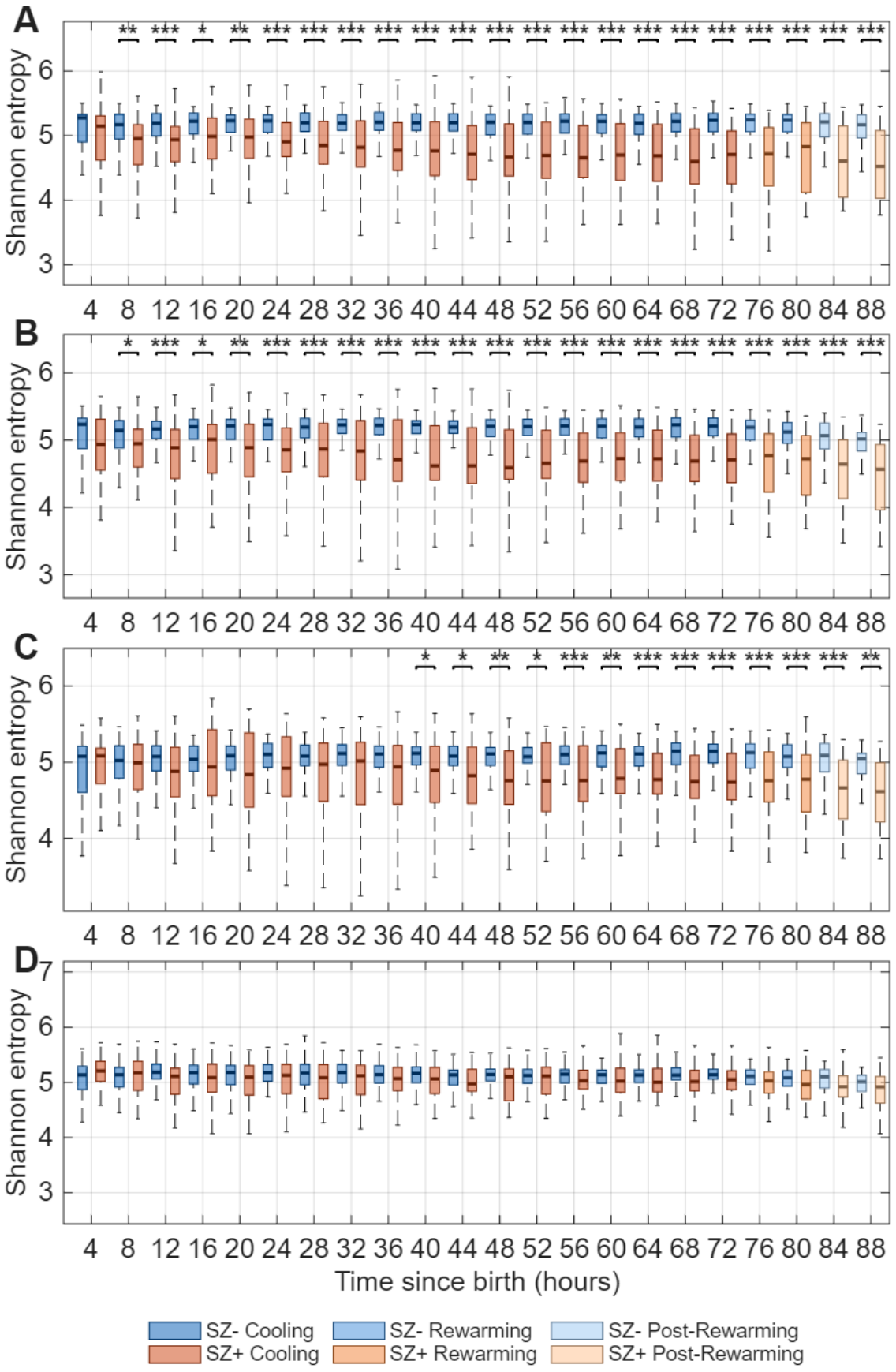
A
B
C
D
Shannon entropy
Time since birth (hours)
SZ- Cooling
SZ+ Cooling
SZ- Rewarming
SZ+ Rewarming
SZ- Post-Rewarming
SZ+ Post-Rewarming

Figure 3: Frequency band specific Shannon entropy results for SZ+ and SZ- groups over 6-90 hrs postnatal.

A, B, C, and D show the results for Shannon entropy in the delta, theta, alpha and beta frequency bands, respectively. Within each two-hour time bin, the median of each metric across all channels (Fp1, Fp2, T3, T4, C3, C4, O1, O2 and Cz) and epochs was calculated. These two-hour epochs were then pooled into four-hour time bins for visualization. The middle line in the boxes represents the median value, while the upper and lower limits of the boxes represent the IQR. The statistical significance test was the two-sided Wilcoxon rank sum test followed by FDR correction using the Benjamini & Yekutieli (2001) method [39]. The brackets and stars at the top of the plots represent a statistically significant result for that 2-hour time bin. One star (*) represents $p < 0.05$, two stars (**) represent $p < 0.01$ and three stars (***) represent $p < 0.001$. The color of the box plots changes to lighter shades when a majority of the epochs move from the cooling phase to the rewarming phase and then from the rewarming phase to the post-rewarming phase (normothermia). Abbreviations: SZ+ = neonates exhibiting electrographic or electroclinical seizures, SZ- = neonates not exhibiting seizures, IQR = interquartile range, FDR = false discovery rate.

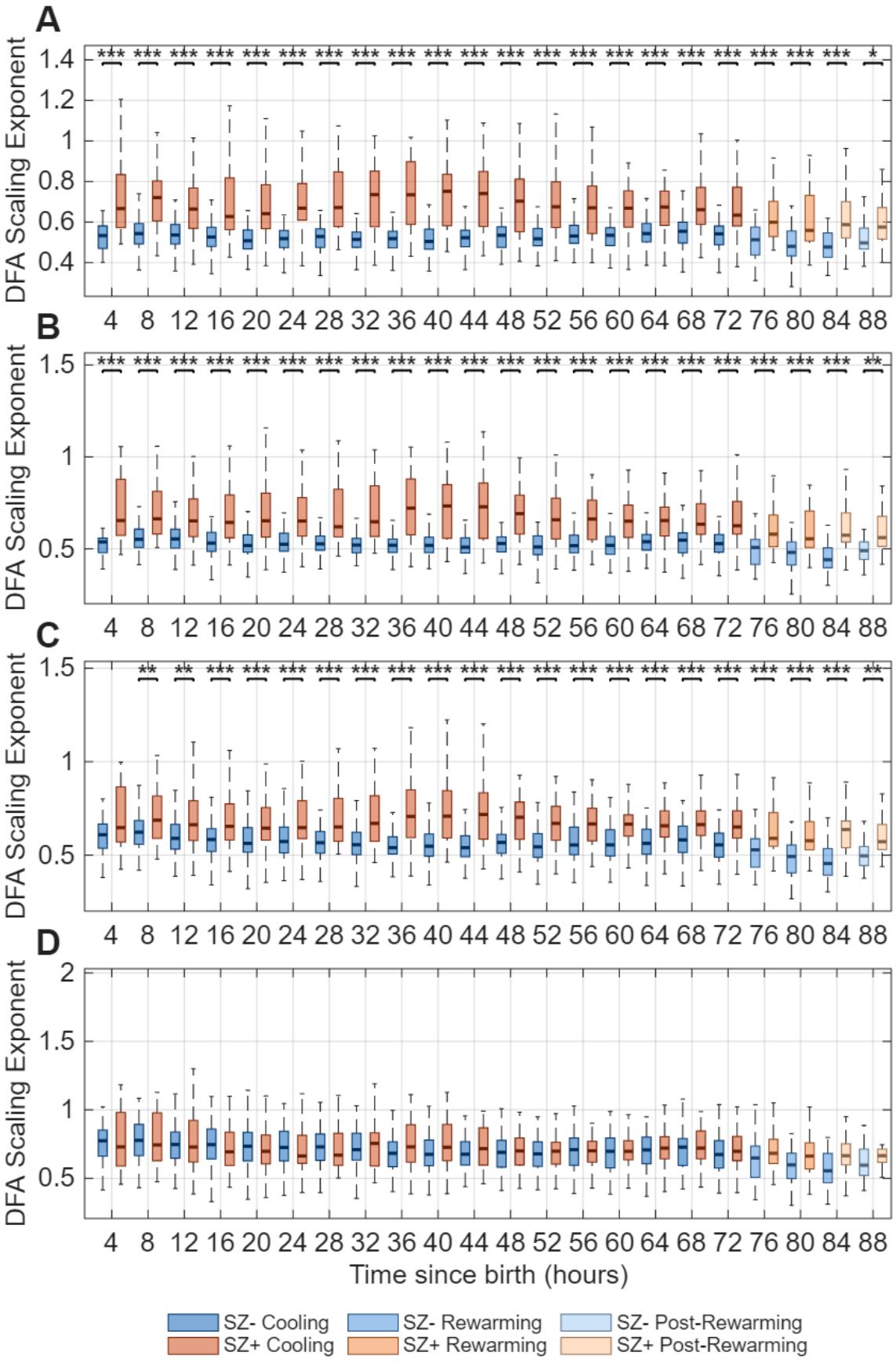
A
B
C
D
DFA Scaling Exponent
Time since birth (hours)
SZ- Cooling
SZ+ Cooling
SZ- Rewarming
SZ+ Rewarming
SZ- Post-Rewarming
SZ+ Post-Rewarming

Figure 4: Frequency band specific DFA scaling exponent results for SZ+ and SZ- groups over 6-90 hrs postnatal.

A, B, C, and D show the results for the DFA scaling exponent in the delta, theta, alpha and beta frequency bands, respectively. Within each two-hour time bin, the median of each metric across all channels (Fp1, Fp2, T3, T4, C3, C4, O1, O2 and Cz) and epochs was calculated. These two-hour epochs were then pooled into four-hour time bins for visualization. The middle line in the boxes represents the median value, while the upper and lower limits of the boxes represent the IQR. The statistical significance test was the two-sided Wilcoxon rank sum test followed by FDR correction using the Benjamini & Yekutieli (2001) method [39]. The brackets and stars at the top of the plots represent a statistically significant result for that 2-hour time bin. One star (*) represents $p < 0.05$, two stars (**) represent $p < 0.01$ and three stars (***) represent $p < 0.001$. The color of the box plots changes to lighter shades when a majority of the epochs move from the cooling phase to the rewarming phase and then from the rewarming phase to the post-rewarming phase (normothermia). Abbreviations: SZ+ = neonates exhibiting electrographic or electroclinical seizures, SZ- = neonates not exhibiting seizures, IQR = interquartile range, DFA = detrended fluctuation analysis, FDR = false discovery rate.

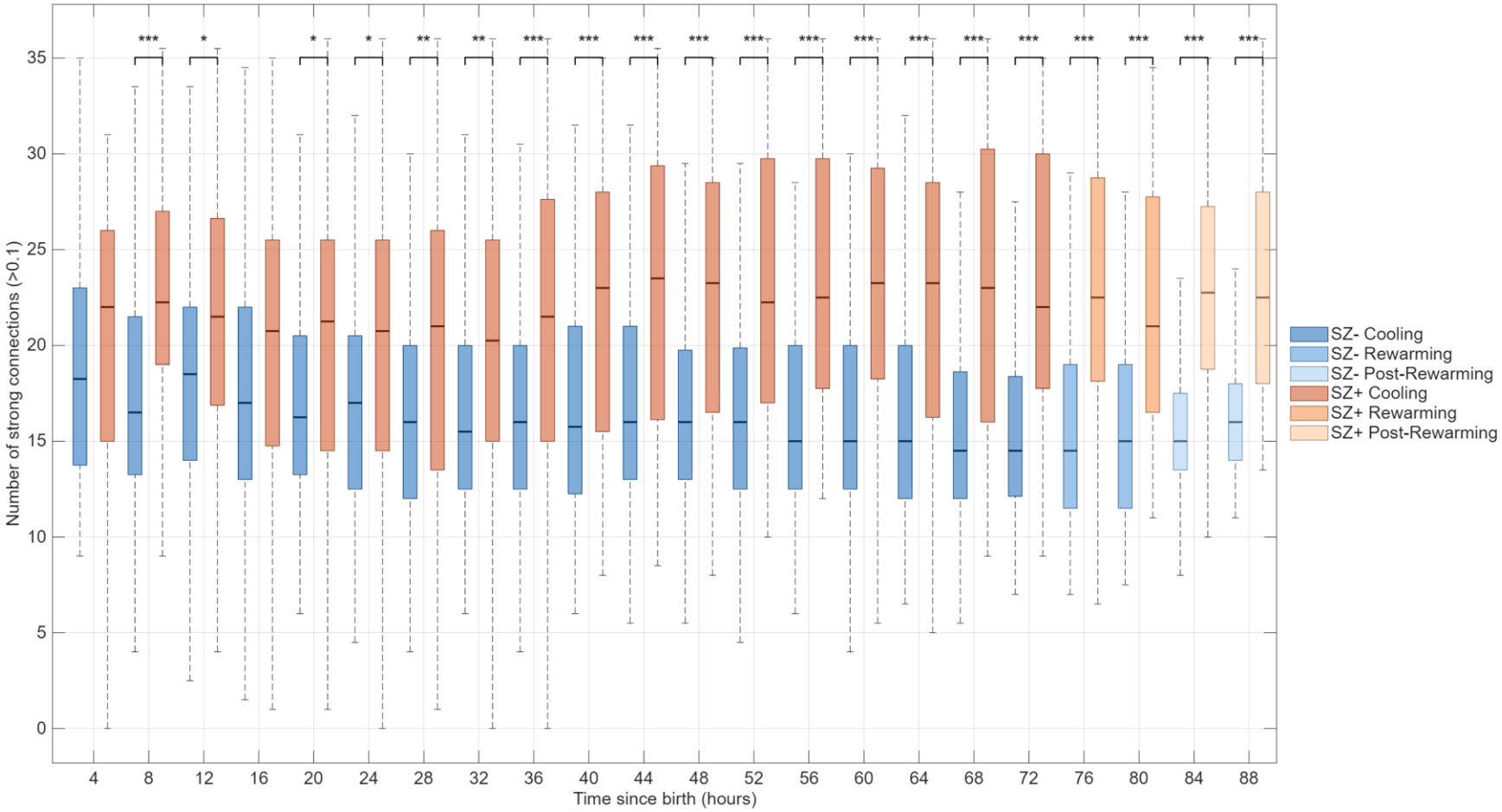

Figure 5: Number of strong cross correlation connections for SZ+ and SZ- groups over 6-90 hrs postnatal.
Within each two-hour time bin, the median of each metric across all channels (Fp1, Fp2, T3, T4, C3, C4, O1, O2 and Cz) and epochs was calculated. These two-hour epochs were then pooled into four-hour time bins for visualization. The middle line in the boxes represents the median value, while the upper and lower limits of the boxes represent the IQR. The brackets and stars at the top of the plots represent a statistically significant result for that 2-hour time bin. One star (*) represents $p < 0.05$, two stars (**) represent $p < 0.01$ and three stars (***) represent $p < 0.001$. The statistical significance test was the two-sided Wilcoxon rank sum test followed by FDR correction using the Benjamini & Yekutieli (2001) method [39]. The color of the box plots changes to lighter shades when a majority of the epochs move from the cooling phase to the

rewarming phase and then from the rewarming phase to the post-rewarming phase (normothermia). Abbreviations: SZ+ = neonates exhibiting electrographic or electroclinical seizures, SZ- = neonates not exhibiting seizures, IQR = interquartile range, FDR = false discovery rate.

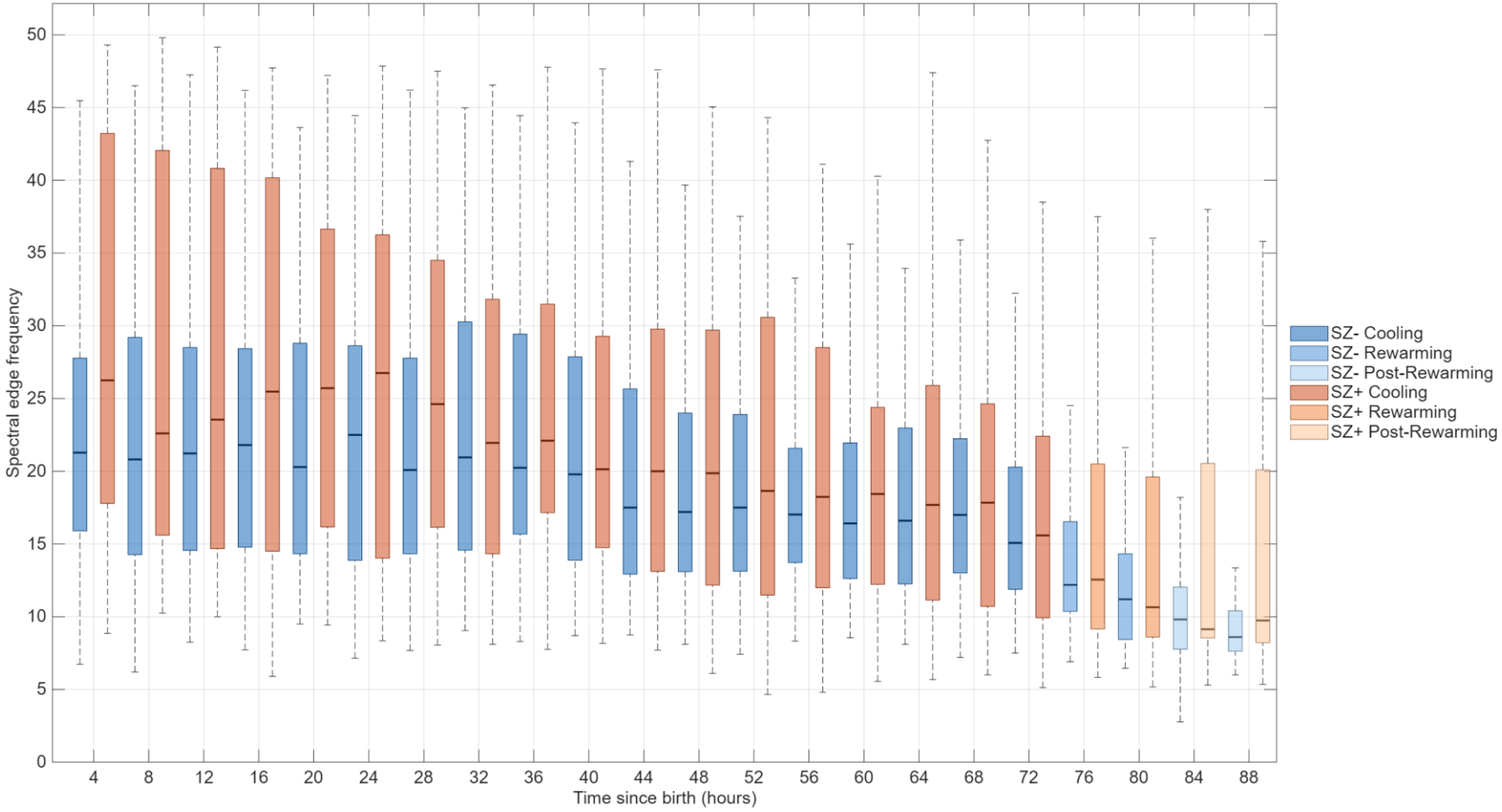

Figure 6: Spectral edge frequency results for SZ+ and SZ- groups over 6-90 hrs postnatal.
Within each two-hour time bin, the median of each metric across all channels (Fp1, Fp2, T3, T4, C3, C4, O1, O2 and Cz) and epochs was calculated. These two-hour epochs were then pooled into four-hour time bins for visualization. The middle line in the boxes represents the median value, while the upper and lower limits of the boxes represent the IQR. No statistically significant results were detected between the SZ+ and SZ-

groups. The statistical significance test was the two-sided Wilcoxon rank sum test followed by FDR correction using the Benjamini & Yekutieli (2001) method [39]. The color of the box plots changes to lighter shades when a majority of the epochs move from the cooling phase to the rewarming phase and then from the rewarming phase to the post-rewarming phase (normothermia). Abbreviations: SZ+ = neonates exhibiting electrographic or electroclinical seizures, SZ- = neonates not exhibiting seizures, IQR = interquartile range, FDR = false discovery rate.

2.3 A similar set of computational EEG metrics exhibit significant differences between NE grades

When comparing the metrics across the NE grades (mild, moderate, and severe), only the mild vs. severe and moderate vs. severe differences were consistently significant, while the mild vs. moderate comparison was not significant for most metrics except for a few metrics where there was a short timeframe of significance. Figure 7 summarizes the metrics and their significant differences across 6-90 hrs postnatal.

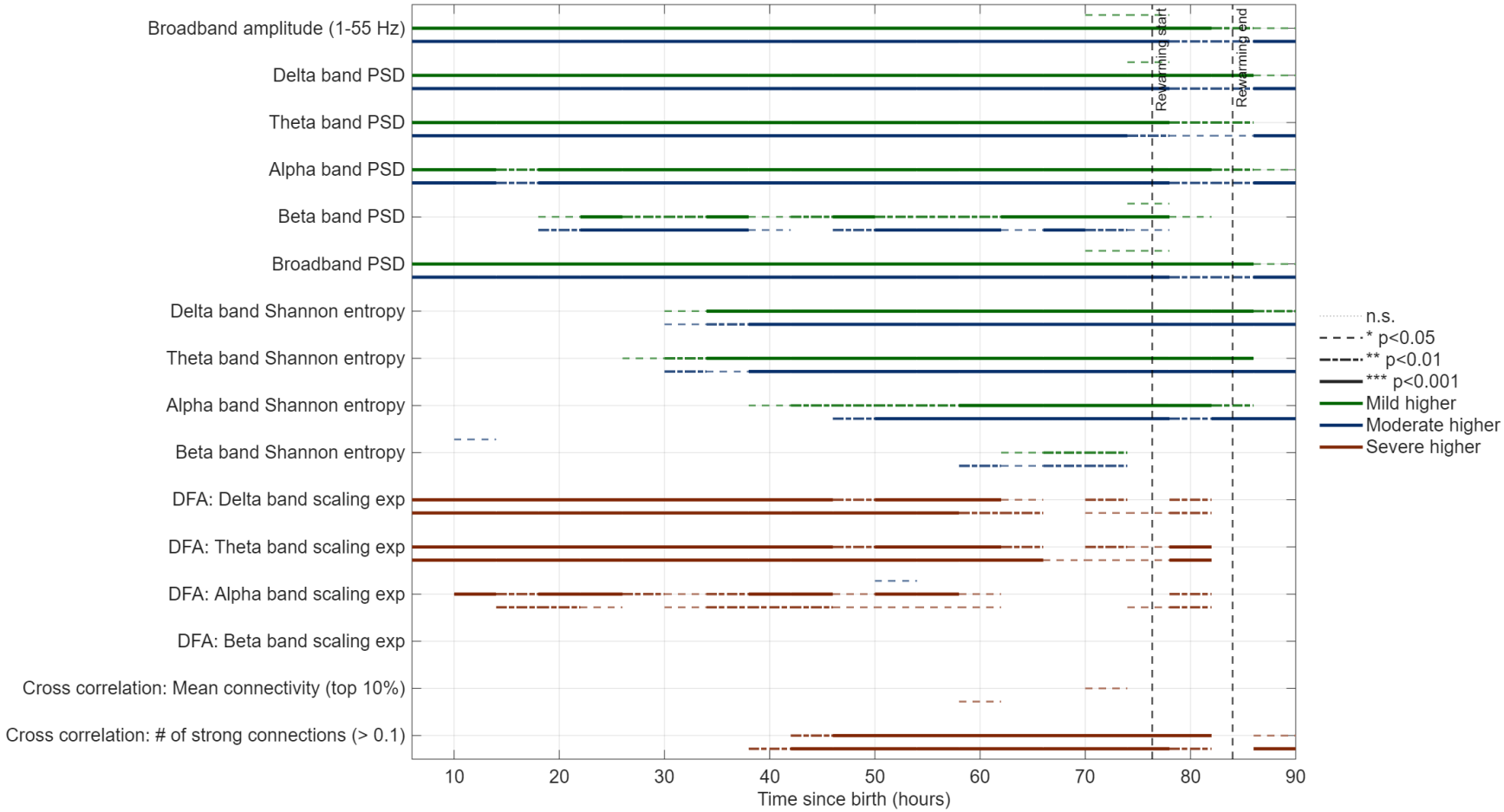

Figure 7: Summary of significant differences between NE grades (mild, moderate, severe).
Within each two-hour time bin, the median of each metric across all channels (Fp1, Fp2, T3, T4, C3, C4, O1, O2 and Cz) and epochs was calculated. These two-hour epochs were then pooled into four-hour time bins for visualization. The statistical significance test was a Kruskal-Wallis test. Pairwise comparisons were then performed using Dunn-Sidak correction to determine which pairs of groups differed significantly, followed by FDR correction using the Benjamini & Yekutieli (2001) method [39]. No statistical significance between any of the groups is represented by no line; the dashed lines represent a significance level of $p < 0.05$ (*); the dash-dot lines represent a significance level of $p < 0.01$ (**); the solid line represents a significance level of $p < 0.001$ (***). The top line seen in broadband amplitude, PSD in the delta, beta and broadband, beta band Shannon entropy and alpha band DFA scaling exponent represent significant differences between the mild and moderate

groups; the middle line represents significant differences between the mild and severe groups; the bottom line represents significant differences between the moderate and severe groups. For the remaining metrics with two lines, the top line represents significant differences between the mild and severe groups; the bottom line represents significant differences between the moderate and severe groups. A green line is used when the metric is higher for the mild grade than the severe grade. A blue line is used when the metric is higher for the moderate grade than the severe grade. A red line is used when the metric is higher for the severe grade than for the moderate or mild grade. For each metric, the mild vs. severe comparison is the upper line, while the moderate vs. severe comparison is the lower line. Abbreviations: PSD = power spectral density, DFA = detrended fluctuation analysis, n.s. = no significance.

The results for comparison of NE grades mirrored those for the comparison of SZ+ and SZ- groups. Broadband amplitude and delta, theta, alpha, beta, and broadband PSD differed significantly between the mild and severe and the moderate and severe groups for the majority of the timeframe (Figure 7, Figure 8, Figure 14, Figure 16). For these metrics, the mild and moderate groups had higher values than the severe group. Delta, theta, and alpha Shannon entropy differed significantly in the mid-to-later part of the timeframe, with the mild and moderate groups having higher values than the severe group (Figure 7, Figure 9). Theta and alpha permutation entropy differed significantly between the mild and severe and the moderate and severe groups at the beginning of the timeframe, with the severe group having higher values (Figure 19). Delta, theta and alpha DFA scaling exponent differed significantly across the beginning and middle of the timeframe, with the severe group having higher values than both mild and moderate grades (Figure 7, Figure 10). The number of strong connections (>0.1) from the cross-correlation analysis differed significantly in the later part of the timeframe, with the severe group having higher values than the mild and moderate NE grades (Figure 7, Figure 11).

Overall, the NE grade comparisons showed fewer statistically significant results than the SZ+/SZ- comparisons, and for most metrics, significance declined during rewarming and post-rewarming.

For some metrics, the mild, moderate and severe NE groups had different trends over time. The severe NE grade had much larger variation than the mild and moderate grades in PSD for the delta, theta and alpha bands (Figure 8A-C), and to a lesser extent in Shannon entropy for these same bands (Figure 9A-C). PSD values appeared to increase over the timeframe for the severe NE group in the delta and theta bands (Figure 8A-B), while Shannon entropy in these same bands appeared to decrease (Figure 9A-B). DFA scaling exponent showed the same pattern as PSD, with the severe grade showing larger variation than the mild and moderate grades, most notably in the delta, theta, and alpha bands (Figure 10A-C).

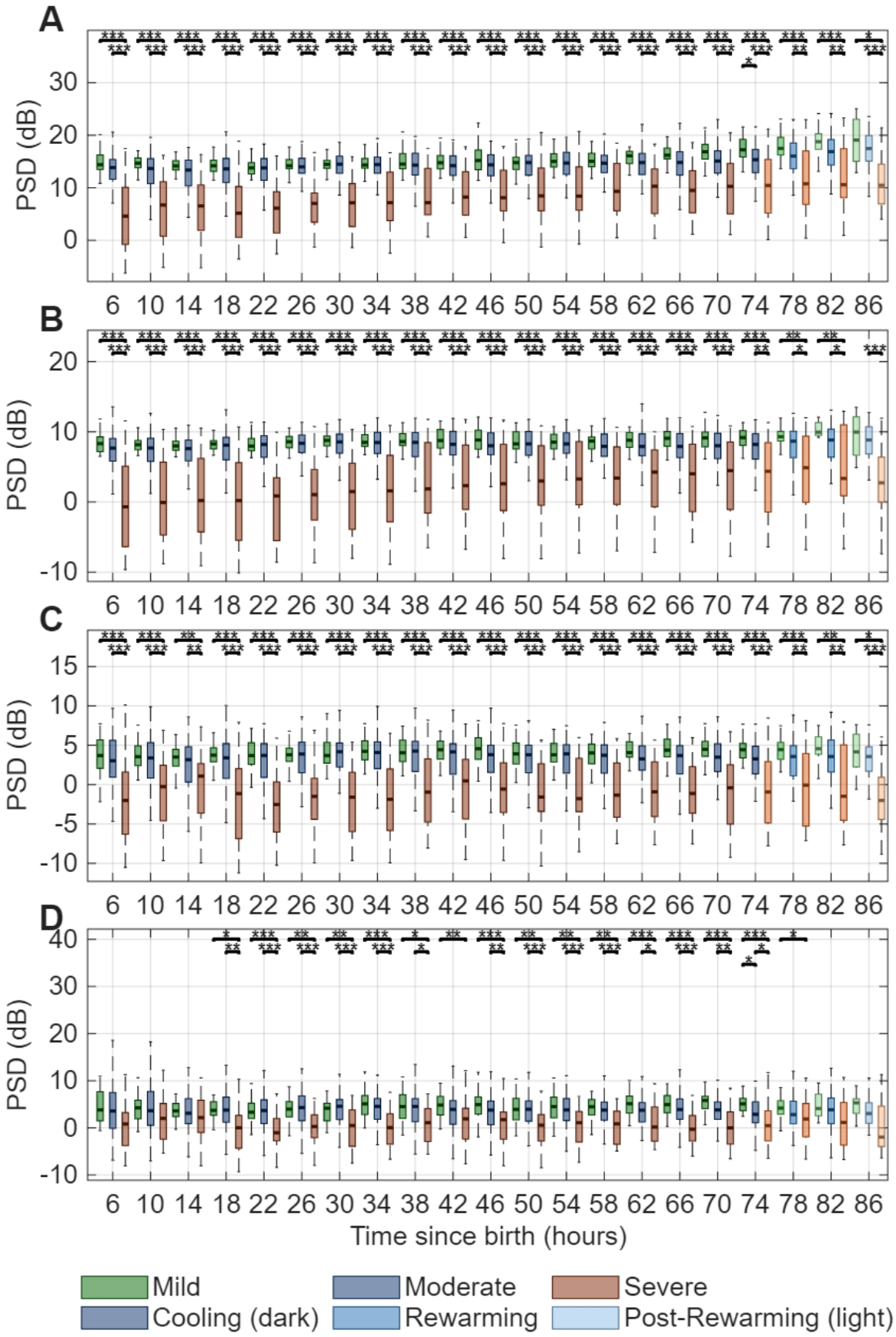
A
B
C
D
PSD (dB)
Time since birth (hours)
Mild
Moderate
Severe
Cooling (dark)
Rewarming
Post-Rewarming (light)

Figure 8: Frequency band specific PSD results for mild, moderate, and severe NE groups over 6-90 hrs postnatal.

A, B, C, and D show the results for PSD in the delta, theta, alpha and beta frequency bands, respectively. Within each two-hour time bin, the median of each metric across all channels (Fp1, Fp2, T3, T4, C3, C4, O1, O2 and Cz) and epochs was calculated. These two-hour epochs were then pooled into four-hour time bins for visualization. The middle line in the boxes represents the median value, while the upper and lower limits of the boxes represent the IQR. The statistical significance test was a Kruskal-Wallis test. Pairwise comparisons were then performed using Dunn-Sidak correction to determine which pairs of groups differed significantly, followed by FDR correction using the Benjamini & Yekutieli (2001) method [39]. The brackets and stars at the top of the plots represent a statistically significant result for that 2-hour time bin. One star (*) represents $p < 0.05$, two stars (**) represent $p < 0.01$ and three stars (***) represent $p < 0.001$. The color of the box plots changes to lighter shades when a majority of the epochs move from the cooling phase to the rewarming phase and then from the rewarming phase to the post-rewarming phase (normothermia). For A and D: The upper bracket represents differences between the mild and severe groups; the middle bracket represents differences between the moderate and severe groups; the lowest bracket represents differences between the mild and moderate groups. For B and C: The upper bracket represents differences between the mild and severe groups; the lower bracket represents differences between the moderate and severe groups. Abbreviations: PSD = power spectral density, NE = neonatal encephalopathy, IQR = interquartile range, FDR = false discovery rate.

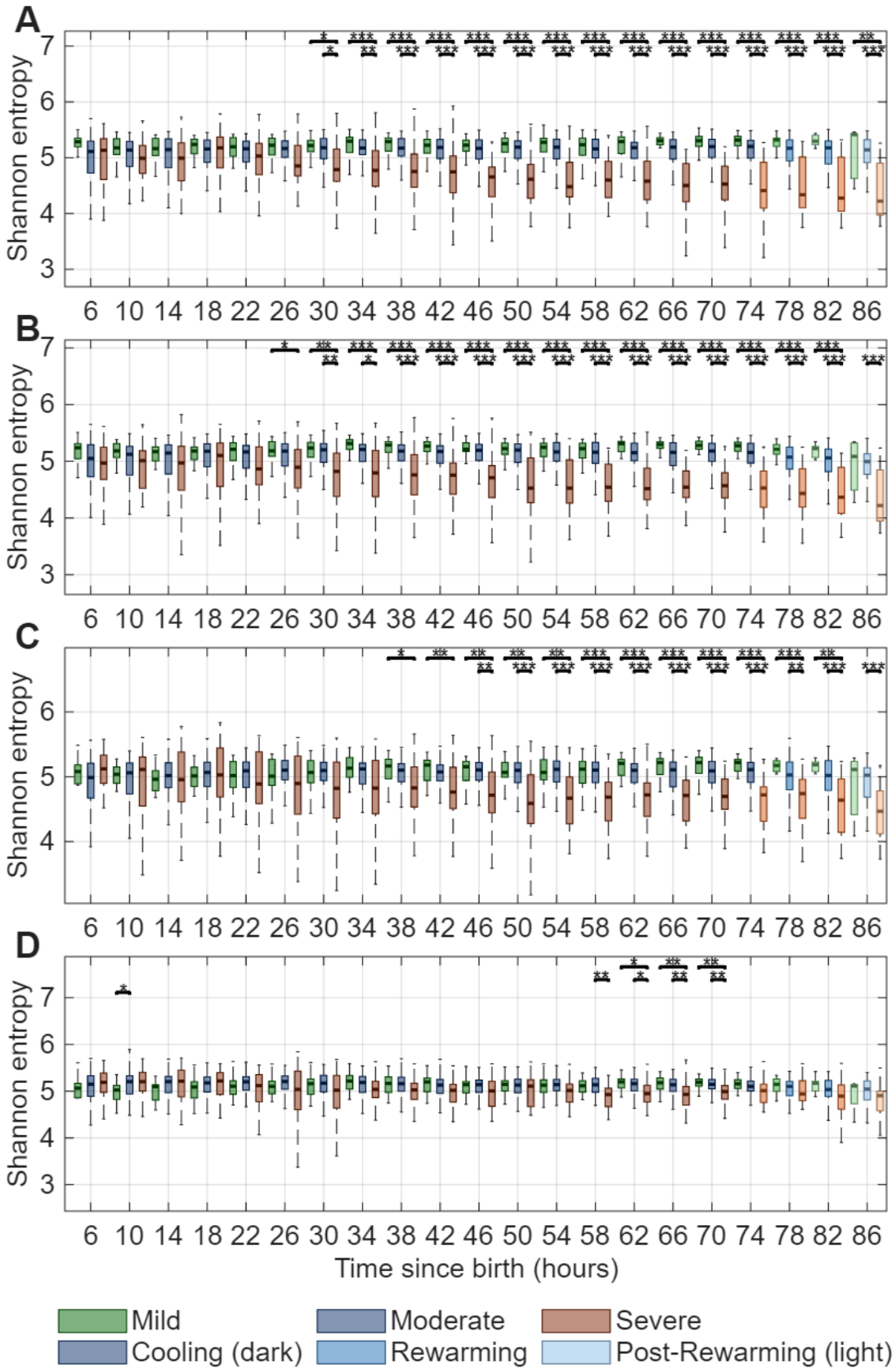
A
B
C
D
Shannon entropy
Time since birth (hours)
Mild
Moderate
Severe
Cooling (dark)
Rewarming
Post-Rewarming (light)

Figure 9: Frequency band specific Shannon entropy results for mild, moderate, and severe NE groups over 6-90 hrs postnatal.

A, B, C, and D show the results for Shannon entropy in the delta, theta, alpha and beta frequency bands, respectively. Within each two-hour time bin, the median of each metric across all channels (Fp1, Fp2, T3, T4, C3, C4, O1, O2 and Cz) and epochs was calculated. The middle line in the boxes represents the median value, while the upper and lower limits of the boxes represent the IQR. The statistical significance test was a Kruskal-Wallis test. Pairwise comparisons were then performed using Dunn-Sidak correction to determine which pairs of groups differed significantly, followed by FDR correction using the Benjamini & Yekutieli (2001) method [39]. The brackets and stars at the top of the plots represent a statistically significant result for that 2-hour time bin. One star (*) represents $p < 0.05$, two stars (**) represent $p < 0.01$ and three stars (***) represent $p < 0.001$. The color of the box plots changes to lighter shades when a majority of the epochs move from the cooling phase to the rewarming phase and then from the rewarming phase to the post-rewarming phase (normothermia). For A, B and C: The upper bracket represents differences between the mild and severe groups; the lower bracket represents differences between the moderate and severe groups. For D: The upper bracket represents differences between the mild and severe groups; the middle bracket represents differences between the moderate and severe groups; the lowest bracket represents differences between the mild and moderate groups. Abbreviations: NE = neonatal encephalopathy, IQR = interquartile range, FDR = false discovery rate.

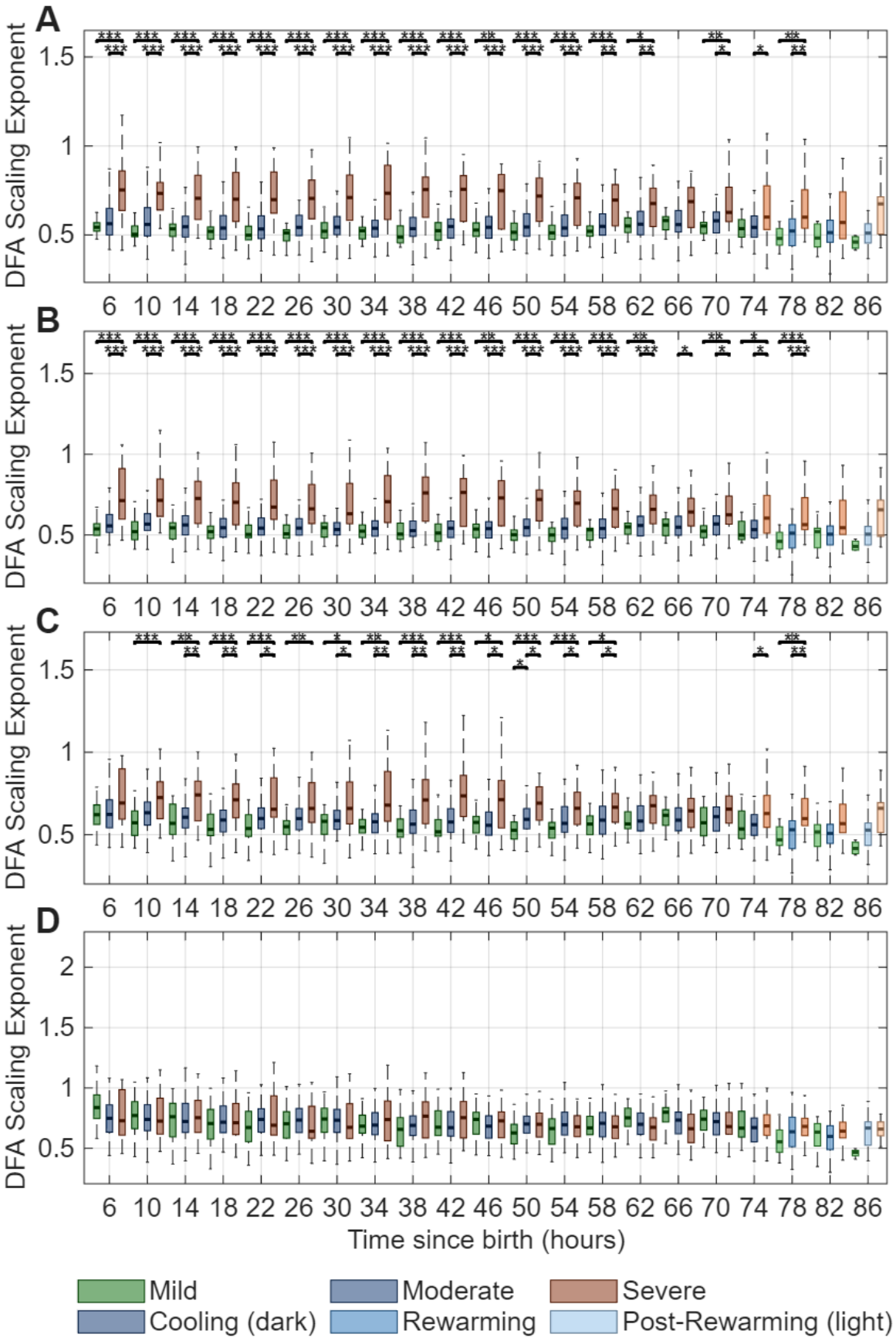
A
B
C
D
DFA Scaling Exponent
Time since birth (hours)
Mild
Moderate
Severe
Cooling (dark)
Rewarming
Post-Rewarming (light)

Figure 10: Frequency band specific DFA scaling exponent results for mild, moderate, and severe NE groups over 6-90 hrs postnatal.

A, B, C, and D show the results for DFA scaling exponent in the delta, theta, alpha and beta frequency bands, respectively. Within each two-hour time bin, the median of each metric across all channels (Fp1, Fp2, T3, T4, C3, C4, O1, O2 and Cz) and epochs was calculated. The middle line in the boxes represents the median value, while the upper and lower limits of the boxes represent the IQR. The statistical significance test was a Kruskal-Wallis test. Pairwise comparisons were then performed using Dunn-Sidak correction to determine which pairs of groups differed significantly, followed by FDR correction using the Benjamini & Yekutieli (2001) method [39]. The brackets and stars at the top of the plots represent a statistically significant result for that 2-hour time bin. One star (*) represents $p < 0.05$, two stars (**) represent $p < 0.01$ and three stars (***) represent $p < 0.001$. The upper bracket represents differences between the mild and severe groups; the middle bracket represents differences between the moderate and severe groups; the lowest bracket represents differences between the mild and moderate groups. The color of the box plots changes to lighter shades when a majority of the epochs move from the cooling phase to the rewarming phase and then from the rewarming phase to the post-rewarming phase (normothermia). For A and B: The upper bracket represents differences between the mild and severe groups; the lower bracket represents differences between the moderate and severe groups. For C: The upper bracket represents differences between the mild and severe groups; the middle bracket represents differences between the moderate and severe groups; the lowest bracket represents differences between the mild and moderate groups. Abbreviations: DFA = detrended fluctuation analysis, NE = neonatal encephalopathy, IQR = interquartile range, FDR = false discovery rate.

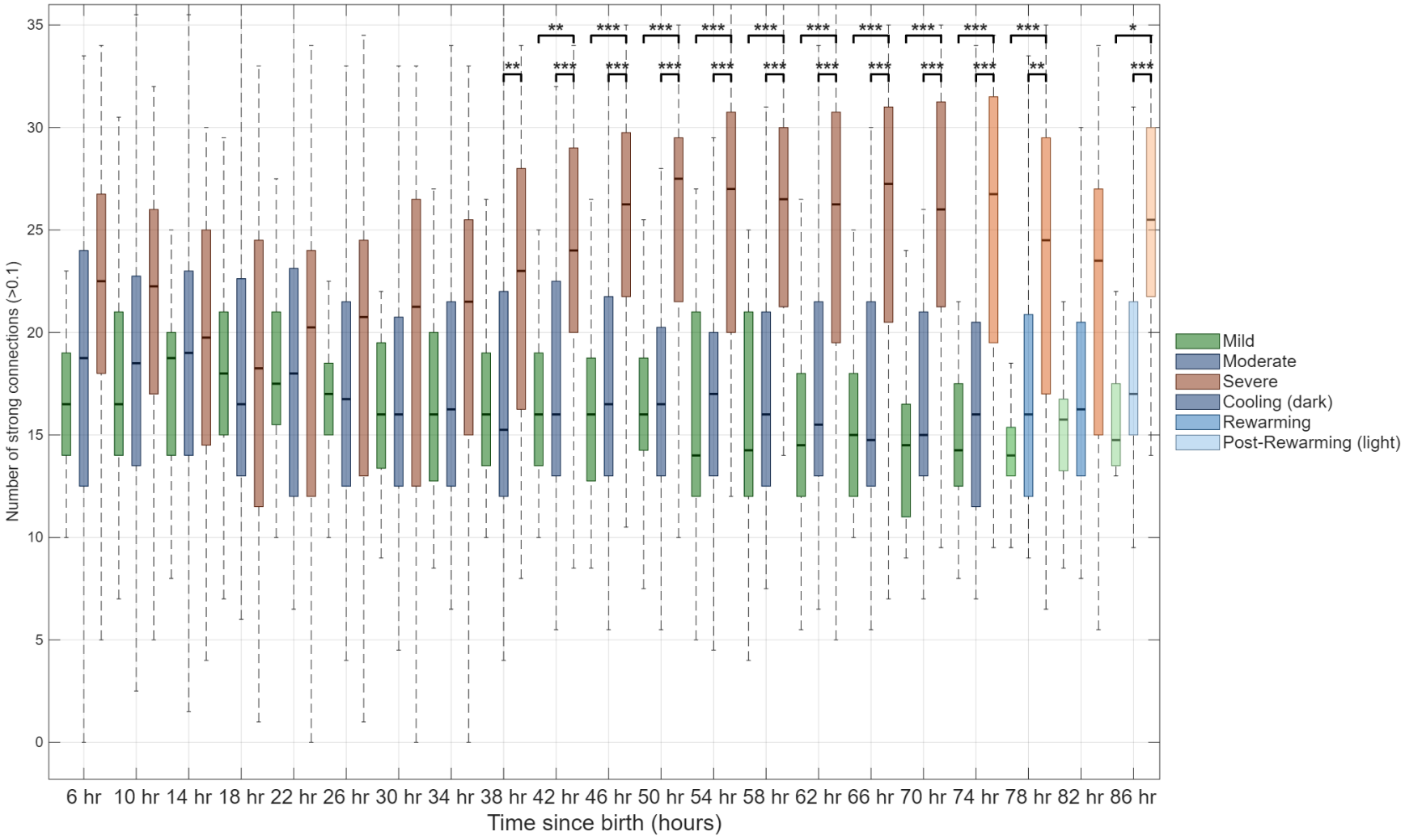

Figure 11: Number of strong cross correlation connections for mild, moderate, and severe NE groups over 6-90 hrs postnatal.

Within each two-hour time bin, the median of each metric across all channels (Fp1, Fp2, T3, T4, C3, C4, O1, O2 and Cz) and epochs was calculated. The middle line in the boxes represents the median value, while the upper and lower limits of the boxes represent the IQR. The statistical significance test was a Kruskal-Wallis test. Pairwise comparisons were then performed using Dunn-Sidak correction to determine which pairs of groups differed significantly, followed by FDR correction using the Benjamini & Yekutieli (2001) method [39]. The brackets and stars at the top of the plots represent a statistically significant result for that 2-hour time bin. One star (*) represents $p < 0.05$, two stars (**) represent $p < 0.01$ and three stars (***) represent $p < 0.001$. The upper bracket represents differences between the mild and severe groups while the lower bracket represents differences between the moderate and severe groups. The color of the box plots changes to lighter shades when a majority of the epochs move from the cooling phase to the rewarming phase and then from the rewarming phase to the post-rewarming phase (normothermia). Abbreviations: NE = neonatal encephalopathy, IQR = interquartile range, FDR = false discovery rate.

2.4 Significant differences remain between SZ- and SZ+ groups when the dataset is limited to the moderate NE grade

As seen in Table 2, there is an uneven distribution of SZ+ and SZ- neonates in the mild, moderate, and severe NE grades. The mild grade is dominated by the SZ- neonates while the severe grade is dominated by the SZ+ neonates. To determine if the NE grades were influencing the significant differences between the SZ+ and SZ- neonates, we ran the metrics within just the moderate NE grade which reduced the total patient population to 55 neonates total with 13 SZ+ and 42 SZ- (Figure 12).

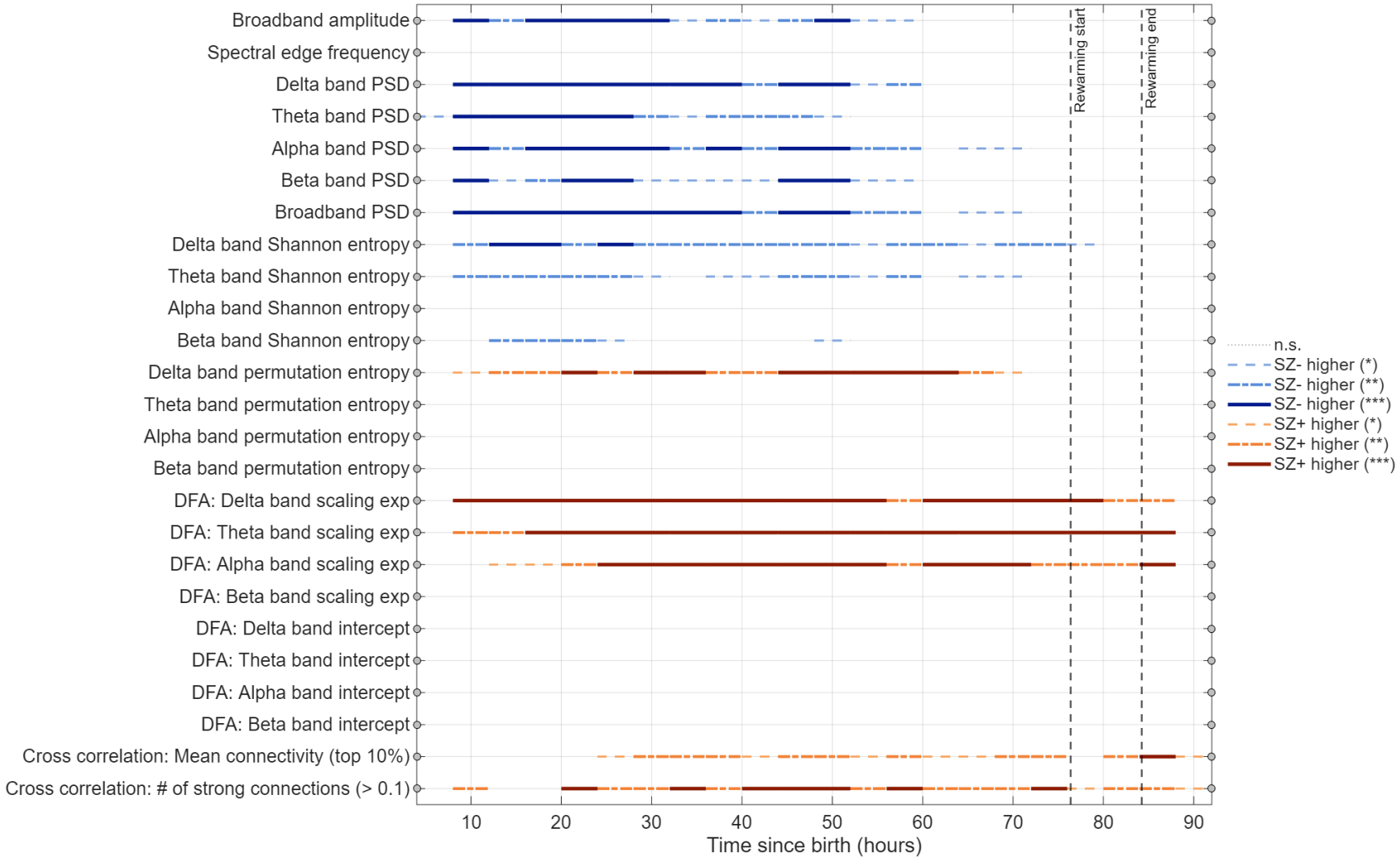

Figure 12: Summary of significant differences between the SZ+ and SZ- groups in moderate NE grade over 6-90 hrs postnatal.
Within each two-hour time bin, the median of each metric across all channels (Fp1, Fp2, T3, T4, C3, C4, O1, O2 and Cz) and epochs was calculated. The statistical significance test was the two-sided Wilcoxon rank sum test, followed by FDR correction using the Benjamini & Yekutieli (2001) method [39]. Line color indicates which group had higher values; within each color, the shade and line style together indicate significance

strength. No significant difference between groups is represented by no line; the lightest color with the dashed line represents a significance level of $p < 0.05$ (*); the middle color shade with the dot-dash line represents a significance level of $p < 0.01$ (**); the darkest color shade with the solid line represents a significance level of $p < 0.001$ (***). The blue lines represent results in which the SZ- group had higher values, while the orange lines represent results in which the SZ+ group had higher values. The vertical lines labeled "Rewarming start" and "Rewarming end" indicate where the majority of patients transition from the cooling phase to the rewarming phase, and then from the rewarming phase to the post-rewarming phase (normothermia). Abbreviations: SZ+ = neonates exhibiting electrographic or electroclinical seizures, SZ- = neonates not exhibiting seizures, PSD = power spectral density, DFA = detrended fluctuation analysis, n.s. = no significance, FDR = false discovery rate.

The broadband amplitude, PSD in the delta, theta, alpha, beta and broadband, Shannon entropy in the delta and theta bands, permutation entropy in the delta band, DFA scaling exponent in the delta, theta and alpha band as well as the number of strong cross correlation connections and mean connectivity all showed statistically significant differences across the timeframe.

CHAPTER 3: DISCUSSION

Here we have demonstrated that fourteen EEG metrics measured during TH in the 6-90 hr postnatal window differentiated neonates with HIE who developed seizures from those who did not. These metrics were broadband amplitude; PSD in the delta, theta, alpha, beta and broadband ranges; Shannon entropy in the delta and theta ranges; Permutation entropy in the delta band; DFA scaling exponent in the delta, theta and alpha ranges; and the number of strong cross-correlation connections and mean connectivity. All of the metrics except mean connectivity also differed significantly by NE grade (mild vs. severe and moderate vs. severe). We also tested significant differences between the SZ+ and SZ- groups in only the moderate NE grade group to determine if the NE grades were driving the results. The results of the moderate NE grade group comparison show that the statistically significant differences were not driven primarily by the NE grade. Due to the decreased power (from the decreased sample size), we would expect less statistical significance across the timeframe, which was what we observed for most metrics.

3.1 Amplitude and PSD

Previous studies showed that delta power and amplitude from EEG recorded within the first three days of life differentiate between HIE severity grades, with more severe groups showing lower values [3-5]. This is consistent with our findings when stratifying by NE grades (Figure 8, Figure 14). To our knowledge, this is the first study to test amplitude and frequency-band-specific PSD as differentiators of seizure development in neonates with HIE. We found that these metrics differentiated the SZ+ and SZ- groups across most frequency ranges (delta, theta, alpha, beta and broadband) and across the majority of the 6-90 hr postnatal timeframe (Figure 2, Figure 13, Figure 15).

3.2 Spectral edge frequency

SEF was tested in previous literature as a candidate differentiator of EEG/HIE severity grade [3]; however, it was one of the few quantitative measures that did not differentiate across grades. This contrasted with amplitude, relative delta power, skewness, kurtosis and discontinuity, which did differentiate severity [3]. This is consistent with our findings, where SEF did not consistently differentiate between the SZ+ and SZ- groups (Figure 6), but amplitude and EEG PSD did differentiate them. This suggests that seizures are associated with a broadband increase in power across all frequency bands rather than a shift in the distribution of power, which is why amplitude and PSD differentiated the groups while SEF, a measure of relative spectral distribution, did not. In contrast, SEF did differ significantly in the NE grade comparison between the mild and severe and moderate and severe groups at the beginning of the timeframe (Figure 17). This would indicate a shift in the distribution of power between the NE grades in this part of the timeframe.

3.3 Entropy

Entropy-based EEG measures (sample, permutation, spectral, and approximate entropy) have been used to differentiate neonatal HIE outcomes, with delta band entropy measures performing best [6]. This is consistent with our findings, where Shannon entropy differentiated between NE grades for the delta, theta and alpha bands in the mid-to-later part of the 6-90 hr timeframe (Figure 9). Similarly, we found that Shannon entropy in the delta and theta bands differentiated the SZ+ and SZ- groups in the mid-to-later part of the timeframe (Figure 3), and permutation entropy showed differences between SZ+ and SZ- in the delta band in the mid-to-later part of the timeframe (Figure 18). Theta and alpha permutation entropy differed significantly between the mild and severe and the moderate and severe groups for a short

timespan at the beginning of the timeframe (Figure 19). This was unique because for the other metrics and in prior studies [6], the delta band was the most useful frequency band for discerning differences between groups.

3.4 DFA scaling exponent and intercept

The DFA scaling exponent has been tested as a differentiator of neonatal EEG background severity, though conventional single-value DFA did not achieve strong predictive performance, leading the authors to pursue a multi-factorial DFA approach instead [8]. This is in contrast with our findings, where the DFA scaling exponent differentiated the SZ+/SZ- groups across the delta, theta, and alpha bands (Figure 4), and the NE grades across the delta, theta, and alpha bands (Figure 10). This discrepancy may be explained by differences in study design: [8] analyzed only four 2-hour epochs per neonate and grouped the neonates by visual assessment of the EEG background severity, whereas our analysis spanned the full cooling-through-rewarming timeframe and used NE grade and seizure occurrence as comparison groups. DFA intercept did differentiate between the SZ+/SZ- groups in the beginning of the timeframe for the delta band (Figure 20) as well as briefly in the beginning of the timeframe for all frequency bands between the NE grades comparing severe to mild/moderate (Figure 21).

3.5 Cross correlation connectivity

Stronger functional connectivity networks, measured using cross correlation analysis, have been measured in infants (median age ~11 months) with infantile epileptic spasms syndrome compared to healthy age-matched controls [23]. In longitudinal data, the onset of Lennox-Gastaut syndrome in young children (median age ~25 months) was associated with high connectivity strength, and the strength decreased in patients who subsequently began treatment and exhibited resolution of the slow spike-and-wave pattern [24]. This is consistent with our

results where we saw that the severe NE grade had a higher number of strong connections than the mild and moderate NE grades (Figure 7, Figure 11). Our study also showed evidence that across the majority of the 6-90 hr timeframe, the mean connectivity and number of strong connections differed significantly between the SZ+ and SZ- groups, with the SZ+ group having higher values (Figure 1, Figure 5, Figure 22).

3.6 Limitations

This thesis had several limitations. First, we grouped the neonates into seizure vs. no seizure as well as into three NE grades. However, it would have been more clinically relevant to classify the neonates based on their follow-up (1-year or 2-year) neurological scores or epilepsy diagnosis. If this follow-up data were available, we would have been able to determine whether any of the metrics included in our study were predictive of the development of epilepsy or other poor cognitive outcomes.

Second, the patients included in this study were all from one hospital and the sample size was limited to 96 patients. This limits the generalizability of our results to patients and populations outside of this hospital and future studies should expand upon this dataset to gain a broader understanding of these results.

Third, some of the neonates in our dataset were administered anti-seizure medications (ASMs) during the EEG recording; however, the time of administration was not available. The ASMs may have an impact on the EEG recording, so the lack of documented administration timing means medication effects cannot be accounted for in the analysis.

Fourth, ictal periods were included in the EEG recordings for this study. Typically, interictal data would be isolated to avoid the EEG characteristics of a seizure skewing the results. Root mean square amplitude has been shown to be a strong classifier of neonatal seizures, with

higher values observed during seizures than during non-seizure periods [40]. Therefore, any bias introduced by not excluding the seizure data likely worked against, rather than in favor of, our finding of lower amplitude and PSD in the SZ+ group. This suggests that our results are conservative, rather than being inflated.

Fifth, in our analysis, all of the metrics were aggregated across channels using the median value to characterize global EEG activity. For future studies, channel-specific results should be analyzed to identify spatial trends in addition to the temporal analysis of the data.

3.7 Future work

Several extensions of this work would help determine whether these metrics are ready for clinical application. Expanding the dataset beyond a single medical center would help establish generalizability. Incorporating additional cross-frequency and network-based connectivity measures, such as phase-amplitude coupling and the weighted phase lag index, could capture dynamics not reflected in the current set of metrics. As long-term follow-up data become available, testing if these metrics can predict outcomes such as epilepsy would show their utility beyond acute seizure risk. Finally, accounting for ASM administration timing would help uncover any medication effects from the EEG trends associated with seizure risk.

CHAPTER 4: Summary and Conclusions

Neonates with HIE are at elevated risk of seizures during the period after birth [2], and seizures themselves have been associated with additional brain injury [18-20]. While prior EEG studies have shown that measures such as amplitude, delta power, and entropy can differentiate HIE severity grade [3-8], no previous study had tested whether these same metrics could distinguish neonates who go on to develop seizures from those who do not. This thesis addressed

that gap by retrospectively analyzing continuous EEG recordings from 96 neonates with HIE undergoing therapeutic hypothermia at CHOC. Six categories of EEG metrics including amplitude, spectral edge frequency, power spectral density, Shannon and permutation entropy, detrended fluctuation analysis scaling exponent, and cross-correlation connectivity were computed across the first 6-90 hours after birth and compared between neonates with and without seizures, as well as across NE severity grades (mild, moderate, severe).

Several EEG metrics differentiated SZ+ from SZ- neonates across the majority of the timeframe. Broadband amplitude and PSD in the delta, theta, alpha, beta, and broadband ranges were lower in the SZ+ group; Shannon entropy in the delta and theta bands was also lower in SZ+ neonates during the mid-to-late portion of the recording; the permutation entropy in the delta band was higher in the SZ+ neonates; the DFA scaling exponent in the delta, theta, and alpha bands was higher in SZ+ neonates; and cross-correlation connectivity, measured using both the number of strong connections and the mean connectivity strength, was higher in SZ+ neonates across the majority of the timeframe. SEF did not differentiate the groups.

Many of these same metrics also differentiated NE grade, though consistent differences were found only between the severe and mild grades and between the severe and moderate grades. There were no consistent statistically significant differences found between mild and moderate grades. Broadband amplitude, PSD (delta, theta, alpha, beta, broadband), and Shannon entropy (delta, theta, alpha) were lower in the severe group, while DFA scaling exponent (delta, theta, alpha) and cross-correlation connectivity were higher in the severe group. The novel finding that many of these metrics differentiate seizure development extends the existing literature in a new, clinically meaningful direction.

Together, these results suggest that background EEG activity is systematically altered in neonates who exhibit seizures, and this alteration is measurable using relatively simple, computationally efficient metrics. Because differences in amplitude, PSD, entropy, DFA, and connectivity were detectable across much of the 6-90 hour window rather than confined to a narrow period, these metrics may have value as an early, continuous indicator of seizure risk in neonates with HIE, potentially supporting closer monitoring or earlier intervention in this population.

This study has several limitations, discussed in detail in Chapter 3, including the absence of long-term neurodevelopmental follow-up data, undocumented ASM administration timing, the inclusion of ictal epochs in the analysis, the use of channel-averaged rather than spatially resolved metrics, and the exclusion of cross-frequency and network-based connectivity measures such as phase-amplitude coupling and the weighted phase lag index. Future work should address these directly by testing whether the metrics identified here predict longer-term outcomes such as epilepsy diagnosis and incorporating spatial and additional connectivity-based analyses.

Overall, this thesis provides evidence that computational EEG metrics recorded during the cooling and rewarming period of therapeutic hypothermia can differentiate neonates with HIE who develop seizures from those who do not, extending prior work on EEG-based severity classification into a new and clinically important application. These findings lay the groundwork for future studies aimed at developing EEG-based tools to support earlier identification and management of seizure risk in this vulnerable patient population.

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APPENDIX A: Additional Figures

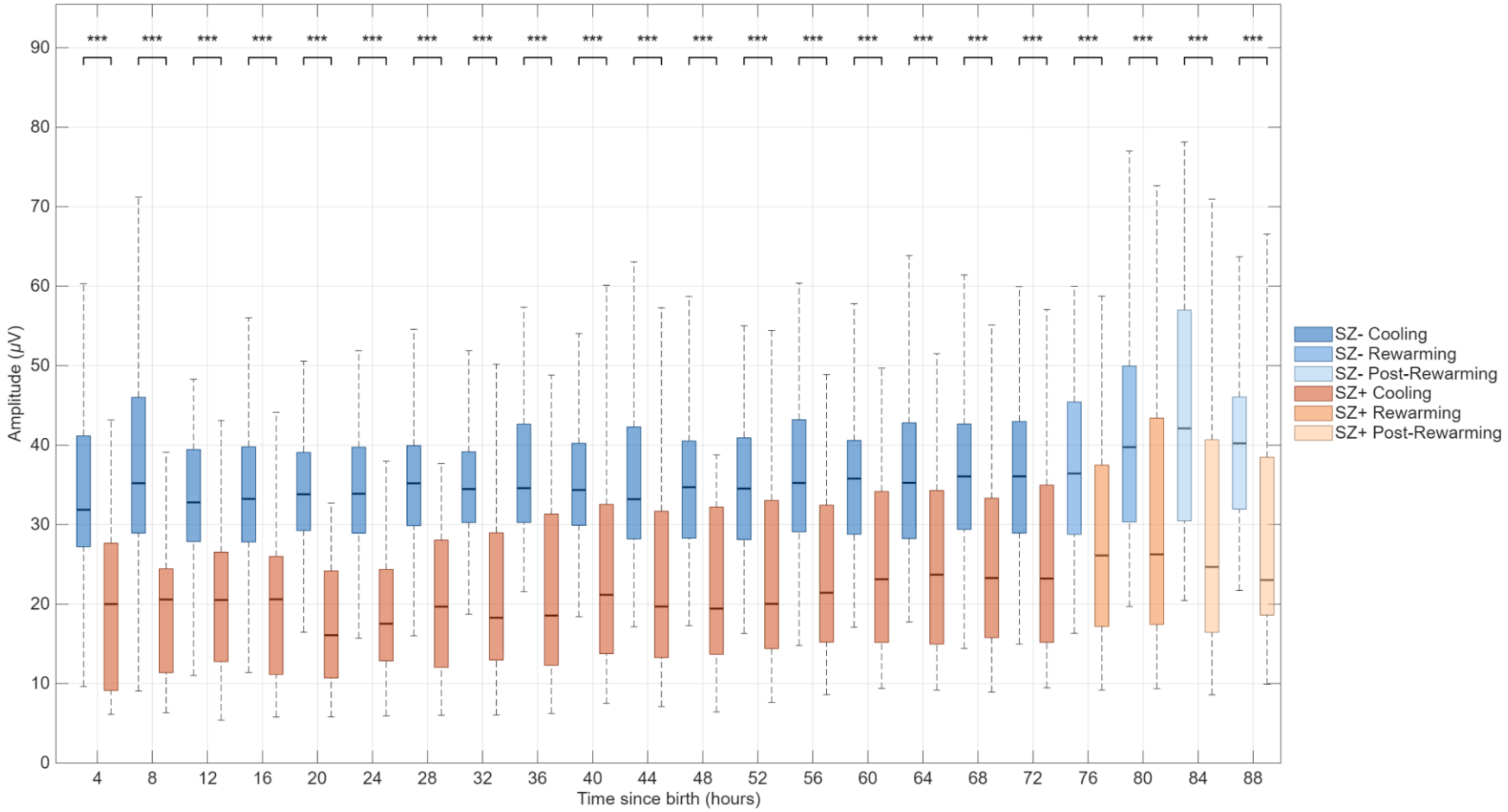

Figure 13: Amplitude results for SZ+ and SZ- groups over 6-90 hrs postnatal.
Within each two-hour time bin, the median of each metric across all channels (Fp1, Fp2, T3, T4, C3, C4, O1, O2 and Cz) and epochs was calculated. These two-hour epochs were then pooled into four-hour time bins for visualization. The middle line in the boxes represents the median value, while the upper and lower limits of the boxes represent the IQR. The statistical significance test was the two-sided Wilcoxon rank sum test followed by FDR correction using the Benjamini & Yekutieli (2001) method [39]. The brackets and stars at the top of the plots represent a

statistically significant result for that 2-hour time bin. One star (*) represents p < 0.05, two stars (**) represent p < 0.01 and three stars (***) represent p < 0.001. The color of the box plots changes to lighter shades when a majority of the epochs move from the cooling phase to the rewarming phase and then from the rewarming phase to the post-rewarming phase (normothermia). Abbreviations: SZ+ = neonates exhibiting electrographic or electroclinical seizures, SZ- = neonates not exhibiting seizures, IQR = interquartile range, FDR = false discovery rate.

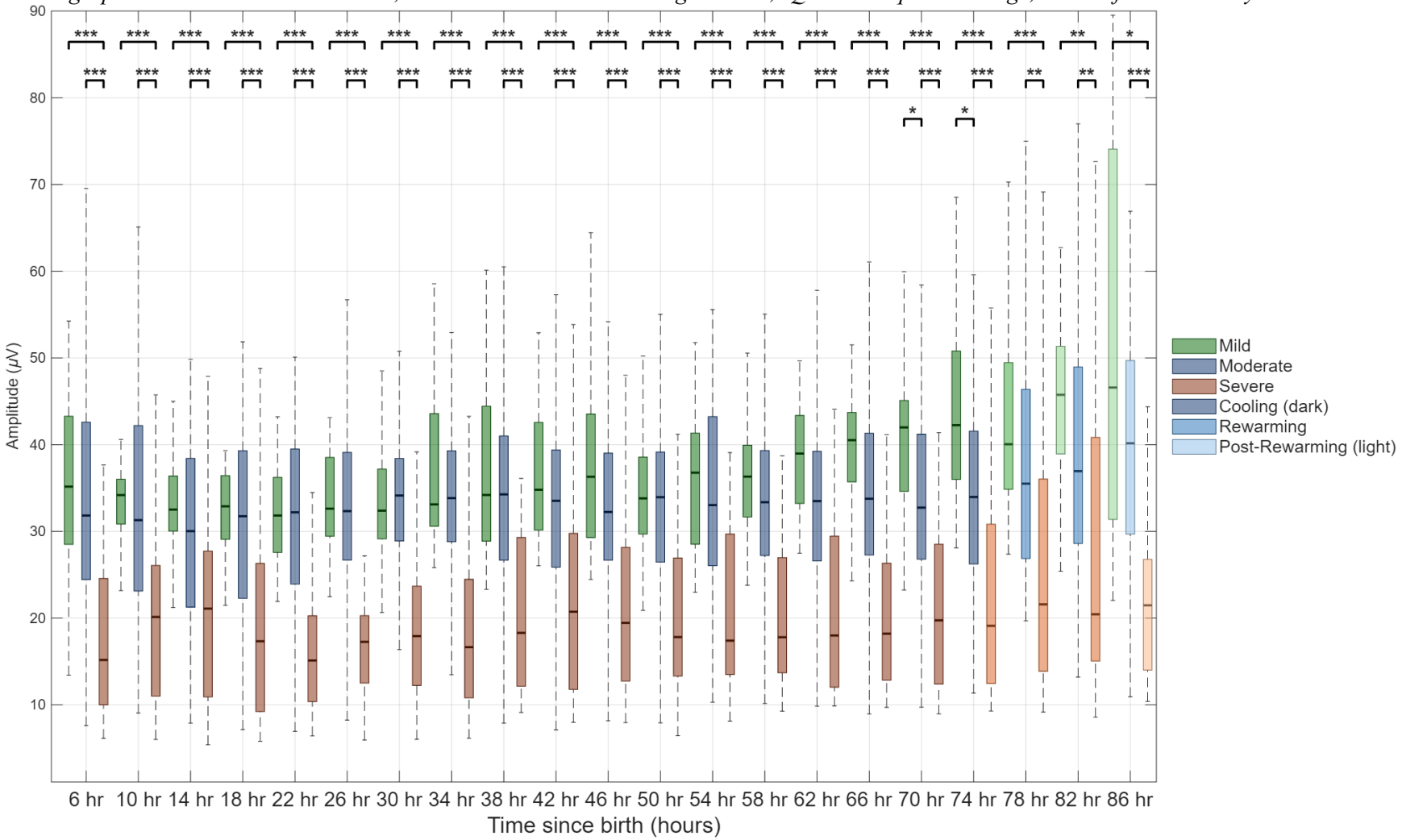

Figure 14: Amplitude results for mild, moderate, and severe NE groups over 6-90 hrs postnatal.

Within each two-hour time bin, the median of each metric across all channels (Fp1, Fp2, T3, T4, C3, C4, O1, O2 and Cz) and epochs was calculated. These two-hour epochs were then pooled into four-hour time bins for visualization. The middle line in the boxes represents the median value, while the upper and lower limits of the boxes represent the IQR. The statistical significance test was a Kruskal-Wallis test. Pairwise comparisons were then performed using Dunn-Sidak correction to determine which pairs of groups differed significantly, followed by FDR correction using the Benjamini & Yekutieli (2001) method [39]. The brackets and stars at the top of the plots represent a statistically significant result for that 2-hour time bin. One star (*) represents $p < 0.05$, two stars (**) represent $p < 0.01$ and three stars (***) represent $p < 0.001$. The upper bracket represents differences between the mild and severe groups; the middle bracket represents differences between the moderate and severe groups; the lowest bracket represents differences between the mild and moderate groups. The color of the box plots changes to lighter shades when a majority of the epochs move from the cooling phase to the rewarming phase and then from the rewarming phase to the post-rewarming phase (normothermia). Abbreviations: PSD = power spectral density, NE = neonatal encephalopathy, IQR = interquartile range, FDR = false discovery rate.

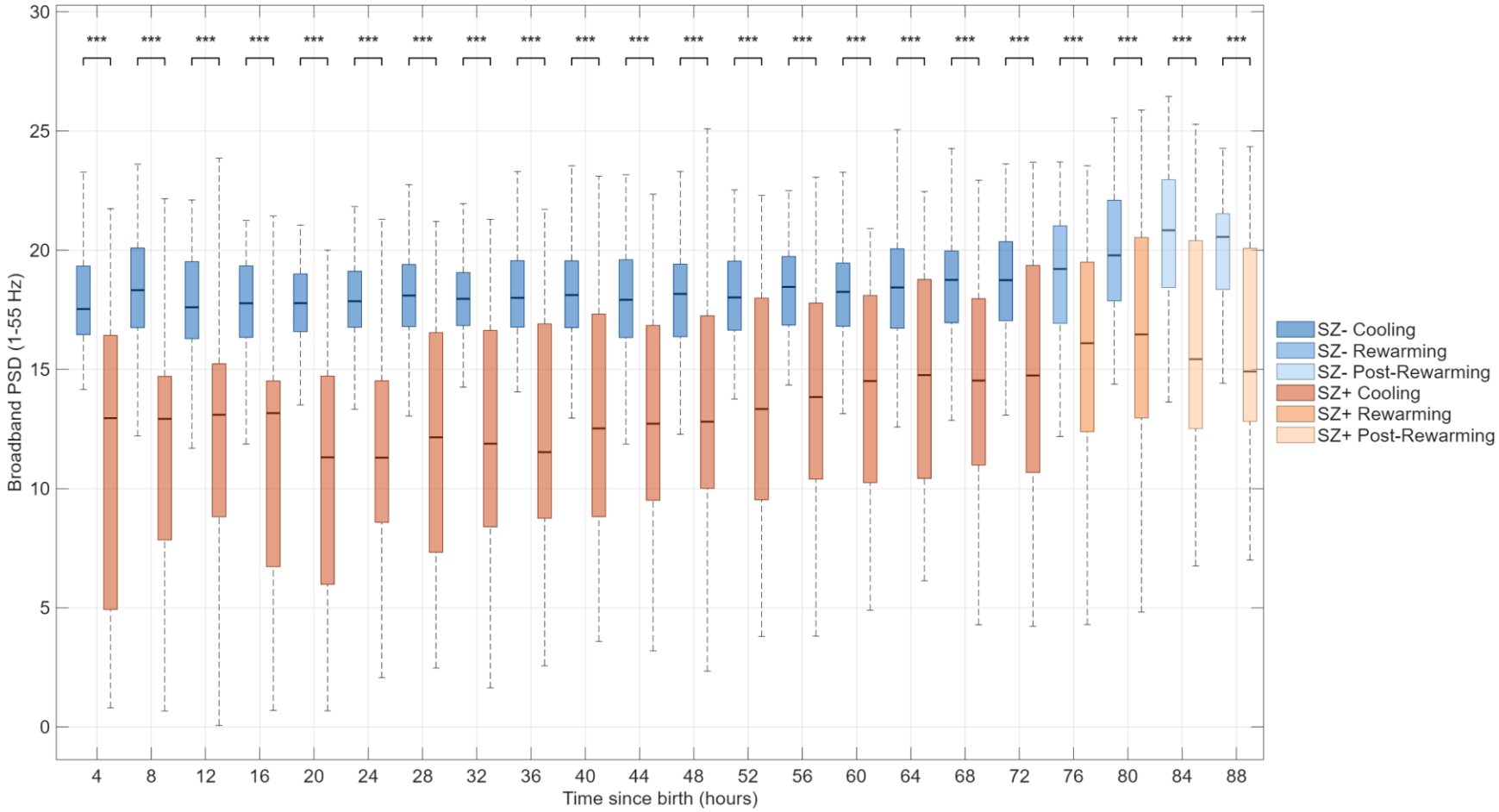

Figure 15: Broadband PSD results for SZ+ and SZ- groups over 6-90 hrs postnatal.
Within each two-hour time bin, the median of each metric across all channels (Fp1, Fp2, T3, T4, C3, C4, O1, O2 and Cz) and epochs was calculated. These two-hour epochs were then pooled into four-hour time bins for visualization. The middle line in the boxes represents the median value, while the upper and lower limits of the boxes represent the IQR. The statistical significance test was the two-sided Wilcoxon rank sum test followed by FDR correction using the Benjamini & Yekutieli (2001) method [39]. The brackets and stars at the top of the plots represent a statistically significant result for that 2-hour time bin. One star (*) represents $p < 0.05$, two stars (**) represent $p < 0.01$ and three stars (***) represent $p < 0.001$. The color of the box plots changes to lighter shades when a majority of the epochs move from the cooling phase to the

rewarming phase and then from the rewarming phase to the post-rewarming phase (normothermia). Abbreviations: SZ+ = neonates exhibiting electrographic or electroclinical seizures, SZ- = neonates not exhibiting seizures, IQR = interquartile range, FDR = false discovery

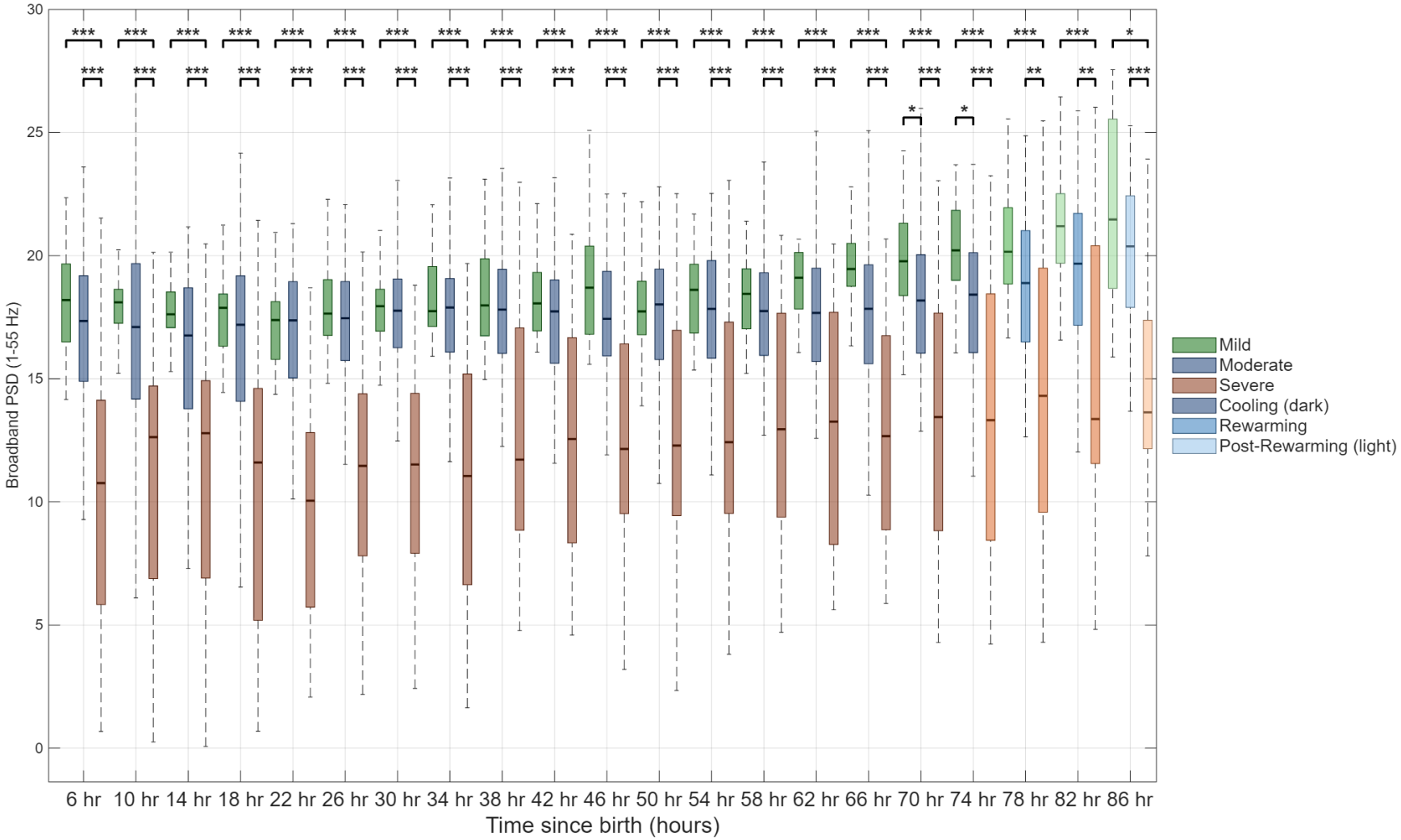

Figure 16: Broadband PSD results for mild, moderate, and severe NE groups over 6-90 hrs postnatal.
Within each two-hour time bin, the median of each metric across all channels (Fp1, Fp2, T3, T4, C3, C4, O1, O2 and Cz) and epochs was calculated. These two-hour epochs were then pooled into four-hour time bins for visualization. The middle line in the boxes represents the median

value, while the upper and lower limits of the boxes represent the IQR. The statistical significance test was a Kruskal-Wallis test. Pairwise comparisons were then performed using Dunn-Sidak correction to determine which pairs of groups differed significantly, followed by FDR correction using the Benjamini & Yekutieli (2001) method [39]. The brackets and stars at the top of the plots represent a statistically significant result for that 2-hour time bin. One star (*) represents $p < 0.05$, two stars (**) represent $p < 0.01$ and three stars (***) represent $p < 0.001$. The upper bracket represents differences between the mild and severe groups; the middle bracket represents differences between the moderate and severe groups; the lowest bracket represents differences between the mild and moderate groups. The color of the box plots changes to lighter shades when a majority of the epochs move from the cooling phase to the rewarming phase and then from the rewarming phase to the post-rewarming phase (normothermia). Abbreviations: NE = neonatal encephalopathy, IQR = interquartile range, FDR = false discovery rate.

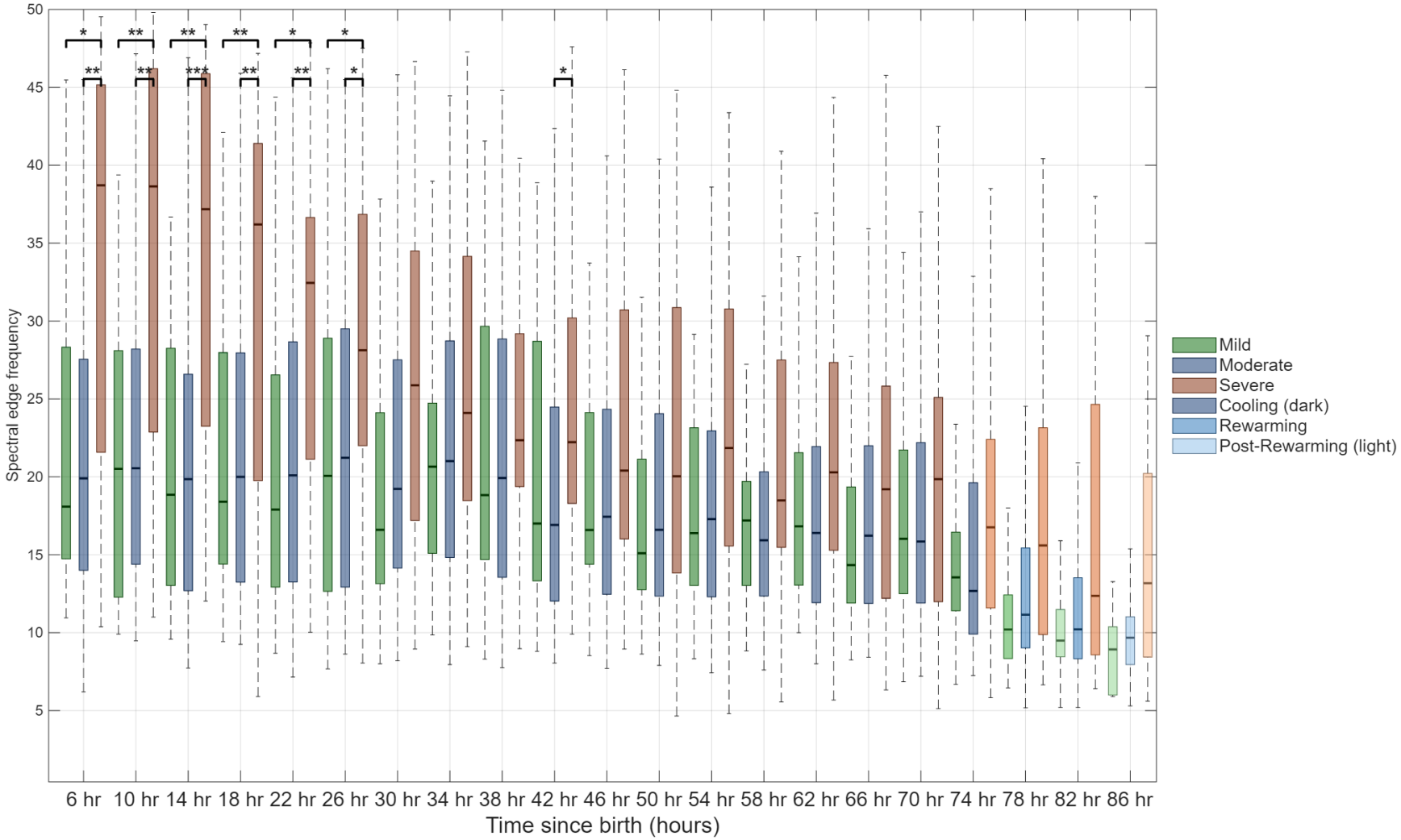

Figure 17: Spectral edge frequency results for mild, moderate, and severe NE groups over 6-90 hrs postnatal.
Within each two-hour time bin, the median of each metric across all channels (Fp1, Fp2, T3, T4, C3, C4, O1, O2 and Cz) and epochs was calculated. These two-hour epochs were then pooled into four-hour time bins for visualization. The middle line in the boxes represents the median value, while the upper and lower limits of the boxes represent the IQR. The statistical significance test was a Kruskal-Wallis test. Pairwise comparisons were then performed using Dunn-Sidak correction to determine which pairs of groups differed significantly, followed by FDR

correction using the Benjamini & Yekutieli (2001) method [39]. The brackets and stars at the top of the plots represent a statistically significant result for that 2-hour time bin. One star (*) represents $p < 0.05$, two stars (**) represent $p < 0.01$ and three stars (***) represent $p < 0.001$. The upper bracket represents differences between the mild and severe groups while the lower bracket represents differences between the moderate and severe groups. The color of the box plots changes to lighter shades when a majority of the epochs move from the cooling phase to the rewarming phase and then from the rewarming phase to the post-rewarming phase (normothermia). Abbreviations: NE = neonatal encephalopathy, IQR = interquartile range, FDR = false discovery rate

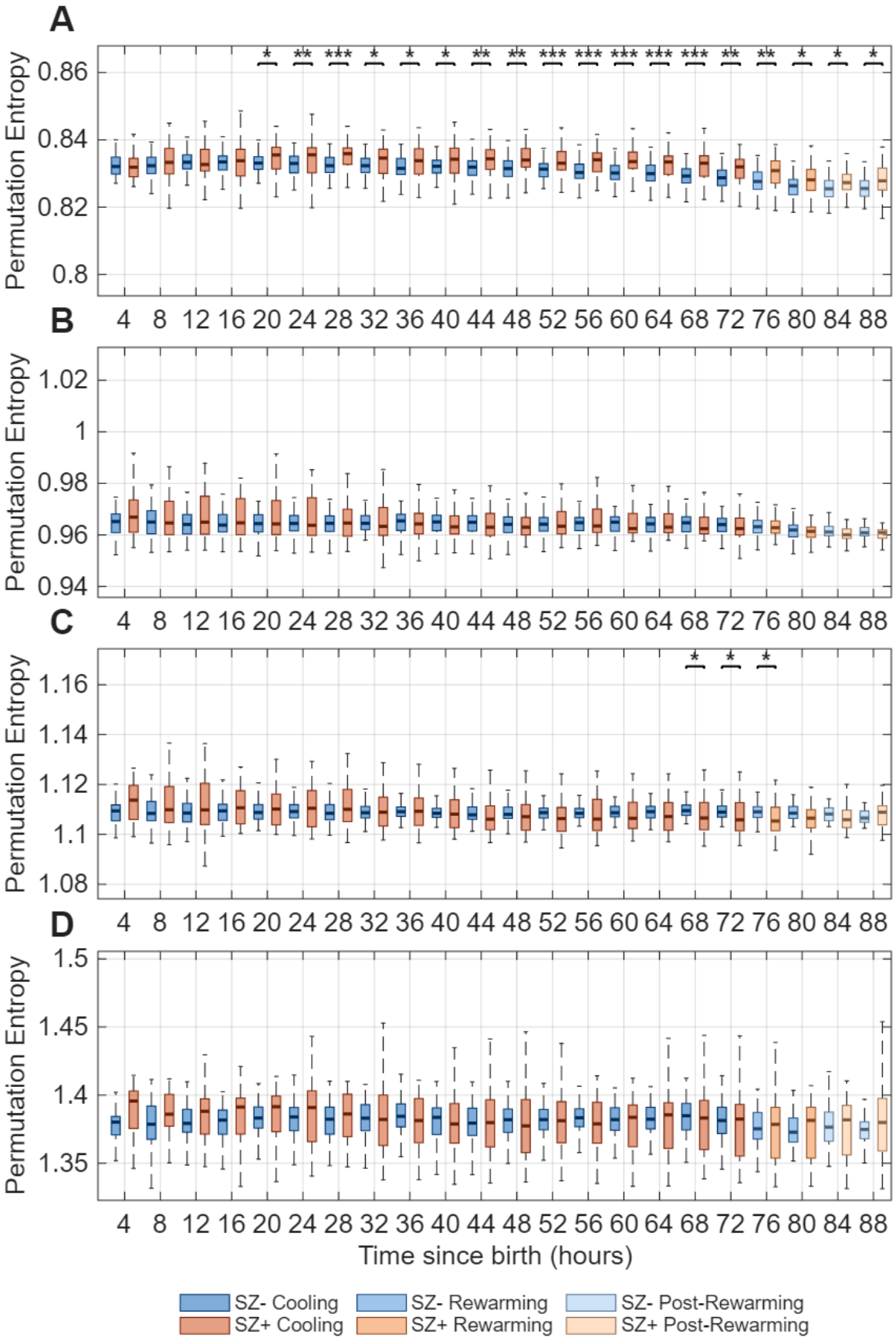
A
B
C
D
Permutation Entropy
0.86
0.84
0.82
0.8
1.02
1
0.98
0.96
0.94
1.16
1.14
1.12
1.1
1.08
1.5
1.45
1.4
1.35
4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68 72 76 80 84 88
Time since birth (hours)
SZ- Cooling
SZ+ Cooling
SZ- Rewarming
SZ+ Rewarming
SZ- Post-Rewarming
SZ+ Post-Rewarming

Figure 18: Frequency band specific permutation entropy results for SZ+ and SZ- groups over 6-90 hrs postnatal.

A, B, C, and D show the results for the permutation entropy in the delta, theta, alpha and beta frequency bands, respectively. Within each two-hour time bin, the median of each metric across all channels (Fp1, Fp2, T3, T4, C3, C4, O1, O2 and Cz) and epochs was calculated. These two-hour epochs were then pooled into four-hour time bins for visualization. The middle line in the boxes represents the median value, while the upper and lower limits of the boxes represent the IQR. The statistical significance test was the two-sided Wilcoxon rank sum test followed by FDR correction using the Benjamini & Yekutieli (2001) method [39]. The brackets and stars at the top of the plots represent a statistically significant result for that 2-hour time bin. One star (*) represents $p < 0.05$, two stars (**) represent $p < 0.01$ and three stars (***) represent $p < 0.001$. The color of the box plots changes to lighter shades when a majority of the epochs move from the cooling phase to the rewarming phase and then from the rewarming phase to the post-rewarming phase (normothermia). Abbreviations: SZ+ = neonates exhibiting electrographic or electroclinical seizures, SZ- = neonates not exhibiting seizures, IQR = interquartile range, DFA = detrended fluctuation analysis, FDR = false discovery rate.

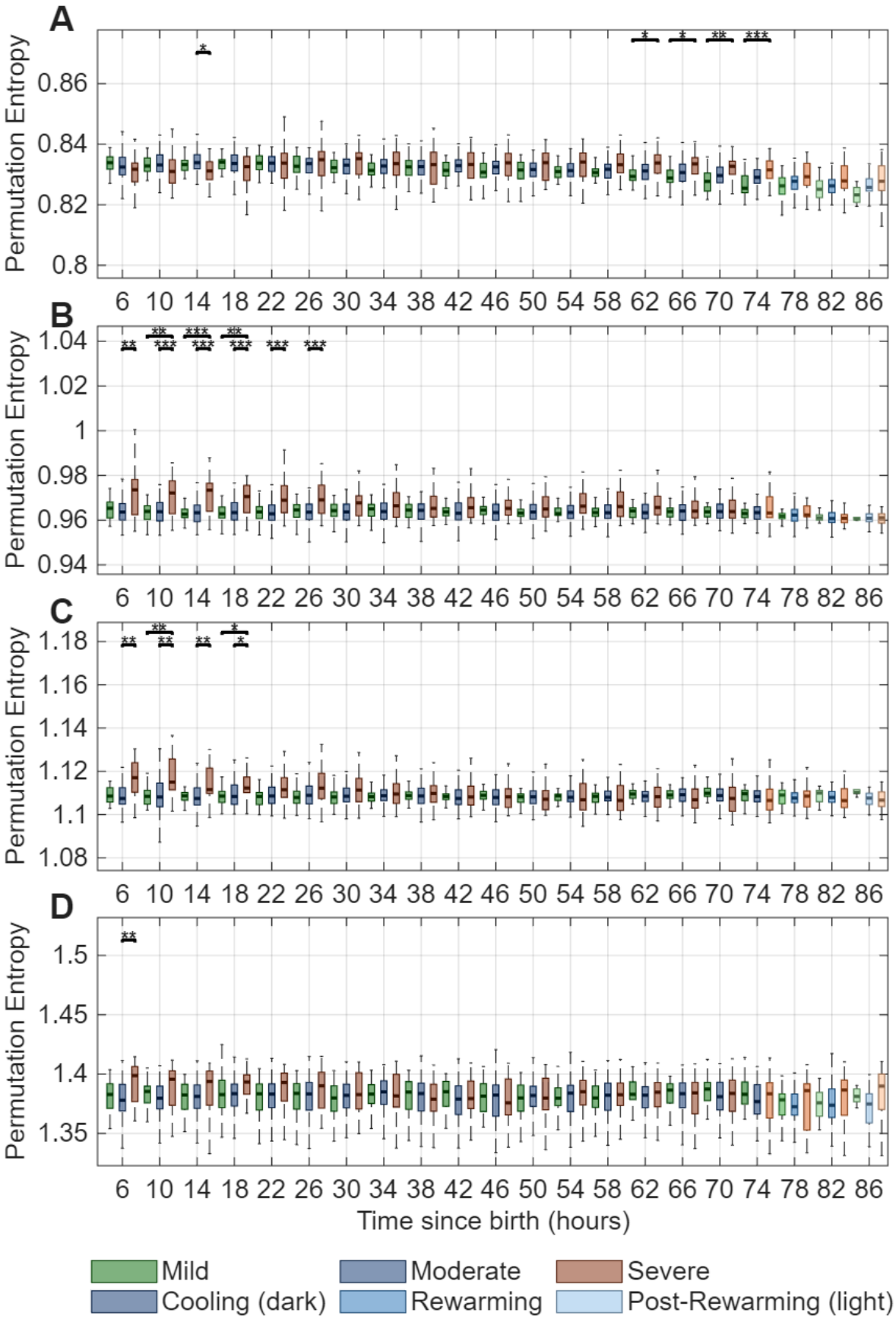
A
B
C
D
Permutation Entropy
0.86
0.84
0.82
0.8
1.04
1.02
1
0.98
0.96
0.94
1.18
1.16
1.14
1.12
1.1
1.08
1.5
1.45
1.4
1.35
6 10 14 18 22 26 30 34 38 42 46 50 54 58 62 66 70 74 78 82 86
Time since birth (hours)
Mild
Moderate
Severe
Cooling (dark)
Rewarming
Post-Rewarming (light)

Figure 19: Frequency band specific permutation entropy results for mild, moderate, and severe NE groups over 6-90 hrs postnatal.
A, B, C, and D show the results for permutation entropy in the delta, theta, alpha and beta frequency bands, respectively. Within each two-hour time bin, the median of each metric across all channels (Fp1, Fp2, T3, T4, C3, C4, O1, O2 and Cz) and epochs was calculated. These two-hour epochs were then pooled into four-hour time bins for visualization. The middle line in the boxes represents the median value, while the upper and lower limits of the boxes represent the IQR. The statistical significance test was a Kruskal-Wallis test. Pairwise comparisons were then performed using Dunn-Sidak correction to determine which pairs of groups differed significantly, followed by FDR correction using the Benjamini & Yekutieli (2001) method [39]. The brackets and stars at the top of the plots represent a statistically significant result for that 2-hour time bin. One star (*) represents $p < 0.05$, two stars (**) represent $p < 0.01$ and three stars (***) represent $p < 0.001$. The upper bracket represents differences between the mild and severe groups while the lower bracket represents differences between the moderate and severe groups. The color of the box plots changes to lighter shades when a majority of the epochs move from the cooling phase to the rewarming phase and then from the rewarming phase to the post-rewarming phase (normothermia). Abbreviations: PSD = power spectral density, NE = neonatal encephalopathy, IQR = interquartile range, FDR = false discovery rate.

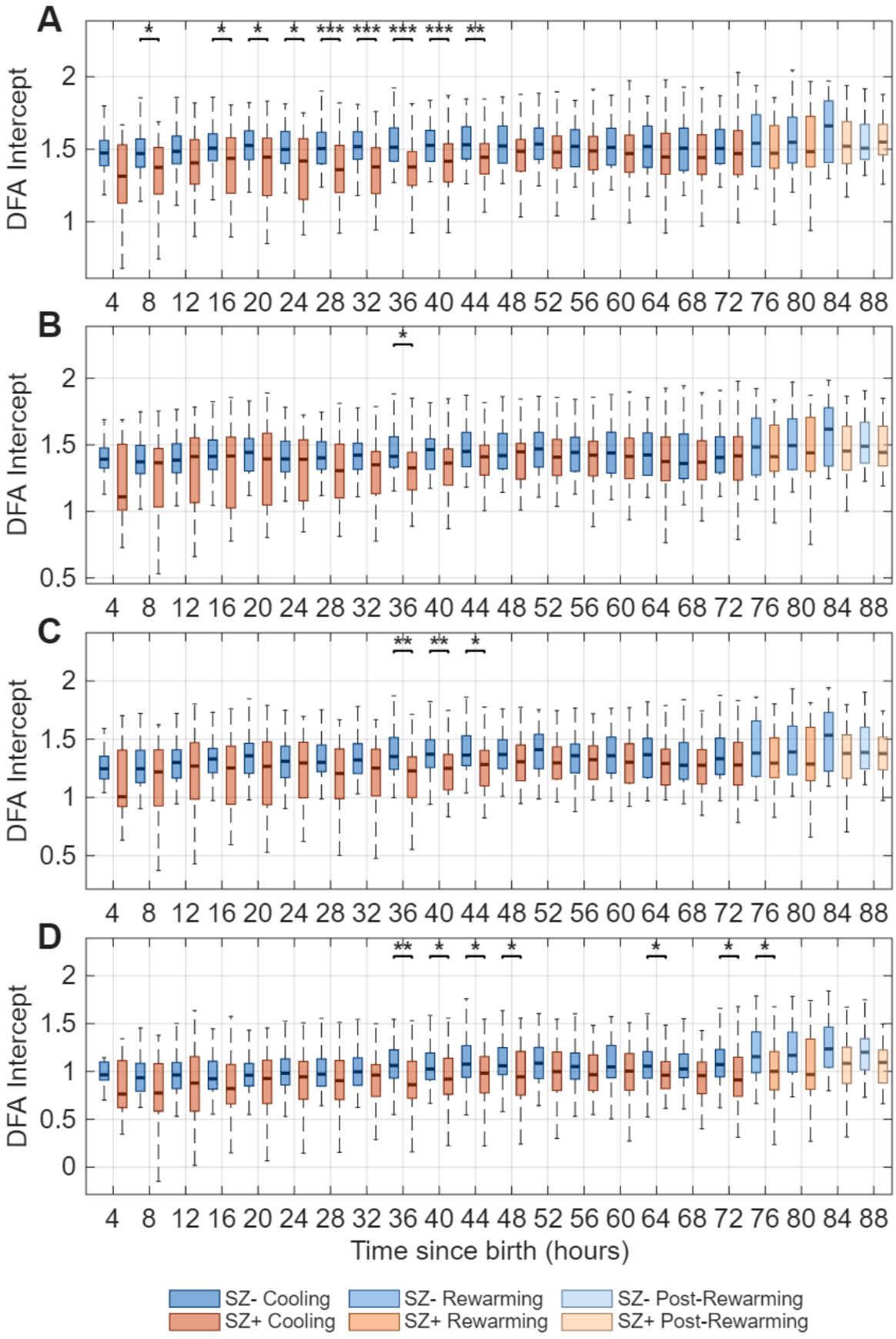
A
B
C
D
DFA Intercept
Time since birth (hours)
SZ- Cooling
SZ+ Cooling
SZ- Rewarming
SZ+ Rewarming
SZ- Post-Rewarming
SZ+ Post-Rewarming

Figure 20: Frequency band specific DFA intercept results for SZ+ and SZ- groups over 6-90 hours postnatal.

A, B, C, and D show the results for the DFA intercept in the delta, theta, alpha and beta frequency bands, respectively. Within each two-hour time bin, the median of each metric across all channels (Fp1, Fp2, T3, T4, C3, C4, O1, O2 and Cz) and epochs was calculated. These two-hour epochs were then pooled into four-hour time bins for visualization. The middle line in the boxes represents the median value, while the upper and lower limits of the boxes represent the IQR. The statistical significance test was the two-sided Wilcoxon rank sum test followed by FDR correction using the Benjamini & Yekutieli (2001) method [39]. The brackets and stars at the top of the plots represent a statistically significant result for that 2-hour time bin. One star (*) represents $p < 0.05$, two stars (**) represent $p < 0.01$ and three stars (***) represent $p < 0.001$. The color of the box plots changes to lighter shades when a majority of the epochs move from the cooling phase to the rewarming phase and then from the rewarming phase to the post-rewarming phase (normothermia). Abbreviations: SZ+ = neonates exhibiting electrographic or electroclinical seizures, SZ- = neonates not exhibiting seizures, IQR = interquartile range, DFA = detrended fluctuation analysis, FDR = false discovery rate.

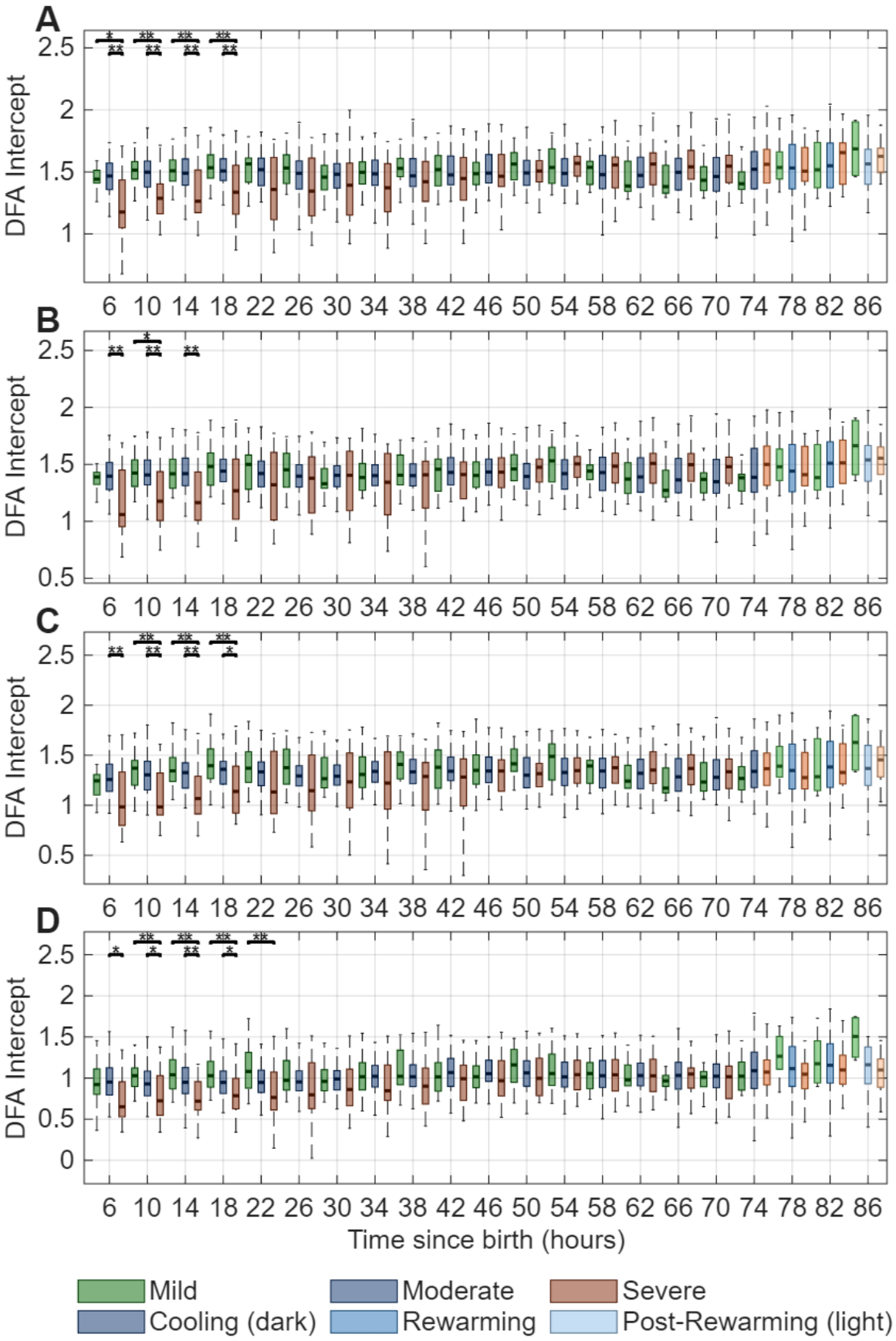
A
B
C
D
DFA Intercept
Time since birth (hours)
Mild
Moderate
Severe
Cooling (dark)
Rewarming
Post-Rewarming (light)

Figure 21: Frequency band specific DFA intercept results for mild, moderate, and severe NE groups over 6-90 hours postnatal.
A, B, C, and D show the results for DFA intercept in the delta, theta, alpha and beta frequency bands, respectively. Within each two-hour time bin, the median of each metric across all channels (Fp1, Fp2, T3, T4, C3, C4, O1, O2 and Cz) and epochs was calculated. These two-hour epochs were then pooled into four-hour time bins for visualization. The middle line in the boxes represents the median value, while the upper and lower limits of the boxes represent the IQR. The statistical significance test was a Kruskal-Wallis test. Pairwise comparisons were then performed using Dunn-Sidak correction to determine which pairs of groups differed significantly, followed by FDR correction using the Benjamini & Yekutieli (2001) method [39]. The brackets and stars at the top of the plots represent a statistically significant result for that 2-hour time bin. One star (*) represents $p < 0.05$, two stars (**) represent $p < 0.01$ and three stars (***) represent $p < 0.001$. The upper bracket represents differences between the mild and severe groups while the lower bracket represents differences between the moderate and severe groups. The color of the box plots changes to lighter shades when a majority of the epochs move from the cooling phase to the rewarming phase and then from the rewarming phase to the post-rewarming phase (normothermia). Abbreviations: PSD = power spectral density, NE = neonatal encephalopathy, IQR = interquartile range, FDR = false discovery rate.

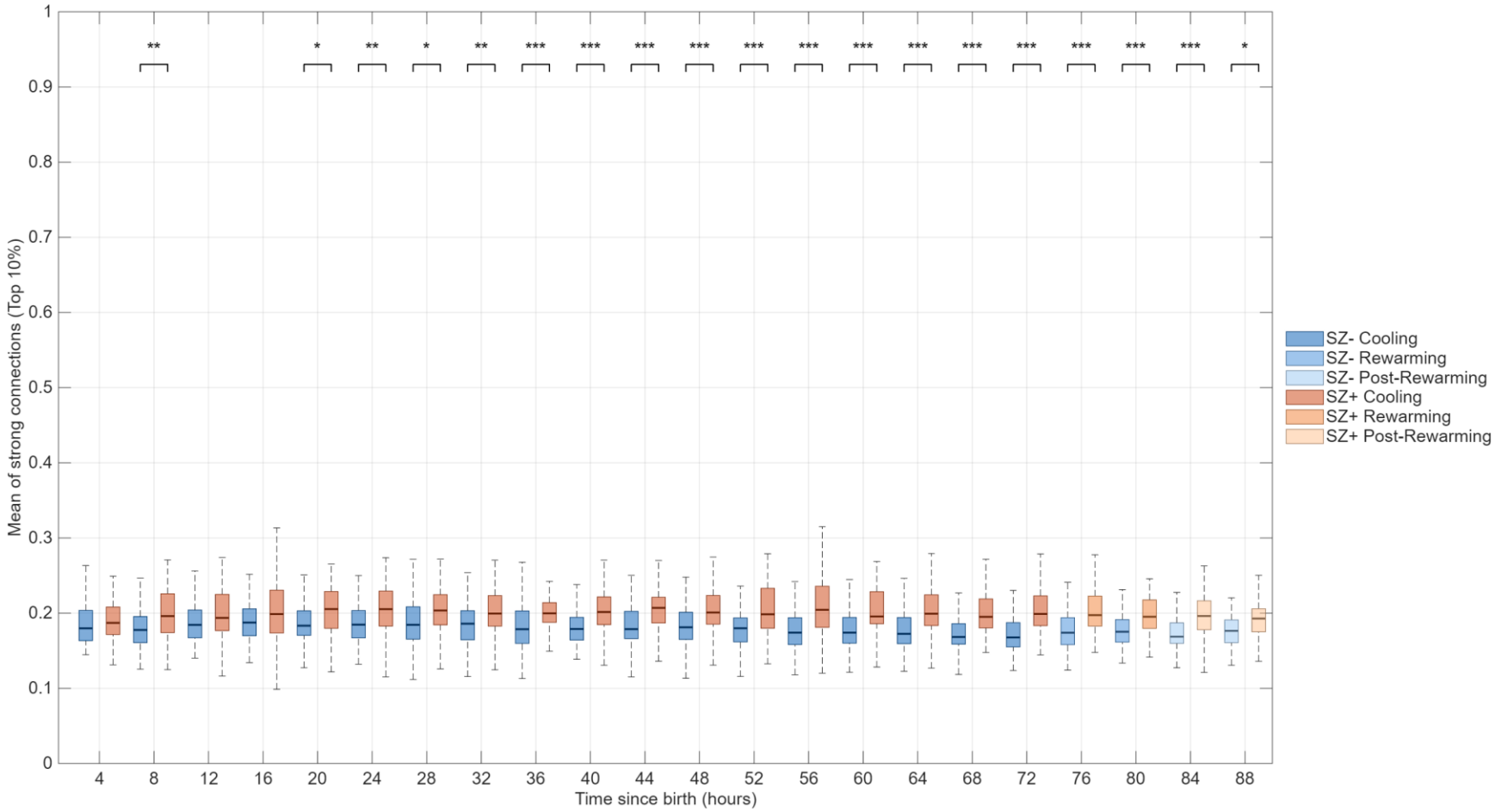

Figure 22: Mean connectivity (top 10%) for SZ+ and SZ- groups over 6-90 hrs postnatal.
Within each two-hour time bin, the median of each metric across all channels (Fp1, Fp2, T3, T4, C3, C4, O1, O2 and Cz) and epochs was calculated. The middle line in the boxes represents the median value, while the upper and lower limits of the boxes represent the IQR. The brackets and stars at the top of the plots represent a statistically significant result for that 2-hour time bin. One star (*) represents $p < 0.05$, two stars (**) represent $p < 0.01$ and three stars (***) represent $p < 0.001$. The statistical significance test was the two-sided Wilcoxon rank sum test followed by FDR correction using the Benjamini & Yekutieli (2001) method [39]. The color of the box plots changes to lighter shades when a majority of the epochs move from the cooling phase to the rewarming phase and then from the rewarming phase to the post-rewarming phase

(normothermia). Abbreviations: SZ+ = neonates exhibiting electrographic or electroclinical seizures, SZ- = neonates not exhibiting seizures, IQR = interquartile range, FDR = false discovery rate.

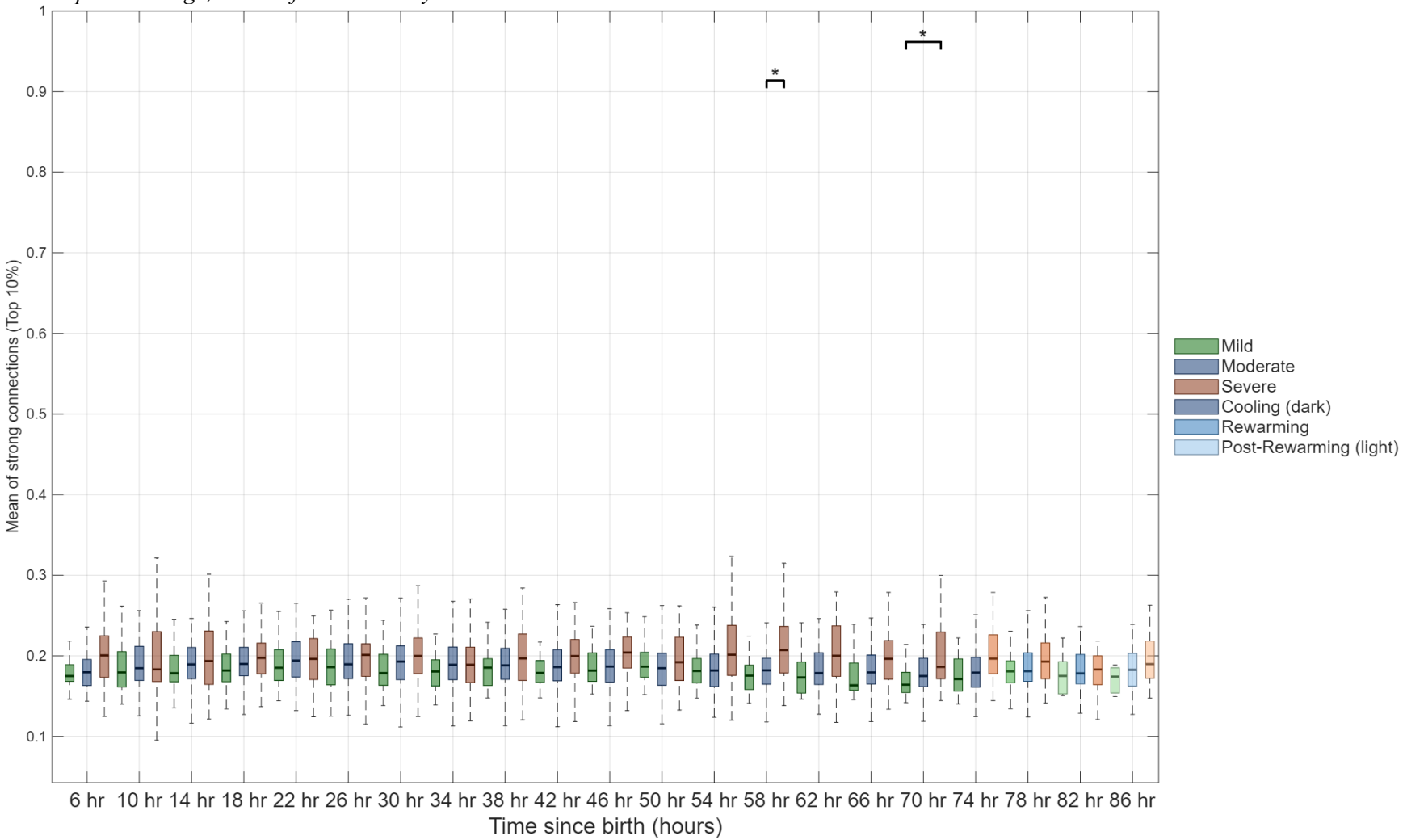

Figure 23: Mean connectivity (top 10%) for mild, moderate, and severe NE groups over 6-90 hrs postnatal.
Within each two-hour time bin, the median of each metric across all channels (Fp1, Fp2, T3, T4, C3, C4, O1, O2 and Cz) and epochs was calculated. These two-hour epochs were then pooled into four-hour time bins for visualization. The middle line in the boxes represents the median

value, while the upper and lower limits of the boxes represent the IQR. The statistical significance test was a Kruskal-Wallis test. Pairwise comparisons were then performed using Dunn-Sidak correction to determine which pairs of groups differed significantly, followed by FDR correction using the Benjamini & Yekutieli (2001) method [39]. The brackets and stars at the top of the plots represent a statistically significant result for that 2-hour time bin. One star (*) represents $p < 0.05$, two stars (**) represent $p < 0.01$ and three stars (***) represent $p < 0.001$. The upper bracket represents differences between the mild and severe groups; the lowest bracket represents differences between the moderate and severe groups. The color of the box plots changes to lighter shades when a majority of the epochs move from the cooling phase to the rewarming phase and then from the rewarming phase to the post-rewarming phase (normothermia). Abbreviations: NE = neonatal encephalopathy, IQR = interquartile range, FDR = false discovery rate.

APPENDIX B: PRISMA

The literature search for the review summary in Table 1 was documented following PRISMA guidelines [41]. The platform used for the search was PubMed and the search terms were as follows: “EEG power neonates hie”, “EEG entropy neonates hie”, and “EEG DFA neonates”. No other limits were used in the search. The search was originally performed in June 2026 and was re-created on August 28th, 2026. A manual review process was used to determine if there were any duplicates in the list of reports. The PRISMA 2020 flow diagram (Figure 24) and the checklist (Table 3) were completed.

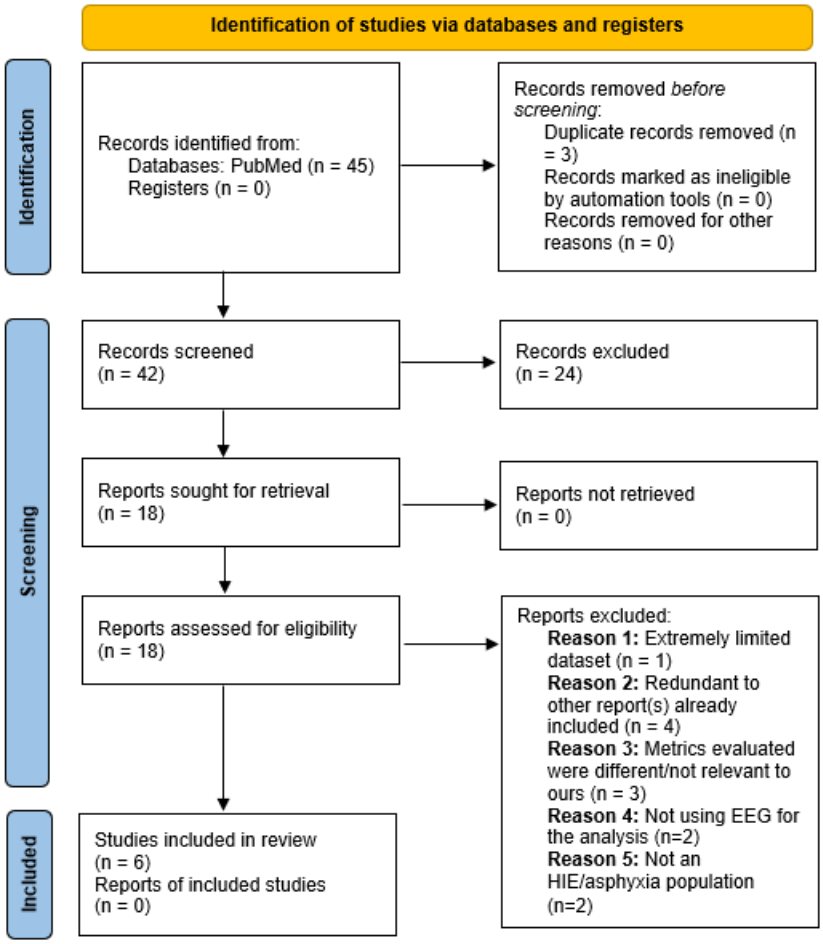

Figure 24: PRISMA 2020 flow diagram for literature summary in Table 1

Table 3: PRISMA checklist for literature summary in Table 1

Section/topic	#	Checklist item	Location(s) Reported
INFORMATION SOURCES AND METHODS			
Database name	1	Name each individual database searched, stating the platform for each.	Appendix B
Multi-database searching	2	If databases were searched simultaneously on a single platform, state the name of the platform, listing all of the databases searched.	N/A
Study registries	3	List any study registries searched.	N/A
Online resources and browsing	4	Describe any online or print source purposefully searched or browsed (e.g., tables of contents, print conference proceedings, web sites), and how this was done.	N/A
Citation searching	5	Indicate whether cited references or citing references were examined, and describe any methods used for locating cited/citing references (e.g., browsing reference lists, using a citation index, setting up email alerts for references citing included studies).	N/A
Contacts	6	Indicate whether additional studies or data were sought by contacting authors, experts, manufacturers, or others.	N/A
Other methods	7	Describe any additional information sources or search methods used.	N/A
SEARCH STRATEGIES			
Full search strategies	8	Include the search strategies for each database and information source, copied and pasted exactly as run.	Appendix B
Limits and restrictions	9	Specify that no limits were used, or describe any limits or restrictions applied to a search (e.g., date or time period, language, study design) and provide justification for their use.	Appendix B
Search filters	10	Indicate whether published search filters were used (as originally designed or modified), and if so, cite the filter(s) used.	N/A
Prior work	11	Indicate when search strategies from other literature reviews were adapted or reused for a substantive part or all of the search, citing the previous review(s).	N/A
Updates	12	Report the methods used to update the search(es) (e.g., rerunning searches, email alerts).	N/A
Dates of searches	13	For each search strategy, provide the date when the last search occurred.	Appendix B
PEER REVIEW			
Peer review	14	Describe any search peer review process.	N/A
MANAGING RECORDS			
Total Records	15	Document the total number of records identified from each database and other information sources.	Appendix B
Deduplication	16	Describe the processes and any software used to deduplicate records from multiple database searches and other information sources.	Appendix B