Temporal dynamics and maturation of resting-state activity in preterm infants

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Abstract:

By interfering with the normal sequence of mechanisms serving the brain maturation, premature birth and related stress can alter perinatal experiences, with potential long-term consequences on a child's neurodevelopment. The early characterization of brain functioning and maturational changes is thus of critical interest in premature infants who are at high risk of atypical outcomes and could benefit from early diagnosis and dedicated interventions. Using high-density electroencephalography (HD-EEG), we recorded resting-state brain activity in extreme and very preterm infants at the equivalent age of pregnancy term (n=43), and longitudinally 2-months later (n=33), compared with full-term born infants (n=14). We characterized the maturation of brain activity by using microstate analysis (a method to quantify the spatiotemporal dynamics of the spontaneous transient network activity) while controlling for vigilance states. The comparison of premature and full-term infants first showed slower dynamics as well as altered spatio-temporal properties of resting-state activity in preterm infants. Maturation of functional networks between term-equivalent age and 2 months later in preterms was translated by the emergence of richer dynamics, manifested in part by faster temporal activity (shorter duration of microstates) as well as an evolution in the spatial organization of the dominant microstates. The inter-individual differences in the temporal dynamics of brain activity at term-equivalent age were further impacted by gestational age at birth and sex (with slower microstate dynamics in infants with lower birth age and in boys) but not by other considered risk factors. This study highlights the potential of the microstate approach to reveal maturational properties of the emerging resting-state network activity in premature infants.

Key words:

Electroencephalography (EEG), Resting-state, Microstates, Prematurity, Brain development

Abbreviations:

BOLD: blood oxygenation level dependent

(m)CA: (months of) corrected age

(HD-)EEG: (high-density-) electroencephalography

(w)GA: (weeks of) gestational age

(f)MRI: (functional) magnetic resonance imaging

M / F: male / female

MS: microstate

(w)PMA: (weeks of) post-menstrual age

TEA: term equivalent age

1. Introduction:

The last trimester of pregnancy is a critical phase of brain development, with vast growth of long-distance axonal connectivity, starting myelination in a few white matter regions, rapid expansion of the cortex, synaptogenesis, and dendritic formation (Kostović & Judaš, 2015). Pre- and perinatal insults such as preterm birth might impair the progression of these mechanisms, and potentially disrupt the whole trajectory of neurodevelopment, interfering with post-natal acquisitions leading to long-term consequences including lower cognitive, motor and behavioral outcomes (Arpi & Ferrari., 2013; Bhutta et al., 2002). In particular, infants born before 32 weeks of gestational age (GA) are at a higher risk of adverse neurodevelopmental outcomes (Marlow et al., 2005; Pierrat et al., 2017;2021).

Magnetic Resonance Imaging (MRI) investigations have associated prematurity with alterations in anatomical maturation of the brain, at the macro and microstructural scales, when comparing preterm infants at the term-equivalent age (TEA) with full-term neonates. These studies have described differences in cortical growth (Ajayi-Obe et al., 2000; Thompson et al., 2019), gyrification (Dubois et al., 2019; Engelhardt et al., 2015), microstructural organization (Bouyssi-Kobar et al., 2018; Dimitrova et al., 2021; Gondova et al.,2023), and white matter connectivity (Ball et al.,2014; Neumane et al. ,2022), that could persist into childhood (e.g. Kelly et al, 2023).

However, the functional correlates of such anatomical alterations are still poorly understood despite the common impairments observed at the behavioral level in children born preterm. A few neuroimaging techniques are available in vulnerable premature infants to characterize such functional characteristics at the cerebral level. Notably, in the past decade functional MRI (fMRI) has allowed identifying precursors of spatially organized functional brain networks in preterms, which show reduced maturation in terms of long-range connectivity compared to full-terms (Doria et al., 2010) and alterations of network-level properties (Fenn-Moltu et al., 2023) that persist until term-equivalent age (Gozdas et al., 2018) and childhood (Wehrle et al., 2018). Yet, understanding how these alterations affect the efficiency of brain networks for processing environmental information requires investigating the dynamic aspects of

the brain activity that go beyond the temporal resolution of the Blood Oxygenation Level Dependent (BOLD) effect captured by fMRI.

While limited in spatial resolution, Electroencephalography (EEG) can complement this picture by offering insights into the fast dynamics of the brain activity and by being easily implementable in vulnerable infants. Previous EEG investigations of preterms have indeed identified differences in the occurrence of discontinuous high-amplitude bursts (Whitehead et al., 2018), in activity synchronization (Yrjölä et al., 2020), spectral density (Guyer et al., 2019), and in the latency (Kaminska et al., 2017) and scalp distribution of sensory-evoked responses (Maître et al. ,2017; Whitehead et al., 2019), with aspects of these alterations persisting into term equivalent age and beyond (Guyer et al., 2019). These investigations indicate that several dimensions of the brain activity such as temporal, scalp distribution, amplitude or signal strength, and the frequency contents can be impacted by prematurity. Nevertheless, methods such as spectral analysis inspect the brain activity over several seconds, limiting their direct interpretation of the temporal properties of brain dynamics. Clinical evaluations also involve assessments of sleep structure and maturational figures in EEG over relatively long timescales (Dereymaeker et al., 2017), while the brain activity experiences fast changes in its functional organization (Baker et al., 2014), supporting development and learning during sleep (Li et al., 2017). These encourage the use of methods that capture multidimensional information about the brain activity that also remain sensitive to the temporal aspects of the fast brain dynamics, in order to provide reliable markers of functional alterations in preterm infants.

Initially proposed for investigating adult brain activity (Lehmann et al., 1987; Michel & Koenig, NeuroImage, 2018), microstate analysis aims at identifying short time-segments in the continuous recordings, where the scalp topography (i.e. the distribution of electric potentials originating from the brain activity over the scalp) remains quasi-stable before transitioning into a new scalp topography. Each of these transient topographies are defined as a microstate (MS), representing the spontaneously occurring transient network activity. Microstates are thus sensitive to both temporal and scalp topographical dynamics of the brain activity.

Recently, the interest in using the microstate approach in infants has emerged, while eliciting various methodological questions (Bagdasarov et al., 2024; Brown et al., 2024; Khazaei et al., 2021). But studies in neonates are rare, focusing on the impact of sleep states on brain dynamics (Khazaei et al., 2021) and on habituation mechanisms to pain (Rupawala et al., 2023), with recent reports indicating MS dynamics change in the preterm period towards the term-age (Hermans et al., 2023). In particular, the period of the first months after the term birth remains unexplored with microstate approach so far, whereas maturational processes including myelination and growth of short cortico-cortical connections show intense progression that potentially relates to accelerated brain functional dynamics and evolution of network spatial distributions. High-density EEG (HD-EEG) recordings might then benefit the characterization of spatio-temporal properties of the brain activity, to better reflect the anatomo-functional changes in the early developmental period.

In addition to investigating brain dynamics in the first months after term birth, the potential impact of perinatal risk factors such as prematurity on MS-related characteristics still needs to be understood given the emerging evidence for the early onset of cerebral atypicalities in preterm-born infants. A better understanding of the heterogeneity of atypicalities (Dimitrova et al., 2020) also requires considering developmental trajectories over the early postnatal

period of intense neuroplasticity, when interventions building on compensatory mechanisms might improve the neurodevelopmental outcomes (Livingston & Happe, 2017). All this raises interest in implementing reliable early diagnostic markers in preterms and calls for longitudinal assessments (as opposed to single time evaluations) in order to also evaluate developmental changes and potential improvements.

In this study, we thus aimed to characterize the functional maturation of brain activity dynamics in high-risk preterm infants using HD-EEG recordings. We used the microstate approach to study the maturation of neurophysiological activity between two time-points during development: at term-equivalent age and two months later. First, we compared preterm and full-term born infants in order to identify how the spatio-temporal properties of brain dynamics are impacted by prematurity, while controlling for vigilance states. Next, we implemented a dedicated approach to reliably characterize and compare the organization of resting-state activity in preterms across the two ages. Then, we evaluated how the metrics describing the dynamics of such a functional organization mature over two months after TEA, and investigated to which extent the interindividual variability in these dynamics are related to perinatal clinical risk factors.

2. Methods:

The study design and procedures (protocol DEVine, CEA 100 054) was reviewed and approved by the ethics committee (Comité de Protection des Personnes, CPP Ile de France 3) and parents were informed about the study and gave written consent to participate in the study with their babies.

2.1. Participants:

The preterm cohort included a group of 43 extreme and very preterm infants (mean gestational age GA at birth: 27.1±1.7 weeks, range: [24.1-30.9] weeks) who were tested longitudinally at two time points : First, at the term-equivalent age (0 months Corrected Age - 0mCA : 41±0.8 weeks of post-menstrual age - w PMA, range: [38.4-42.3] weeks; 20 females, 23 males), and next at 2 months corrected age (2mCA: 50.5±0.7w PMA, range: [49.1-52] weeks; 16 females, 17 males, leading to 33 infants at 2mCA since 10 over 43 did not return for the second exam). To favor a relatively homogeneous group, two additional infants with EEG data were not included in the study analyses due to lack of clinical examination data and presence of severe brain injury identified on MRI.

Table 1 summarizes the neonatal characteristics of the preterm infants (see Supplementary information, SI Table 1 for the details of individual characteristics). Based on previous studies, we selected the clinical factors supposed to play a key role on the neurodevelopment of premature neonates, including GA at birth (considering three GA groups: [24w-26w],]26w-28],]28w,32w]), sex, birth weight (correcting for GA and considering values below the 10th percentile as a marker of intrauterine growth restriction) as well as non-neurological complications during the NICU period: chronic lung disease, need for mechanical ventilatory support / oxygen therapy for more than 21 days, necrotizing enterocolitis, need for parenteral nutrition longer than 3 weeks, and experiencing sepsis (Brouwer et al., 2017; Neumane et al., 2022). From these last 5 factors, we derived a binary factor of neonatal morbidities associated with prematurity, to summarize the presence of at least one of them (or the absence of all five).

All preterm infants also had an MRI at term-equivalent age which allowed us to measure the Kidokoro score, summarizing the brain development and regional abnormalities (Kidokoro et al., 2013). Kidokoro total scores (for the whole brain) were binarized to indicate the presence of any abnormality (scores <= 3 considered as "normal" vs scores >=4 relating to "mild" abnormalities; for the analyses, we did not consider any infant with score >=8 corresponding to moderate and severe abnormalities, in order to favor a relatively homogeneous group).

The full-term cohort included a group of 14 infants (mean gestational age at birth: 40.1±1.1 weeks, range: [37.6-41.6] weeks) who were tested first a few days/weeks after birth (mean age at test : 41.6±1.1w PMA, range: [39.7-43.3] weeks; 7 females, 7 males) and 2 months later (mean age at test : 50.1±0.9w PMA, range: [48.9-51.9] weeks; 7 females, 6 males, leading to 13 infants at 2mCA. since 1 over 14 infants did not return for the second exam). The preterm and full-term cohorts were not statistically different in terms of sex and corrected age at EEG examinations.

2.2. EEG recording

High-density 128-channel Electroencephalography (HD-EEG, EGI(R)) were recorded during rest while infants were staying calm in a baby cot, or occasionally in the parent's arms when it was easier to keep the infant rested, for approximately 10min. Sampling rate of the recordings was 1000 Hz. A camera recorded the infant's behavior. The experimenter marked the approximate awake/sleep (eye movement) state of infants during the recording. These served the later expert evaluation for sleep scoring (see below).

2.3. Preprocessing

EEG recordings were first bandpass filtered between [0.2-45] Hz and then preprocessed using an automated pipeline for infants' continuous EEG (APICE) (Flo et al., 2022). In brief, preprocessing consisted of detecting artifacts on the continuous data where "bad times" and "bad channels" were first defined. Bad times were identified as those with more than 30% of the rejected channels and lasting at least 100 ms. Bad channels were those presenting artifacts during more than 30% of the good times. Artifacts were corrected using target principal component analysis on segments shorter than 100 ms and spatial spherical spline to interpolate bad channels. Finally, artifacts were detected again, and bad times and channels were re-defined. Because the preprocessing was not perfect, recordings were also visually inspected after this step, and obvious remaining artifacts were marked manually. At the end of this step, segments shorter than 8 seconds were rejected from further analyses.

All EEG recordings were down-sampled to 250 Hz and re-referenced to the common-average montage before further processing.

2.4. Sleep scoring

For assessing the vigilance state, recordings were first transformed into a low-density bipolar montage as in the standard clinical settings. Different vigilance states were then scored by an expert in neonatal electrophysiology. At 0mCA, the recordings were categorized into awake, active (continuous) and quiet (noncontinuous) sleep. At 2mCA, they were categorized into awake, stage1 and stage2 sleep. At 0mCA, quiet sleep was determined mainly based on the detection of discontinuous traces, while active sleep was recognized by continuous traces as well as the

presence of maturative figures such as frontal sharp waves. At 2mCA, stage2 was determined mainly by the presence of sleep spindles, while stage1 was classified by the presence of slow-wave activity. Awake activity was similar to that of active sleep/stage1, but was distinguished based on the infant behavior (using camera recording information) as well as the presence of more abundant movement-related activity. After sleep scoring and preprocessing, we recovered on average 2.6/6.5/3 minutes of recordings for awake/active sleep/quiet sleep at 0mCA per infant, and 4.5/5.7/4.5 minutes of recordings for awake/stage1 sleep/stage2 sleep at 2mCA per infant. Since sleep staging is inherently different for the two age groups, we only considered comparable states for further age group comparisons (see below): active sleep at 0mCA vs sleep stage1 at 2mCA, or quiet sleep at 0mCA vs sleep stage2 at 2mCA.

2.5. Microstate analysis

Microstate analysis was performed using the Microstate EEGlab toolbox (Poulsen et al., 2018), through the following steps: For each subject, 1000 random EEG samples (topographical maps) were taken from the global field power maxima. These EEG samples were then concatenated across all subjects and partitioned into a fixed number of microstate classes using modified K-means clustering (Pascual-Marqui et al. 1995) without differentiating opposite polarity topographies (polarity invariance). This allowed identifying the microstate classes (i.e. template microstates) at the group-level. To decide on the final number of microstate classes, we estimated how well the microstate templates fitted the data by varying the number of microstate classes between 3 and 15. We measured the global explained variance in the data as a function of the number of template microstates. As described in section 3.1 (Figure 1.a), we selected 7 microstate classes as the gain in the explained variance was less than 1 % when more microstate classes were considered. This number was also in agreement with a previous study describing 7 microstates in newborns (Khazaei et al., 2021), allowing to identify comparable measures.

The first analysis aimed at comparing MS characteristics between the preterm and full-term groups in order to identify the potential main effect of prematurity on MS development. To do so, we focused on identifying the group-level template microstates across both preterm and full-term infants, separately for each vigilance state and each age group (0mCA: active sleep/quiet sleep; 2mCA: stage1/stage2; we did not include awake state in this analysis since very few infants in the full-term group had adequate data for this condition). Identifying the template microstates across all infants required accounting for the imbalanced number of subjects in the two groups (more infants in the preterm than the full-term group), in order to avoid biasing the measures to one group. This was achieved by creating five subsets of preterms, where each subset had the same number of infants as the full-term group, including infants from various GA at birth, allowing repetitions across subsets while ensuring that each infant appeared at least in one subset. We iteratively identified the 7 template microstates for each of the five preterm subsets completed by the full-term neonates, and the final set of template microstates was obtained by averaging the template microstates across the five iterations.

In a second step, analyses focused on the whole preterm group alone in order to evaluate the impact of clinical factors related to prematurity as well as maturational changes between 0 and 2mCA in preterms. The 7 template microstates were identified at the preterm group-level, separately for each vigilance state and each age.

The procedures above identified group-level template microstates for each of the planned analyses, which were then projected back into each individual infant recording while using a 30 ms temporal smoothing. This allowed parsing the continuous EEG recordings of each individual infant into a sequence of microstates, which in turn provided statistical metrics describing the microstate dynamics. These metrics included duration (the average life-time of a microstate when activated), occurrence (the frequency of appearance per second), coverage (the fraction of recording time a given microstate is active), explained variance (the similarity of EEG samples to a given microstates). Only results of the analyses for MS duration and explained variance are presented in the main manuscript, while other metrics are presented in Supplementary Information. We also considered transition probabilities between different microstates, measuring how frequently one microstate is followed by another microstate.

2.6. Statistical analyses:

2.6.1. Comparing microstates between the preterm and full-term infants: Impact of prematurity

We first evaluated whether the microstates approach is sensitive enough for capturing the effects of prematurity on the brain activity dynamics. To this end, we compared the metrics of microstate dynamics between preterm and full-term infants separately for each age group and sleep state by conducting Analyses of Variance (ANOVA) with each microstate metric (duration/explained variance/coverage/occurrence) as dependent variable, group (preterm/full-term) as between-subject factor, microstate (MS 1 to 7) as within-subject factor and the two-way interaction group x microstate. This involved conducting 4 ANOVAs per metric (2 age group, 2 sleep states since wakefulness was not considered).

2.6.2. Comparing microstates between 0mCA and 2mCA: Maturation of brain dynamics in preterms

We assessed how microstates change with maturation between 0 and 2mCA, focusing on the preterm group because the results from previous analysis suggested group differences, and the reduced number of full-term infants limited analyses on this single group which was not the main scope of this study focused on prematurity. We first compared the two ages at the level of template microstates identified for each preterm group (0 / 2mCA) and by measuring their similarity in terms of spatial distribution (Figure 2.a.b). This was achieved by computing the spatial correlation, i.e. Pearson correlation between the vectors of activity values across channels, between the template microstates of the two age groups. This resulted in a correlation matrix, where each value indicated the similarity index between a pair of microstates of the two age-groups. To set a threshold for determining similarity of microstates as high or low, we also calculated the spatial correlation between the template microstates within each age group, reasoning that the maximum value of within-group spatial correlations can be considered as the highest spatial similarity between independent states, below which microstates can be considered as independent. Based on this value, we thresholded the two-age-correlation matrix and merged the template microstates with high-similarity by averaging them. This resulted in a set of averaged templates, shared between the two age groups, in addition to age-specific templates corresponding to the remaining templates with low similarity between the two ages (Figure 2.c). As indicated in the results section, this analysis identified microstates shared between two age groups: 5 for active sleep/stage1, 6 for quiet sleep/stage2 and 5 for wakefulness. When projecting back the template

microstates to each individual infant, we used the shared and age-specific template microstates for each age group (Figure 2.c.d).

This approach allowed us to compare microstate metrics between the two age groups, focusing on the shared microstates between the two age groups. Again, we conducted an Analysis of Variance (ANOVA) for each of the four metrics as dependent variable, age group (0mCA/2mCA) as between-subject factor, microstate as within-subject factor, and the two-way interaction age group x microstate. This involved conducting 3 ANOVAs per metric (3 vigilance states).

Note that few infants had longitudinal data for the same vigilance state: only for active sleep/stage1, an adequate number of infants with longitudinal data (n=21) allowed us to run an additional ANOVA with age group as within-subject factor (i.e. repeated measure) to evaluate whether the results were consistent with the previous analysis with age group as between-subject factor.

Besides, to investigate whether there is a structure in the successive activation of microstates, we quantified the transition probabilities of microstates, indicating how frequently microstates of a certain class are followed by microstates of other classes (Transition probability of X⇒Y: number of times X is followed by Y divided by the number of total transitions). Since transition probabilities between microstates also depend on the base coverage rate of each microstate, we divided (i.e. normalized) the transition probabilities by the coverage rate of the 'destination' microstates (i.e. the microstate that the transition goes into). To assess whether each microstate has favorable microstates to transition into at each age, we compared the normalized transition probability between a pair of microstates (i.e. an initial microstate and a destination microstate) and the alternative transitions from the initial microstate using paired t-tests: the transition was considered favorable if higher than at least half of the alternative transitions. To have comparable results between the two ages and evaluate whether such transitioning patterns change with development, we focused on the shared microstates between the two age-groups for each vigilance state. And we corrected t-test statistics for multiple comparisons using the False Discovery Rate (FDR) approach.

2.6.3. Relating microstates at 0mCA and their maturation between 0-2mCA to clinical factors in preterms

To determine how neonatal characteristics at birth and clinical factors during the perinatal period might impact brain maturation, we finally explored the relationships between microstates metrics and the 5 following prematurity risk factors: 1) groups of GA at birth ([24-26] /]26-28] /]28-32]w), 2) sex (males/females), 3) binarized risk of intrauterine growth restriction (yes/no), 4) binarized Kidokoro score (mild/normal) and 5) binarized neonatal morbidity factor (at least one of the 5 identified non-neurological complications / none). To maximize the statistical power and the number of infants, this analysis focused on the conditions of active sleep at 0mCA (n=41) comparable to stage1 sleep at 2mCA (n=22). Among all microstate metrics we focused on duration to facilitate the interpretability of the directions of effects, given previous reports suggested shorter latency of brain responses relate to advances maturation following myelination of pathways in early infancy (Dubois et al, 2008).

A first ANOVA (n=41) assessed the relationship between the MS duration during active sleep at 0mCA (shared and age-specific microstates) as dependent variable, the 5 risk factors as independent between-subject factors, and microstate (microstate 1 to 7) as independent within-subject factor, as well as specified two-way interactions (microstate x each of the 5 factors, and GA group x sex; all other two-way interactions were not included because they involved few data points).

A second ANOVA (n=22) evaluated the relationship between the longitudinal difference in microstate duration from active sleep at 0mCA to stage1 sleep at 2mCA (focusing on shared microstates between the two ages) as dependent variable, risk factors as independent between-subject factors, microstate as independent within-subject factor as well as the two-way interactions microstate x GA group, microstate x sex, and GA group x sex. As risk factors, we here focused on GA group, sex and neonatal morbidity factor, thus not considering Kidokoro score and risk of intrauterine growth restriction, as well as other two-ways interactions because of the reduced number of subjects and the reduced inter-individual variability for these factors limiting reliable testing.

For all statistical tests, when effects were found to be significant, post hoc tests were carried out using t tests.

3. Results:

3.1. Identifying microstates at different vigilance states

In order to decide on the number of microstates, we first identified the microstates at 0mCA and 2mCA without separating the vigilance states in preterms. We incrementally increased the number of microstate classes between 3 and 15 and measured the global explained variance as a function of microstate classes. The gain in the global explained variance was less than 1 % when more than 7 microstate classes were considered (Figure 1.a). We therefore considered 7 microstates for the rest of the analyses and identified 7 template microstates per age and per vigilance states (see below). The global explained variance was overall higher at 0mCA compared to 2mCA ($p<0.001$, Figure 1.a) and increased with progression in sleep depth at both ages (Figure 1.b).

3.2. Comparing microstates between preterm and full-term infants: Impact of prematurity

Table 2 and SI Table2 summarize the results of statistical analyses on the differences in the microstate metrics between the preterm and full-term groups, at 0 and 2mCA and for different sleep states.

At 0mCA all microstate metrics during active sleep showed sensitivity to prematurity. For microstate duration, there was a main effect of group (preterm vs full-term, $p=0.041$) and microstate class ($p<0.001$) and an interaction between group and microstate class ($p=0.025$). For explained variance, coverage and occurrence, there was a main effect of microstate class (all $p<0.001$) and an interaction group x microstate class (Explained variance: $p=0.021$, Coverage: $p=0.005$, Occurrence: $p=0.004$) but no effect of group (all $p>0.1$). Post hoc tests showed that MS3 during active sleep was the most impacted by prematurity, with longer duration ($p=0.001$), higher explained variance ($p<0.001$), coverage ($p<0.001$) and occurrence ($p=0.018$) in preterms than full-terms.

All microstate metrics during quiet sleep at 0mCA showed sensitivity to prematurity as well. There was no significant main effect of group (all $p>0.1$) but a main effect of microstate class (all $p<0.001$) and an interaction between group and microstate class (Duration: $p=0.011$, Explained variance: $p<0.001$, Coverage: $p<0.001$, Occurrence: $p=0.037$). Post hoc tests revealed that MS2 during quiet sleep was the most impacted by prematurity, again with longer duration ($p=0.002$), higher explained variance ($p=0.006$), coverage ($p=0.005$) in preterms than full-terms (Figure 3).

At 2mCA, during stage1 sleep, explained variance, coverage and occurrence also showed sensitivity to prematurity, with a main effect of microstate class ($ps<0.001$) and an interaction between group and microstate class (Explained variance: $p = 0.067$, Coverage: $p =0.016$, Occurrence: $p<0.001$) but no significant main effect of the group (all $p>0.1$). Post hoc tests showed that MS3 during stage1 sleep was most consistently impacted by prematurity showing longer duration ($p=0.019$) and higher explained variance ($p<0.001$), coverage ($p<0.001$) and occurrence ($p=0.004$) in preterms compared to full-terms.

All microstate metrics showed sensitivity to prematurity during stage2 sleep at 2mCA as well (Figure 3). There was no significant main effect of group ($ps>0.1$) on these metrics but a main effect of microstate class ($ps<0.001$) and an interaction between group and microstate class (Duration: $p = 0.094$, Explained variance: $p = 0.027$, Coverage: $p =0.007$, Occurrence: $p<0.001$). Post hoc tests indicated that MS4 during stage2 sleep was the most impacted by prematurity: it had longer duration ($p=0.023$), higher explained variance ($p<0.0016$), coverage ($p=0.019$) and occurrence ($p=0.049$) in preterms vs full-terms.

3.3. Comparing microstates between 0mCA and 2mCA : Maturation of brain dynamics in preterms

The pipeline implemented to compare microstates characteristics at 0 and 2mCA in preterms is presented in Figure 2 as detailed in the methods section. Table 3 and SI Table 3 summarize the results of statistical analyses for the changes in the microstate metrics with development between 0 and 2mCA considering different vigilance states.

For active/stage1 sleep, five microstates out of 7 were shared between 0 and 2mCA and were included in the statistical analyses for age comparison (Table 3a). We observed a main effect of microstate class for all metrics and a main effect of age group for duration, coverage and occurrence, as well as an interaction between age group and microstate class for explained variance and occurrence. The post hoc tests indicated longer microstate durations ($t(1,61) = 7.4$, $p < 0.001$) and lower coverage ($t(1,61) = -2.1$, $p = 0.032$) at 0 compared to 2mCA. The post hoc investigation of interactions between age group and microstate class indicated that the explained variance increased for only one of the microstates (MS2) from 0 to 2mCA ($p=0.03$), and the occurrence increased for all microstates except one (MS4) from 0 to 2mCA ($p<0.001$) (Figure 4.a). When restricting infants to the subgroup of infants with longitudinal data both at 0 and 2mCA for active / stage1 sleep, results were replicated (Table 3b).

For quiet/stage2 sleep, six microstates out of 7 were shared between 0 and 2mCA and were included in the statistical analyses for age comparison (Table 3c). We observed a main effect of microstate class and an interaction between age group and microstate class for all metrics, as well as a main effect of age group for duration and occurrence and an interaction between age group and microstate class for explained variance and occurrence. Post hoc analyses of the interactions between age group and microstate class indicated mixed patterns across microstates, with MS4

being most consistently impacted across several metrics, showing longer duration (p=0.033), higher explained variance, coverages and occurrence (ps<0.001) at 2mCA compared to 0mCA. In contrast, MS2 and MS5 indicated lower explained variance (p=0.022 and p=0.002 respectively) and coverage (p=0.030 and p=0.013 respectively), as well as shorter duration (ps<=0.005) at 2 mCA compared to 0mCA (Figure 4.b).

For simplicity, results for wakefulness are described in Supplementary Information (SI Table4, SI Figure 2 & 3.).

Comparing the normalized transition probability of pairs of microstates indicated that several microstates had significant favorable transitions, whose number varied with age and vigilance state (from 2 to 8, see Figure 5). These transitions were in part stable between 0 and 2mCA for each vigilance state. For active / stage1 sleep, three of the transitions remained stable between the two ages (out of the 8/4 favorable transitions identified at 0/2mCA) (Figure 5.a), while for quiet/stage2 sleep, four transitions remained stable (out of the 6/8 favorable transitions identified at 0/2mCA) (Figure 5.b). For the awake state, only one transition remained stable across age groups and the favorable transitions became more organized with maturation (2 vs 7 favorable transitions identified respectively at 0 vs 2mCA) (Figure 5.c).

3.4. Relating perinatal factors to microstates at 0mCA and their maturation between 0mCA and 2mCA

Finally, we performed statistical analyses to evaluate the impact of perinatal risk factors on the microstate duration during active sleep at 0mCA comparable to stage1 sleep at 2mCA. First, MS duration at 0mCA was impacted by some but not all factors. There was a main effect of sex ($F(1,40) = 12.8$, $p = 0.001$) and microstate ($F(6,203) = 12.2$, $p < 0.001$) as well as an interaction between GA at birth group and microstate class ($F(12,203)= 2.1$, $p = 0.021$) (Figure 6). But there was no main effect of microstate class, GA at birth ($F = 2.2$, $p = 0.125$), intrauterine growth restriction ($F= 3.0$, $p = 0.093$), Kidokoro score and neonatal morbidity and no effect of any considered interactions. The post hoc tests revealed longer duration in males than females ($t = 4.7$, $p <0.001$) and longer duration for groups with lower GA at birth ([24-26] vs.]26-28] weeks) for some MS (ps<0.05).

For longitudinal changes in microstate duration from 0mCA to 2mCA, we did not observe any main effect, despite trends for group of GA at birth ($F= 1.9$, $p = 0.185$) and sex ($F = 2.3$, $p = 0.151$), nor effects of the two-way interactions.

4. Discussion:

In this study, we used an original microstate approach to characterize the dynamics of the resting-state EEG activity in infants while considering different vigilance states. When comparing preterms and full-terms, alterations were identified at the term-equivalent age but also at 2mCA. Comparing infant dynamics at 0 and 2mCA with a robust approach allowed us to identify the presence of both similar (i.e. shared between age groups) and age-specific microstates in all the three vigilance states. For microstates similar between the age groups, we observed a shortening of microstate duration, as well as changes in the coverage, occurrence, explained variance, and the transition patterns of microstates. The inter-individual differences in the temporal dynamics of microstates in preterms were further impacted by gestational age at birth and sex, with longer microstate duration in infants with lower birth age and in males, but not by other clinical risk factors of prematurity.

Methodological considerations for the characterization of microstate dynamics in infants

For studying microstate dynamics in infants, we considered a number of methodological issues. Across all analyses, we ensured that the group-level template microstates were representative of all subjects and were not biased towards a subgroup of infants (see below). First, for identifying group-level templates, we took into account the differences in recording durations across infants by using an iterative method, each time taking a fixed number of data points from each infant recording, therefore not biasing the clustering algorithm to infants with longer recording duration.

Regarding the preterm cohort, a relatively large number of infants was included, with quite uniform sampling of gestational ages at birth. We ensured that the template microstates considering a subgroup of preterms were not biased towards certain GA: consistently, no correlation between GA and the metric of global explain variance was found, indicating that the fit of group-level microstates to each individual infant data was not dependent on gestational age at birth.

Besides, for the preterms vs full-terms comparison, we presumed the template microstates should be similar between the preterm infants at the term-equivalent age and full-term infants, given that the group-level microstates identified in the whole preterm group during sleep were qualitatively similar to those of a previous study in full-term asleep neonates (Khazaei et al., 2021). Yet, to ensure comparing MS metrics between preterms and full-terms relied on an initially-defined unbiased set of templates considering both groups, we implemented a strategy excluding bias towards the larger group of preterm infants. For this, we created subgroups of preterm infants with the same number of infants as the full-term group, ensuring preterm subgroups represented infants with various gestational ages at birth. This allowed us to identify group-level microstates iteratively across each preterm subgroup and full-terms, with a balanced number of infants, and therefore unbiased towards a specific group or GA at birth.

Similarly, we ensured that comparing MS metrics between 0mCA and 2mCA infants relied on an initially-defined unbiased set of microstates. For this comparison, both groups had an adequate number of infants but no previous work studied MS in 2-month-old infants so we could not hypothesize how different the MS were between 0 and 2mCA. Therefore, we implemented a data-driven approach to measure the similarity of the templates between the two age groups and obtained shared and age-group specific MS. This allowed us to compare the MS metrics between the age groups only for the shared template MS which were unbiased towards any group.

Moreover, across all analyses we also took into account that different vigilance states can impact the resting-state brain activity (Dereymaeker et al.,2017), given their crucial role in modulating developmental and learning mechanisms early in life (Li et al., 2017). A previous study in full-term neonates indicated that the spatiotemporal properties of MS change between active and quiet sleep (Khazaei et al., 2021, see also Khazaei et al., 2023). We therefore controlled for the impact of vigilance states by identifying the template microstates separately for each one and performing between-group comparisons of MS metrics only for comparable vigilance states. Moreover, when comparing global explained variance of microstates between different vigilance states, we observed an increase in global explained variance from awake to active/stage1 sleep and further into quiet sleep/stage2. This is consistent with higher global explained variance reported in a previous study of full-term neonates in quiet

compared to active sleep (Khazaei et al., 2021), and these findings might suggest a global reduction in complexity of brain dynamics with progression in sleep. Yet, sleep progression has been shown to impact MS dynamics in a more detailed manner beyond a global change, with differential effects on the dynamics of different microstates, both in neonates (Khazaei et al., 2021, see also Khazaei et al., 2023) and in adults (Brodbeck et al., 2012). Studying the impact of vigilance states on the dynamics of MS was beyond the scope of our study, but a similar strategy as the ones we used for other comparisons (see description above) would be required for comparing the MS templates and metrics across vigilance states.

Besides, our study benefited from HD-EEG recordings that are suggested to improve reliability of MS analysis (Zhang et al., 2021). But interestingly, the microstates we identified in preterms at the term-equivalent age (for active and quiet sleep) were similar to those of full-term asleep neonates reported in the previous study (Khazaei et al., 2021). This indicates that the microstates capturing functional organization of the spontaneous brain activity are quite reproducible across different study settings, neonatal populations and recording systems, and only with a few minutes of recording (Bagdasarov et al., 2023). This opens up the perspective of implementing MS templates by age group, which could be opened up to the scientific and clinical community for future multi-site studies for example.

One limitation of our study relied on the limited sample size of preterms (n=43 but with inter-individual variability in clinical characteristics) and typical full-term infants (n=14). Studying other aspects of MS dynamics maturation, such as their relation to risk factors, was not possible beyond sex and gestational age at birth in our cohort with limited longitudinal evaluations. Future studies in larger cohorts could address such relations by considering more detailed risk factors. But this was beyond the scope of current study.

Brain dynamics are impacted by prematurity and dysmaturation.

Comparing preterm and full-term infants indicated that the dynamics of MS, as captured with several MS metrics, were impacted by prematurity particularly at TEA. These alterations were mainly translated in terms of slower dynamics (i.e. longer duration and lower occurrence rate of MS), which may find their roots in less mature brain structures. This might relate to less advanced myelination of white matter pathways (Neumane et al, 2022), as this mechanism accelerates the conduction of neural impulses and impact the speed of brain responses during development (Dubois et al., 2008), and/or to less complex cortical microstructure with reduced dendritic arborization and synaptogenesis (Dimitrova et al., 2021;Gondova et al, 2023) as this might negatively impact neural activity synchronization (Dupont et al., 2006). Such alterations in preterms at TEA might affect the network-wide activation patterns we measured with the MS approach. A similar deceleration of the brain dynamics has also been recently reported in infants with hypoxic-ischemic encephalopathy (Khazaei et al., 2023), confirming that perinatal insults slow down network-wide brain activity. However, direct links between the structural and functional alterations remain to be established, potentially by individualizing different brain networks that mature progressively during infancy (Dubois et al, 2014, 2016). In our study, we observed that the impact of prematurity was different across MS metrics and MS class (interactions group x MS class), which suggests that not all functional networks are impacted in a similar way. This is in agreement with the asynchronous maturation pattern of brain networks and suggests that different networks have different time windows of vulnerability (Wu et al., 2017) leading to differential susceptibility to prematurity (Neumane et al., 2022).

Beyond prematurity, we also aimed to evaluate to which extent specific perinatal and clinical factors might impact the development of brain dynamics in infants. Previous studies have identified several risk factors related to prematurity as impacting behavioral outcomes of infants (Allen et al., 2008; O'Driscoll et al., 2018; Pierrat et al., 2021). Nevertheless, our sample size limited the possibility to test multiple associations, so we focused on the factors supposed to have a major impact. First, gestational age at birth appeared to modulate the MS dynamics and maturation as infants with lower GA showed slower dynamics, in a comparable way as preterms vs full-terms. In future studies, it would be interesting to characterize such an effect by considering GA as a continuous variable in a larger group of infants including some with moderate prematurity (GA at birth between 32 and 37w). Importantly, GA at birth had a differential impact on different MS dynamics, suggesting that different networks have different time windows of vulnerability over the gestational period (Wu et al., 2017). We also evaluated the impact of sex on MS dynamics and our results (slower dynamics of fast transient network activations in males) seemed consistent with the higher vulnerability of males compared to females over this specific period of development (Perivier et al., 2016). These results further suggested the sensitivity of our MS approach to highlight some inter-individual variability across infants, that might be interesting to target for individualized interventions. Other clinical risk factors of adverse outcomes related to prematurity were also considered (weight at birth, respiratory, intestinal or infectious complications, brain abnormalities) but none were shown to impact the brain dynamics in our study. Nevertheless, this may be related to the lack of large variability in our cohort of infants and to our limited sample size which constrained us to binarize each factor and combine some of them into a composite morbidity score. It is also possible that some of the assessed clinical factors were not reflective of maturational changes captured with EEG dynamics. In particular, the MRI Kidokoro score is supposed to capture macrostructural abnormalities related to global and regional volumetric growth as well as brain lesions but may not be sensitive to diffuse microstructural alterations impacting the network functional activity.

Maturation changes the spatio-temporal brain dynamics in preterm infants.

Comparing brain dynamics between 0m and 2mCA indicated an evolution of the brain dynamics in terms of the appearance of new activity patterns among the dominant microstates, as well as major impact on spatiotemporal properties of microstates. The emergence of age-group specific MS suggests a reorganization of functional network activities following intense maturation of cortical regions and white matter pathways promoting functional specialization of networks (Dubois et al., 2014; Gao et al., 2014; Keunen, Counsell, Benders, 2017). For example, the appearance of a majorly posteriorly-distributed MS at 2mCA in active sleep, might relate to the intense development of the visual system in the first months after birth (Dubois et al.,2008; 2014).

The changes in the MS properties involved a major shortening of MS duration between 0mCA at 2mCA, which is also in agreement with a recent study of preterm infants indicating acceleration of MS dynamics between the preterm and term period [250–300 ms at 30w PMA to 150–200 ms after 37w PMA] (Hermans et al., 2023). Interestingly, the average duration of the MS in our study, 140 ms at 0mCA and 112 ms at 2mCA, is in the intermediate range between the preterm period (Hermans et al., 2023) and childhood with MS durations reported between 60-90 ms (Bagdasarov et al., 2022; Koenig et al., 2002; Takarae et al., 2021), suggesting a continuous change in the duration of MS from birth through childhood. The shortening of MS duration, together with the increase in the

occurrence rate of MS, indicate an acceleration of the brain dynamics which could reflect the intensive myelination of white matter pathways accelerating the conduction of neural impulses (Dubois et al., 2008), and/or maturation of cortical microstructure through the growth of dendritic arborization and synaptogenesis (Dimitrova et al., 2021;Gondova et al, 2023) around the term-equivalent age and first following weeks. Most of the developmental changes concerned microstates with an anterior/posterior distribution of the brain activity, a spatial organization also reported in functional connectivity patterns in newborns and showing some alterations in preterms (Omidvarnia et al., 2014; Tokariev et al., 2019). An interesting direction for future investigations would be addressing how the functional connectivity patterns identified with EEG relate to the dynamics of MS and their maturation in early development (see Khazaei et al., 2023).

Besides metrics concerning individual MS, we also studied the changes in the transition between microstates which offers information into the structure of the temporal dependencies in brain dynamics and their evolution with development. At both ages, the transition between the microstates had a non-random structure, with most microstates having a tendency to transition into their 'favorite' microstates (one or more than one). The pattern of such transitions evolved with development between 0 and 2mCA for certain microstates, while it remained similar for other microstates, with most changes concerning wakefulness. Such changes in the transition pattern of MS could suggest a reorganization of the brain dynamics, showing an evolution in the continuous shifting between transient network activities. The dynamic aspects of brain activity have also been described for functional resting-state networks identified with fMRI at the temporal resolution of the BOLD effect (França et al., 2024), indicating their alterations in preterms at TEA compared to full-terms. Here, we could not compare these dynamic aspects and transition probabilities between the full-term and preterm groups, since the reduced size of the full-term group limited the reliability of statistical testing.

To conclude, this study implemented a dedicated strategy for the quantitative evaluation of brain activity in preterm infants using the microstate approach, highlighting fundamental features that describe the maturation of the brain dynamics and show sensitivity to perinatal risk factors. Such an approach might offer novel potential diagnostic markers useful for prognosis. Although beyond the scope of current study, this encourages future research to perform similar analysis in larger groups of full-term neonates in order to characterize the normative developmental trajectories required for a better identification of atypicalities in preterm infants with a wide inter-individual variability.

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Tables:

Table 1: Summary of neonatal characteristics of the infants at birth. These include groups of GA at birth ([24-26] /]26-28] /]28-32] weeks), sex (males/females), binarized risk of intrauterine growth restriction (yes/no), binarized risk of intrauterine growth restriction (yes/no), binarized Kidokoro score (mild/normal), as well as information regarding five categories of non-neurological complications at NICU. These complications were considered for chronic lung disease (yes/no), use of mechanical ventilation for more than 3 weeks (yes/no), necrotizing enterocolitis (yes/no), parenteral nutrition for more than 3 weeks (yes/no) and sepsis (yes/no). which were then summarized as one single binarized score of neonatal morbidity factor (yes: at least one of the 5 identified non-neurological complications/no: none). See Supplementary Table 1 for the report of individual characteristics.

Subjects	GA at birth	GA category	Sex	Intrauterine growth restriction	MRI Kidokoro score	Chronic lung disease	Mechanical ventilation>3weeks	Necrotizing enterocolitis	Parenteral nutrition>3weeks	Sepsis	Clinical morbidity risk
n = 43	27.1±1.7 w Min = 24.1 w Max = 30.9 w	n = 13/19/11 [24-26] /]26-28] /]28-32]w	n = 23/20 M/F	n = 8/35 Yes/No	n = 7/36 Mild/Normal	n = 25/18 Yes/No	n = 17/26 Yes/No	n = 7/36 Yes/No	n = 20/23 Yes/No	n = 32/11 Yes/No	n = 37/6 Yes/No

Table 2: Comparison of microstates metrics (duration and explained variance) between Preterm (PT) and Full-Term (FT) infants for different sleep states. Significant statistical tests are indicated with asterisk (* p<0.05, and ** p<0.005). Comparisons regarding coverage and occurrence are indicated in SI Table 2.

a. Active / Stage 1 sleep

	0mCA – Active sleep		2mCA – Stage 1 Sleep	
	Duration	Explained Variance	Duration	Explained Variance
Group (PT vs FT)	F(1,48) = 4.4, p = 0.041*	F(1,48) = 0.1, p = 0.818	F(1,28) < 0.1, p = 0.904	F(1,28) =1.0, p = 0.325
Microstate	F(6,288) = 14.2, p < 0.001**	F(6,288) = 29.2, p < 0.001**	F(6,168) = 1.5, p = 0.168	F(6,168) = 5.5, p < 0.001**
Group x Microstate	F(6,288) = 2.5, p = 0.025*	F(6,288) = 2.5, p = 0.021*	F(6,168) = 1.6, p = 0.155	F(6,168) = 2.0, p = 0.067
Posthoc tests	MS1(PT vs FT): t = -0.7, p = 0.496	MS1(PT vs FT): t = -1.3, p = 0.198	MS1(PT vs FT): t = 0.5, p = 0.627	MS1(PT vs FT): t = 0.3, p = 0.797
	MS2(PT vs FT): t = 0.3, p = 0.738	MS2(PT vs FT): t = -0.7, p = 0.479	MS2(PT vs FT): t = 1.3, p = 0.208	MS2(PT vs FT): t = 1.7, p = 0.103
	MS3(PT vs FT): t = 4.2, p = 0.001**	MS3(PT vs FT): t = 4.6, p < 0.001*	MS3(PT vs FT): t = 2.5, p = 0.019*	MS3(PT vs FT): t = 4.2, p < 0.001**
	MS4(PT vs FT): t = 2.0, p = 0.066	MS4(PT vs FT): t = 0.4, p = 0.724	MS4(PT vs FT): t = -1.2, p = 0.286	MS4(PT vs FT): t = -1.1, p = 0.313
	MS5(PT vs FT): t = 0.8, p = 0.422	MS5(PT vs FT): t = -1.1, p = 0.288	MS5(PT vs FT): t = -0.1, p = 0.901	MS5(PT vs FT): t < -0.1, p = 0.991
	MS6(PT vs FT): t = 1.4, p = 0.169	MS6(PT vs FT): t = -0.1, p = 0.935	MS6(PT vs FT): t = 0.9, p = 0.417	MS6(PT vs FT): t = 1.5, p = 0.157
	MS7(PT vs FT): t = 2.1, p = 0.054	MS7(PT vs FT): t = 1.5, p = 0.157	MS7(PT vs FT): t = -0.6, p = 0.570	MS7(PT vs FT): t = -0.8, p = 0.447

b. Quiet / Stage2 sleep

	0mCA – Quiet sleep		2mCA – Stage 2 Sleep	
	Duration	Explained Variance	Duration	Explained Variance
Group (PT vs FT)	F(1,30) = 0.6, p = 0.444	F(1,30) = 0.4, p = 0.550	F(1,30) < 0.1, p = 0.789	F(1,30) < 0.1, p = 0.910
Microstate	F(6180) = 51.2, p < 0.001**	F(6,180)= 43.5, p < 0.001**	F(6,180) = 15.7, p < 0.001**	F(6,180) = 26.7, p < 0.001**
Group x Microstate	F(6,180) = 2.9, p = 0.011*	F(6,180)) = 4.2, p < 0.001**	F(6,180) = 1.8, p = 0.094	F(6,180) = 2.4, p = 0.027*
Posthoc tests	MS1(PT vs FT): t = 0.2, p = 0.871	MS1(PT vs FT): t = -0.5, p = 0.643	MS1(PT vs FT): t = -0.8, p = 0.431	MS1(PT vs FT): t = -1.6, p = 0.130
	MS2(PT vs FT): t = 3.5, p = 0.002**	MS2(PT vs FT): t = 3.0, p = 0.006*	MS2(PT vs FT): t = 1.5, p = 0.162	MS2(PT vs FT): t = 1.0, p = 0.320
	MS3(PT vs FT): t = 0.4, p = 0.674	MS3(PT vs FT): t = -0.3, p = 0.775	MS3(PT vs FT): t = -0.8, p = 0.471	MS3(PT vs FT): t = -1.0, p = 0.357
	MS4(PT vs FT): t = -1.1, p = 0.283	MS4(PT vs FT): t = -1.5, p = 0.161	MS4(PT vs FT): t = 2.4, p = 0.023 *	MS4(PT vs FT): t = 4.0, p < 0.001**
	MS5(PT vs FT): t = -0.3, p = 0.784	MS5(PT vs FT): t = -0.4, p = 0.679	MS5(PT vs FT): t = 1.0, p = 0.338	MS5(PT vs FT): t = 0.9, p = 0.408
	MS6(PT vs FT): t =0.9, p = 0.368	MS6(PT vs FT): t = 0.5, p = 0.616	MS6(PT vs FT): t = 0.3, p = 0.798	MS6(PT vs FT): t = 0.7, p = 0.470
	MS7(PT vs FT): t = 1.5, p = 0.148	MS7(PT vs FT): t = 0.8, p = 0.407	MS7(PT vs FT): t = -1.1, p = 0.288	MS7(PT vs FT): t = -1.6, p = 0.146

Table 3: Maturation of microstates metrics between 0mCA and 2mCA for different vigilance states. Comparisons were made between comparable sleep states, a.b) at active/ stage1 sleep for 0mCA / 2mCA respectively, and c) at quiet / stage2 sleep for 0mCA/2mCA. The statistical tests in a) and c) were made cross-sectionally and in b) longitudinally. Significant statistical tests are indicated with asterisk (* p<0.05, and ** p<0.005).

a. Active / Stage1 sleep

	Duration	Explained Variance
Age (0 vs 2mCA)	F(1,61) = 33.8, p <0.001 **	F(1,61) = 0.1, p = 0.902
Microstate	F(4,244) = 14.1, p <0.001**	F(4,244) = 26.3, p <0.001**
Age x Microstate	F(4,244) = 0.9, p = 0.453	F(4,244) = 2.7, p = 0.029*
Post hoc tests		MS1 (0 vs 2mCA): t = 1.4, p = 0.159 MS2 (0 vs 2mCA): t = -2.2, p = 0.033* MS3 (0 vs 2mCA): t = 1.0, p = 0.334 MS4 (0 vs 2mCA): t = 0.7, p = 0.460 MS5 (0 vs 2mCA): t = -1.3, p = 0.184

c. Active / Stage1 sleep (longitudinal)

	Duration	Explained Variance
Age (0 vs 2mCA)	F(1,20) = 46.1, p <0.001 **	F(1,20) < 0.1, p = 0.803
Microstate	F(4,80) = 8.3, p <0.001**	F(4,80) = 17.5, p <0.001**
Age x Microstate	F(4,100) = 0.6, p = 0.630	F(4,100) = 2.6, p = 0.040*
Post hoc tests		MS1 (0 vs 2mCA): t = 1.7, p = 0.091 MS2 (0 vs 2mCA): t = -1.6, p = 0.107 MS3 (0 vs 2mCA): t = 0.4, p = 0.649 MS4 (0 vs 2mCA): t = 0.8, p = 0.421 MS5 (0 vs 2mCA): t = -1.5, p = 0.141

c. Quiet / Stage2 sleep

	Duration	Explained Variance
Age (0 vs 2mCA)	F(1,42) = 6.1, p = 0.018 *	F(1,42) = 0.99, p = 0.324
Microstate	F(5,210) = 13.1, p < 0.001**	F(5,210) = 31.7, p < 0.001**
Age x Microstate	F(5,210) = 5.9, p < 0.001**	F(5,210) = 11.1, p < 0.001**
Post hoc tests	MS1(0 vs 2mCA): t =1.6 , p = 0.124 MS2(0 vs 2mCA): t = 3.0, p = 0.005** MS3(0 vs 2mCA): t = 0.9, p = 0.389 MS4(0 vs 2mCA): t = -2.2 , p = 0.033* MS5(0 vs 2mCA): t = 4.0, p < 0.001** MS6(0 vs 2mCA): t = 1.4, p = 0.168	MS1(0 vs 2mCA): t = 2.8 , p = 0.009* MS2(0 vs 2mCA): t = 2.4, p = 0.022* MS3(0 vs 2mCA): t = -2.0 , p = 0.051 MS4(0 vs 2mCA): t = -4.9, p < 0.001** MS5(0 vs 2mCA): t = 3.3, p = 0.002** MS6(0 vs 2mCA): t = -1.1, p = 0.257

Figures:

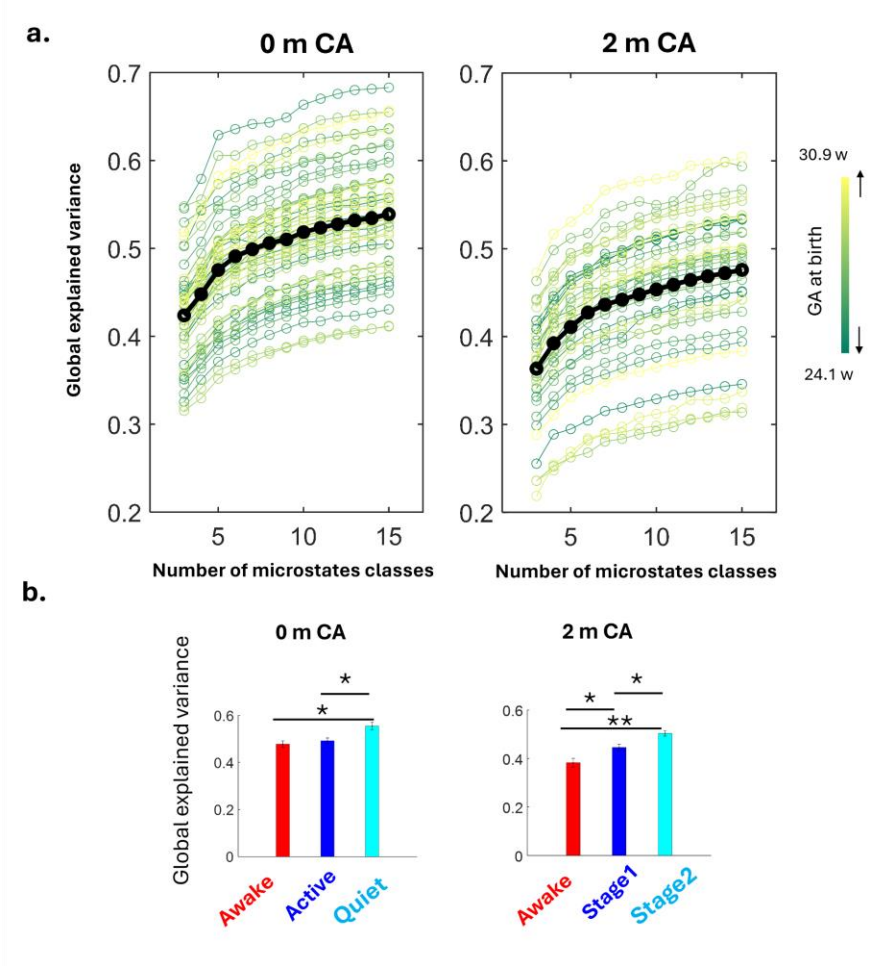


Figure 1 : a) Gain in the global explained variance as a function of the number of microstate classes for preterm infants at 0 months of corrected age (0mCA) and 2mCA in the overall recordings without separating vigilance states. Each individual infant is color-coded based on the gestational age (GA) at birth. For a number of microstates higher than 7, the gain in the explained variance dropped below 1%. **b)** Global explained variance for different vigilance states was measured when the number of microstate classes were fixed at 7. The global explained variance increased with progress in sleep. Significant comparisons are highlighted with asterisks (* $p<0.05$, ** $p<0.005$).

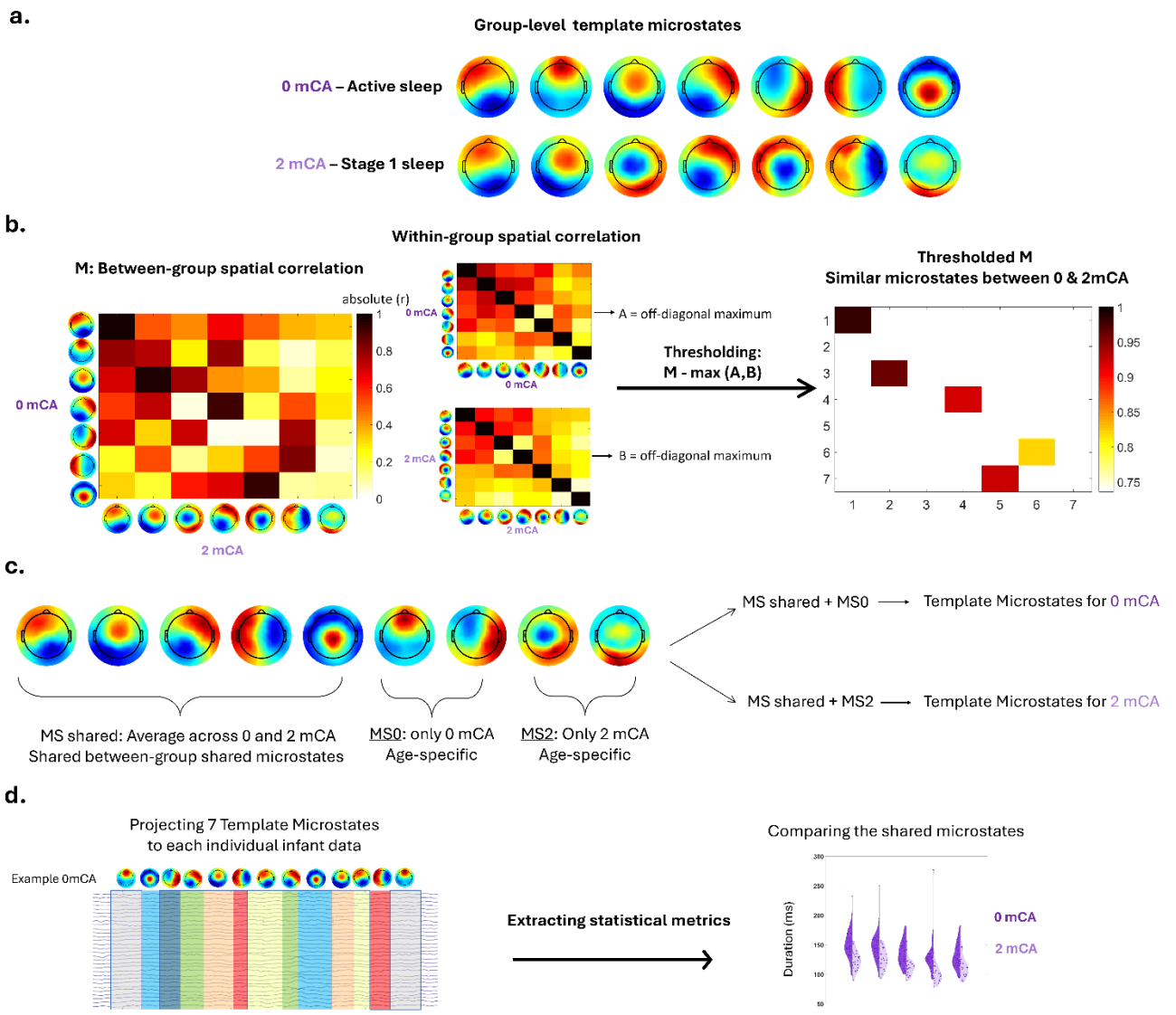


Figure 2 : a) Group-level template microstates are identified separately for each age group in preterm infants. **b)** Similarity between group-level microstates of the two age-groups is computed using spatial correlation irrespective of polarity of the maps. Similarity between group-level microstates are also computed for each group separately (i.e. within-group) and their maximal values are used to threshold the matrix representing the between-group similarity. **c)** Similar microstates between the two age-groups are averaged, constituting the "MS shared: shared microstates". Unsimilar microstates are kept as age-specific for each of the age groups (0/2mCA-> MS0/2). The combination of shared and age-specific microstates constituted the final set of 7 template microstates for each age group, among which shared microstates are comparable. **d)** The final microstate templates for each age group are back projected to each individual infant recording, illustrating activation of different microstates over time. Microstate metrics are extracted for all the microstates and are compared between age groups for the shared microstates.

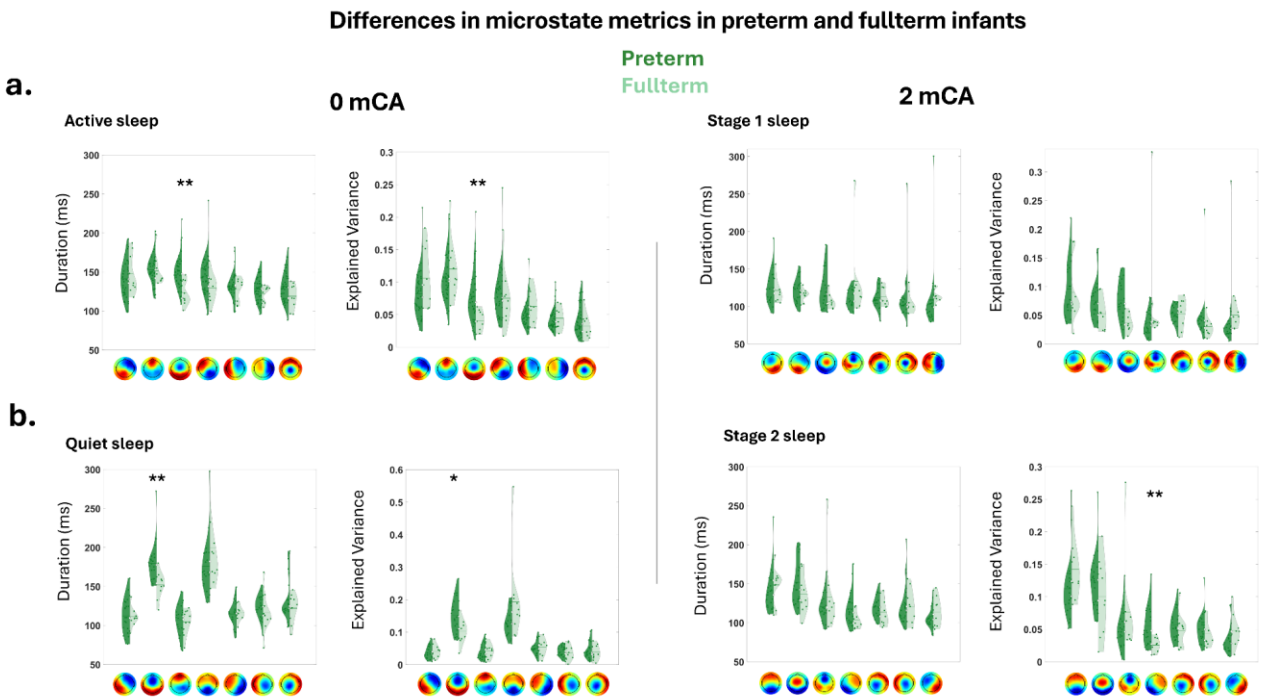


Figure 3 : differences in microstates duration and explained variance between preterm (dark green) and full-term (light green) infants at 0mCA (left panel) and 2mCA (right panel) during different vigilance states: **a)** active/stage1 sleep **b)** quiet/stage2 sleep. Asterisks represent significant differences between preterms and full-terms (** p<0.005, *p<0.05).

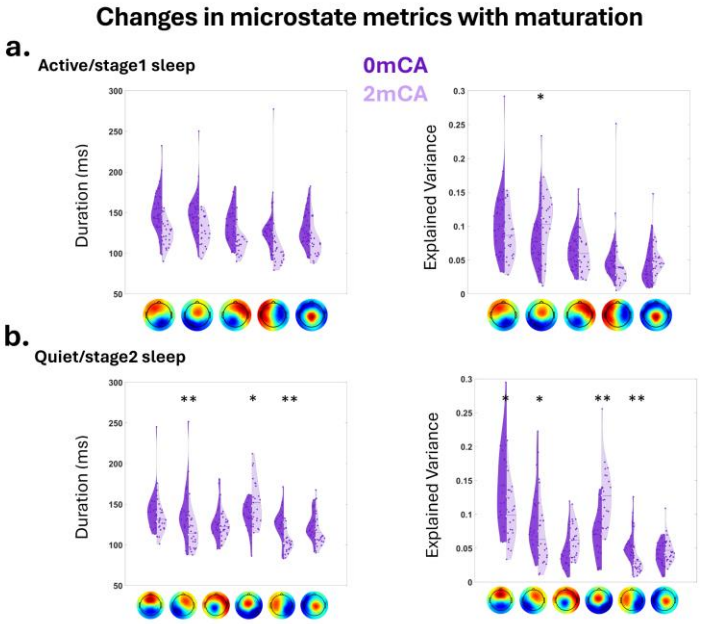


Figure 4 : Evolution in microstate duration and explained variance between 0mCA (dark purple) and 2mCA (light purple) during different vigilance states: a) active/stage1 sleep b) quiet/stage2 sleep. Asterisks represent significant differences between 0mCA and 2mCA infants (** p<0.005, *p<0.05).

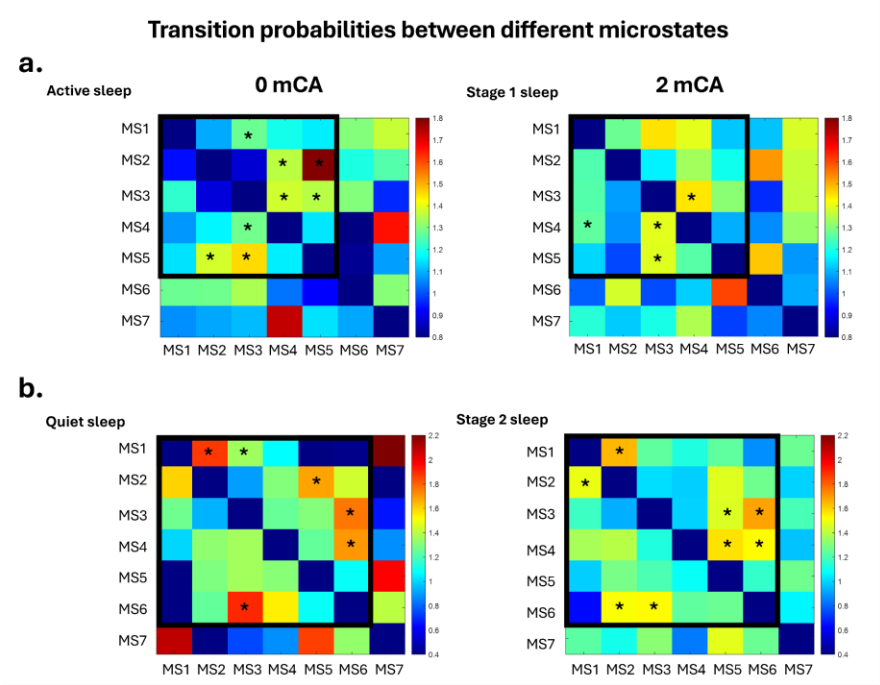


Figure 5 : Normalized transition probabilities between different microstates at different vigilance states in preterms at 0/2mCA (left/right column): **a)** active sleep/stage1 sleep **b)** quiet sleep/stage2 sleep. Asterisks represent significant favorable transitions. The dark squares highlight the MS shared between 0 and 2mCA for each vigilance state.

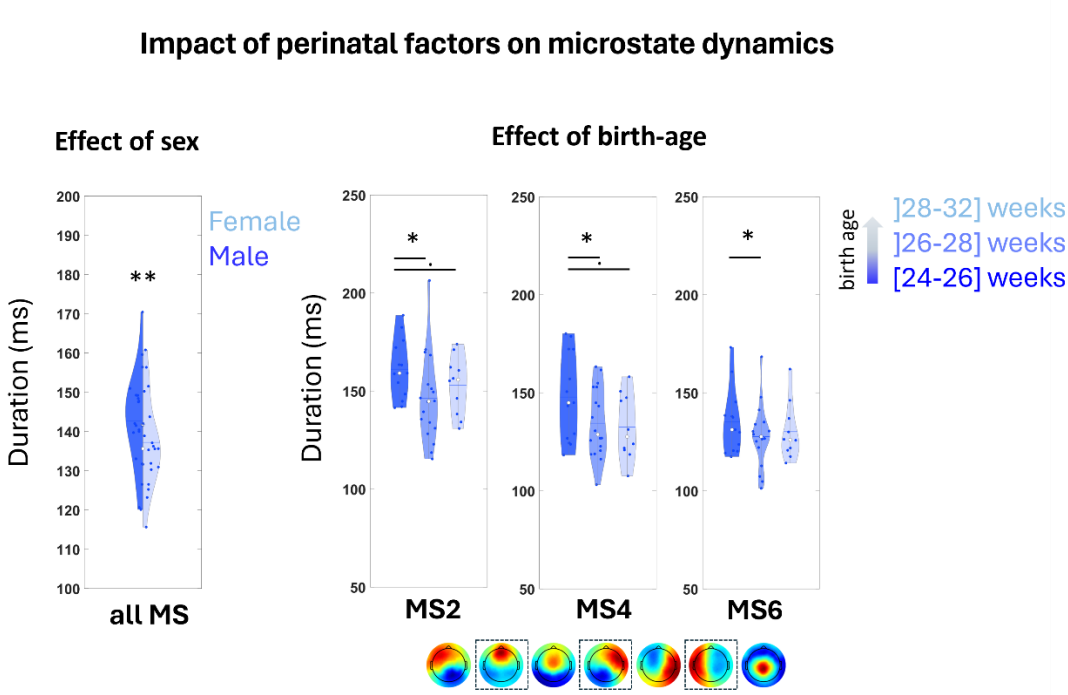


Figure 6 : Impact of perinatal factors on microstates duration. **a)** Sex, considering the average duration over all MS and **b)** groups of GA at birth for specific MS of active sleep**.** Asterisks represent significant differences between sex and GA groups (** p<0.005, *p<0.05, • p<0.1): longer duration in males and in infants with the lowest GA at birth.

Supplementary Information:

1. Participants information

SI Table 1: Detailed Neonatal characteristics and clinical risk factors for each of the preterm infants. These include groups of GA at birth, GA category (1: [24-26] /2:]26-28] / 3:]28-32] weeks), sex (males/females), binarized risk of intrauterine growth restriction (yes/no), binarized Kidokoro score (mild/normal), as well as information regarding five categories of non-neurological complications at NICU. These complications were considered for chronic lung disease (yes/no), use of mechanical ventilation for more than 3 weeks (yes/no), necrotizing enterocolitis (yes/no) and parenteral nutrition for more than 3 weeks (yes/no) and sepsis (yes/no).

Subjects	GA at birth	GA category	Sex	Intrauterine growth restriction	MRI Kidokoro score	Chronic lung disease	Mechanical ventilation>3weeks	Necrotizing enterocolitis	Parenteral nutrition>3weeks	Sepsis
1	26.6	2	M	No	Normal	No	No	No	No	Yes
2	27.0	2	F	No	Normal	No	Yes	Yes	No	No
3	27.3	2	F	No	Normal	No	No	No	No	Yes
4	27.9	2	M	No	Mild	No	No	No	No	No
5	25.7	1	M	No	Normal	No	No	No	No	Yes
6	25.7	1	M	No	Normal	No	No	No	No	Yes
7	25.1	1	F	Yes	Normal	Yes	Yes	No	Yes	Yes
8	26.4	2	M	No	Normal	No	No	No	Yes	Yes
9	26.4	2	F	No	Mild	Yes	No	No	Yes	Yes
10	26.6	2	M	No	Normal	No	No	No	No	Yes
11	26.6	2	M	No	Normal	No	No	No	No	Yes
12	29.9	3	M	Yes	Normal	No	Yes	Yes	Yes	Yes
13	29.7	3	F	No	Normal	No	No	No	No	Yes
14	25.9	1	F	No	Normal	No	No	No	No	Yes
15	25.9	1	M	No	Normal	Yes	No	No	Yes	Yes
16	25.9	1	F	No	Mild	No	No	No	No	Yes
17	27.4	2	M	No	Mild	Yes	No	No	No	Yes
18	30.9	3	M	No	Normal	No	No	No	No	No
19	26.0	1	M	No	Normal	Yes	No	No	Yes	Yes
20	25.1	1	F	No	Normal	Yes	Yes	No	No	Yes
21	27.9	2	M	No	Normal	Yes	No	No	No	No
22	28.1	3	F	Yes	Normal	Yes	Yes	Yes	Yes	Yes
23	27.7	2	M	No	Normal	No	No	No	No	No
24	28.9	3	F	No	Normal	Yes	Yes	No	Yes	Yes
25	29.7	3	M	No	Normal	Yes	Yes	No	Yes	Yes
26	24.3	1	F	No	Normal	Yes	Yes	No	Yes	Yes
27	26.7	2	F	No	Normal	Yes	No	No	No	No
28	25.0	1	F	No	Normal	Yes	Yes	Yes	Yes	Yes
29	27.1	2	M	No	Normal	Yes	Yes	No	Yes	No
30	28.6	3	M	Yes	Normal	Yes	No	No	No	Yes
31	29.4	2	F	No	Normal	Yes	No	No	Yes	Yes
32	27.6	2	M	Yes	Normal	Yes	No	No	No	Yes
33	26.3	2	F	No	Normal	Yes	Yes	No	Yes	Yes
34	26.7	2	M	No	Normal	Yes	No	No	No	Yes
35	28.3	3	M	No	Normal	Yes	Yes	No	Yes	Yes
36	24.6	1	M	Yes	Normal	Yes	Yes	No	Yes	Yes
37	24.1	1	M	No	Mild	Yes	Yes	Yes	Yes	Yes
38	30.6	3	M	Yes	Mild	No	No	No	No	No
39	26.3	2	F	No	Normal	Yes	Yes	Yes	Yes	Yes
40	27.1	2	F	No	Normal	Yes	Yes	No	Yes	No
41	28.1	3	F	No	Mild	No	No	No	No	No
42	25.1	1	F	No	Normal	Yes	Yes	Yes	Yes	Yes
43	30.9	3	F	Yes	Normal	No	No	No	No	No

2. Comparing microstates between preterm and full-term infants: Impact of prematurity on MS coverage and occurrence

SI Table 2: Comparison of microstates coverage and occurrence between Preterm (PT) and Full-Term (FT) infants for different sleep states. Significant statistical tests are indicated with asterisk (* p<0.05, and ** p<0.005).

a. Active / Stage 1 sleep

	0mCA – Active sleep		2mCA – Stage 1 Sleep	
	Coverage	**Occurrence**	**Coverage**	**Occurrence**
Group (PT vs FT)	F(1,48) = 0.4, p = 0.530	F(1,48) = 2.7, p = 0.107	F(1,28) < 0.1, p = 0.963	F(1,28) < 0.1, p = 0.922
Microstate	F(6,288) = 11.0, p < 0.001	F(6,288) = 8.1, p < 0.001**	F(6,168) = 4.1, p < 0.001**	F(6,168) = 9.8, p < 0.001**
Group x Microstate	F(6,288) = 3.2, p = 0.005*	F(6,288) = 3.2, p = 0.004**	F(6,168) = 2.7, p = 0.016*	F(6,168) = 4.5, p < 0.001 **
Posthoc tests	MS1(PT vs FT): t = -1.7, p = 0.114	MS1(PT vs FT): t = -2.6, p = 0.025*	MS1(PT vs FT): t < 0.1, p = 0.986	MS1(PT vs FT): t = -0.2, p = 0.872
	MS2(PT vs FT): t = -1.2, p = 0.245	MS2(PT vs FT): t = -2.2, p = 0.043	MS2(PT vs FT): t = 0.9, p = 0.396	MS2(PT vs FT): t = 0.7, p = 0.499
	MS3(PT vs FT): t = 3.8, p = 0.001*	MS3(PT vs FT): t = 2.6, p = 0.018*	MS3(PT vs FT): t = 4.0, p < 0.001**	MS3(PT vs FT): t = 3.4, p = 0.004**
	MS4(PT vs FT): t = 0.4, p = 0.733	MS4(PT vs FT): t = -0.5, p = 0.627	MS4(PT vs FT): t = -1.4, p = 0.189	MS4(PT vs FT): t = 3.5, p = 0.004**
	MS5(PT vs FT): t = -1.1, p = 0.290	MS5(PT vs FT): t = -2.6, p = 0.024*	MS5(PT vs FT): t = -0.5, p = 0.616	MS5(PT vs FT): t = -0.4, p = 0.715
	MS6(PT vs FT): t = -0.4, p = 0.676	MS6(PT vs FT): t = -1.4, p = 0.171	MS6(PT vs FT): t = 1.6, p = 0.131	MS6(PT vs FT): t = 2.2, p = 0.046*
	MS7(PT vs FT): t = 1.2, p = 0.268	MS7(PT vs FT): t = 0.6, p = 0.565	MS7(PT vs FT): t = -1.7, p = 0.091	MS7(PT vs FT): t = -2.9, p = 0.014*

b. Quiet / Stage2 sleep

	0mCA – Quiet sleep		2mCA – Stage 2 Sleep	
	Coverage	**Occurrence**	**Coverage**	**Occurrence**
Group (PT vs FT)	F(1,30) < 0.1, p = 0.976	F(1,30) = 0.1, p = 0.785	F(1,30) = 1.6, p = 0.211	F(1,30) < 0.1, p = 0.992
Microstate	F(6,180) = 73.24, p < 0.001**	F(6,180) = 70.0, p < 0.001**	F(6,288) = 22,0, p < 0.001**	F(6,180) = 22.7, p < 0.001**
Group x Microstate	F(6,180) = 4.2, p < 0.001**	F(6,180) = 2.3, p = 0.037*	F(6,180) = 3.1, p = 0.007*	F(6,180) = 4.0, p < 0.001**
Posthoc tests	MS1(PT vs FT): t = -0.9, p = 0.362	MS1(PT vs FT): t = -1.4, p = 0.189	MS1(PT vs FT): t = -1.9, p = 0.070	MS1(PT vs FT): t = -2.1, p = 0.049*
	MS2(PT vs FT): t = 3.1, p = 0.005*	MS2(PT vs FT): t = 1.6, p = 0.127	MS2(PT vs FT): t = 1.7, p = 0.122	MS2(PT vs FT): t = 1.3, p = 0.209
	MS3(PT vs FT): t = -0.7, p = 0.496	MS3(PT vs FT): t = -1.2, p = 0.266	MS3(PT vs FT): t = -1.3, p = 0.239	MS3(PT vs FT): t = -2.0, p = 0.061
	MS4(PT vs FT): t = -1.8, p = 0.097	MS4(PT vs FT): t = -2.6, p = 0.016*	MS4(PT vs FT): t = 2.8, p = 0.019*	MS4(PT vs FT): t = 2.1, p = 0.049*
	MS5(PT vs FT): t = -0.6, p = 0.568	MS5(PT vs FT): t = -0.7, p = 0.500	MS5(PT vs FT): t = 1.0, p = 0.321	MS5(PT vs FT): t = 0.8, p = 0.427
	MS6(PT vs FT): t = 1.0, p = 0.358	MS6(PT vs FT): t = 1.1, p = 0.290	MS6(PT vs FT): t = 0.5, p = 0.650	MS6(PT vs FT): t = 0.4, p = 0.710
	MS7(PT vs FT): t = 0.9, p = 0.358	MS7(PT vs FT): t = 0.5, p = 0.629	MS7(PT vs FT): t = -1.9, p = 0.085	MS7(PT vs FT): t = -1.9, p = 0.077

3. Comparing microstates between 0mCA and 2mCA: Impact of maturation on MS coverage and occurrence in preterms

SI Table 3: Maturation of microstates coverage and occurrence between 0mCA and 2mCA during sleep states. Significant statistical tests are indicated with asterisk (* p<0.05, and ** p<0.005).

a. Active / Stage1 sleep

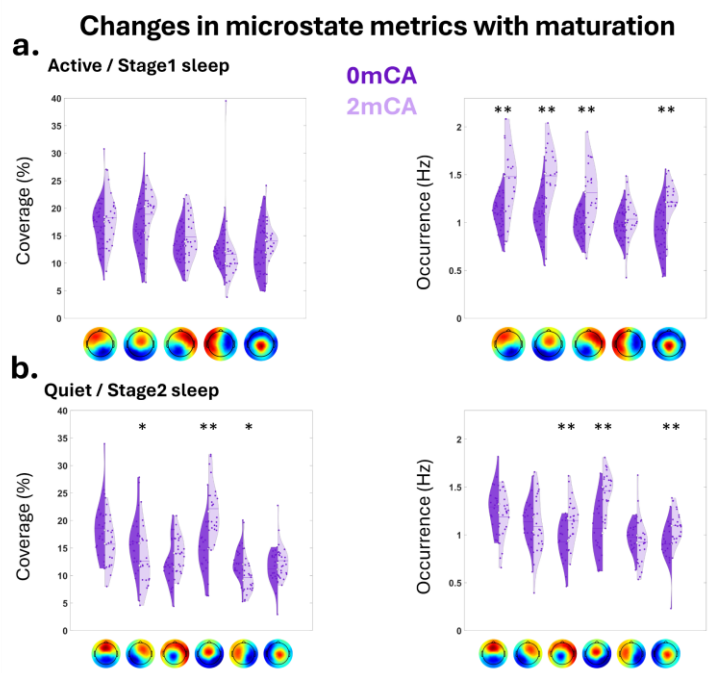
	Coverage	Occurrence
Age (0 vs 2mCA)	F(1,61) = 18.1, p < 0.001**	F(1,61) = 58.4 p < 0.001**
Microstate	F(4,244) = 16.1, p < 0.001**	F(4,244) = 17.4, p < 0.001**
Age x Microstate	F(4,244) = 1.0, p = 0.401	F(4,244) = 5.9, p < 0.001**
Post hoc tests		MS1 (0 vs 2mCA): t = - 4.8, p < 0.001**
		MS2 (0 vs 2mCA): t = -5.0, p < 0.001**
		MS3 (0 vs 2mCA): t = -4.6, p < 0.001**
		MS4 (0 vs 2mCA): t = -1.3, p = 0.207
		MS5 (0 vs 2mCA): t = -4.6, p < 0.001**

b. Active / Stage1 sleep (longitudinal)

	Coverage	Occurrence
Age (0 vs 2mCA)	F(1,20) = 4.2, p = 0.044*	F(1,20) = 80.9, p < 0.001**
Microstate	F(4,80) = 11.6, p <0.001**	F(4,80) = 15.2, p < 0.001**
Age x Microstate	F(4,100) = 1.0, p = 0.405	F(4,100) = 3.6, p = 0.009*
Post hoc tests		MS1 (0 vs 2mCA): t = -4.3, p < 0.001**
		MS2 (0 vs 2mCA): t = -4.6, p < 0.001**
		MS3 (0 vs 2mCA): t = -4.7, p < 0.001**
		MS4 (0 vs 2mCA): t = -1.0, p = 325
		MS5 (0 vs 2mCA): t = -4.2, p < 0.001**

c. Quiet / Stage2 sleep

	Coverage	Occurrence
Age (0 vs 2mCA)	F(1,42) = 1.9, p = 0.169	F(1,42) = 5.9, p = 0.019*
Microstate	F(5,210) = 18.4, p < 0.001**	F(5,210) = 17.6, p < 0.001**
Age x Microstate	F(5,210) = 9.4, p < 0.001**	F(5,210) = 10.2, p < 0.001**
Post hoc tests	MS1(0 vs 2mCA): t = 1.9 , p = 0.059	MS1(0 vs 2mCA): t = 1.4 , p = 0.174
	MS2(0 vs 2mCA): t = 2.2, p = 0.030*	MS2(0 vs 2mCA): t = 0.5, p = 0.587
	MS3(0 vs 2mCA): t = -1.7 , p = 0.104	MS3(0 vs 2mCA): t =-3.1 , p = 0.003**
	MS4(0 vs 2mCA): t = -5.0, p < 0.001**	MS4(0 vs 2mCA): t = -5.4, p < 0.001*
	MS5(0 vs 2mCA): t = 2.6, p = 0.013*	MS5(0 vs 2mCA): t = 0.6 , p = 0.535
	MS6(0 vs 2mCA): t = -1.6, p = 0.118	MS6(0 vs 2mCA): t = -3.2 , p = 0.003**



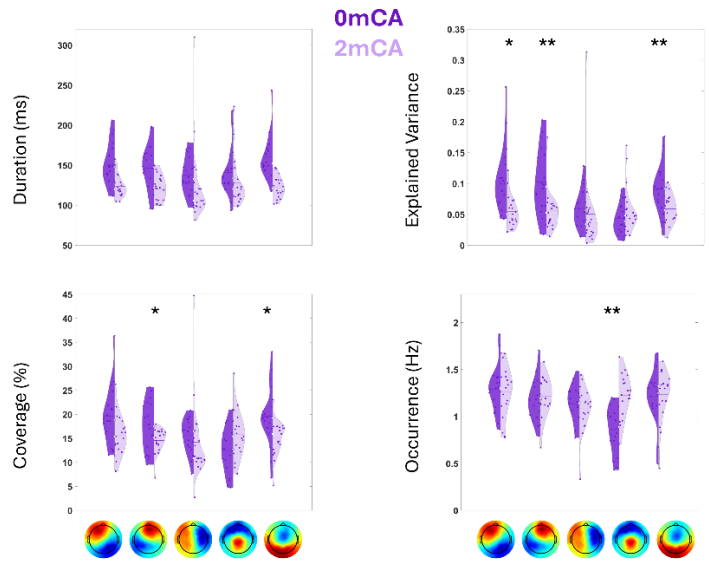
SI Figure 1 : Evolution in microstate metrics between 0mCA (dark purple) and 2mCA (light purple) during different sleep states. Asterisks represent significant differences between 0mCA and 2mCA infants (** p<0.005, *p<0.05).

4. Comparing microstates between 0mCA and 2mCA: Impact of maturation on MS dynamics in preterms during wakefulness

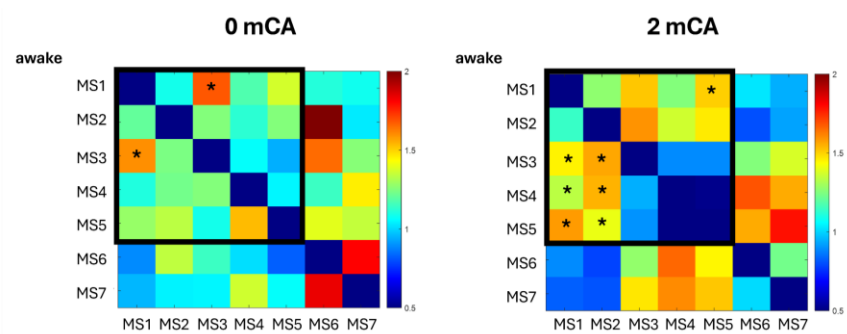
SI Table 4: Maturation of microstates metrics (duration, explained variance, coverage and occurrence) between 0mCA and 2mCA during wakefulness. Significant statistical tests are indicated with asterisk (* p<0.05, and ** p<0.005).

	Duration	Explained Variance	Coverage	Occurrence
Age (0 vs 2mCA)	F(1,33) = 14.9, p < 0.001 **	F(1,33) = 22.5, p < 0.001**	F(1,33) = 32.0, p < 0.001**	F(1,33) = 0.4, p = 0.539
Microstate	F(4,132) = 0.6, p =0.647	F(4,132) = 3.8, p =0.005*	F(4,132) = 2.6, p = 0.038*	F(4,132) = 6.7, p < 0.001**
Age x Microstate	F(4,132) = 1.2, p =0.305	F(4,132) = 3.2, p = 0.016*	F(4,132) = 2.5, p = 0.044*	F(4,132) = 5.0, p < 0.001**
Post hoc tests		MS1(0 vs 2mCA): t = 2.8, p = 0.008*	MS1(0 vs 2mCA): t=1.9, p = 0.063	MS1(0 vs 2mCA): t = -0.1, p = 0.911
		MS2(0 vs 2mCA): t = 3.3, p = 0.003**	MS2(0 vs 2mCA): t= 2.3, p = 0.028*	MS2(0 vs 2mCA): t= -0.1, p = 0.911
		MS3(0 vs 2mCA): t = 0.5, p = 0.643	MS3(0 vs 2mCA): t = 0.7, p = 0.5	MS3(0 vs 2mCA): t= 1.0, p = 0.305
		MS4(0 vs 2mCA): t = -1.3, p = 0.217	MS4(0 vs 2mCA): t = -1.8, p = 0.081	MS4(0 vs 2mCA): t = -3.5, p = 0.001**
		MS5(0 vs 2mCA): t = 3.1, p = 0.004**	MS5(0 vs 2mCA): t = 3.0, p =0.006*	MS5(0 vs 2mCA): t = 0.4, p = 0.673

Changes in microstate metrics with maturation during wakefulness



SI Figure 2 : Evolution in microstate metrics (duration, explained variance, coverage and occurrence)between 0mCA (dark purple) and 2mCA (light purple) during different wakefulness. Asterisks represent significant differences between 0mCA and 2mCA infants (** p<0.005, *p<0.05).



SI Figure 3 : Normalized transition probabilities between different microstates at wakefulness in preterms at 0/2mCA (left/right column). Asterisks represent significant favorable transitions. The dark squares highlight the MS shared between 0 and 2mCA.