TWITCHES DURING N2 AND N3 ARE COUPLED WITH SLEEP SPINDLES BUT NOT DELTA OSCILLATIONS IN 6-MONTH-OLD INFANTS

Taylor G. Christiansen[1], Greta Sokoloff[1,2], Hailey C. Long[1],
Olivia K. Kopp[1], Lydia K. Karr[1], and Mark S. Blumberg[1,2]

[1]Department of Psychological and Brain Sciences
[2]Iowa Neuroscience Institute
University of Iowa
Psychological and Brain Sciences Building
340 Iowa Ave
Iowa City, Iowa 52242

Corresponding author:
Mark S. Blumberg, PhD
Department of Psychological and Brain Sciences
340 Iowa Avenue
G60 PBSP
University of Iowa
Iowa City, IA 52242
Email: mark-blumberg@uiowa.edu

ABSTRACT

Twitches are discrete movements that characterize REM sleep. However, recent work showed that twitches also occur during NREM sleep in human infants beginning around 3 months of age, a time when sleep spindles and the cortical delta rhythm are also emerging. Further, NREM twitches co-occur with sleep spindles, suggesting a unique functional role. Given that NREM sleep is composed of distinct substages, we investigated whether twitching and twitch-spindle coupling are differentially expressed during N2 and N3 sleep. In 6-month-old human infants (n=21; 7 females), we recorded EEG, respiration, and video during daytime sleep. We found that the high intensity of twitching during N2 resembled REM more than N3. In contrast, sleep spindles exhibited similar temporal characteristics during N2 and N3. Also, despite differences in the intensity of twitching during N2 and N3, significant twitch-spindle coupling occurred in both stages. Finally, twitch rate was inversely related to delta power across NREM periods. These findings suggest that although twitching occurs during REM, N2, and N3 sleep at this age, its expression is compatible with some sleep components (e.g., rapid eye movements, sleep spindles) but not others (e.g., cortical delta), highlighting the continuing need to better understand the dynamic organization of sleep and its individual components in early development.

Keywords: infancy, development, sleep spindles, myoclonic twitches, delta oscillations, sensorimotor system

STATEMENT OF SIGNIFICANCE

Twitching has long been recognized as a core component of mammalian REM sleep. However, twitching also occurs during non-REM (NREM) sleep in human infants beginning around 3 months of age. Given that NREM sleep is composed of distinct stages (including N2 and N3), we asked whether twitching and its temporal coupling with sleep spindles—which are brief oscillatory events that are implicated in neuroplasticity and neurodevelopmental disorders—are differentially expressed during the two stages. Even though twitching was more intense during N2 than N3, twitch-spindle coupling occurred in both stages, again suggesting a unique and likely transient functional contribution to sensorimotor development. A necessary next step is to delineate the developmental trajectory of twitching and twitch-spindle coupling beyond 6 months of age.

INTRODUCTION

During infancy, when the brain is undergoing its most rapid development, sleep is the predominant state, with human newborns spending up to 16 hours each day asleep, evenly divided between rapid eye movement (REM) and non-REM (NREM) sleep[1]. The relatively high proportion of REM sleep in early development inspired the ontogenetic hypothesis, which posited a mechanistic relation between REM sleep—especially its phasic events—and brain development. Limb twitches are among the most prevalent phasic components of REM sleep in early development[2]. In rat pups, twitches are spontaneously generated in the brainstem[3] to produce movements throughout the body. These movements trigger sensory responses that cascade throughout the sensorimotor system, likely refining sensorimotor circuits[2] and contributing to the development of cerebellar-dependent internal models[4–6].

Similar to rat pups, human infants exhibit high rates of twitching across the body during REM sleep[7]. However, in contrast to rat pups, human infants between 3 and 6 months of age also increasingly express twitches during NREM sleep[8]. The presence of twitches at high rates during NREM in infants is surprising given the apparent lack of similar reports in other mammals. Additionally, twitching exhibits distinct developmental trajectories during REM and NREM sleep: Specifically, whereas the rate of NREM twitching increases across the first 6 postnatal months, twitching during REM sleep occurs at a high and steady rate throughout this period.

One path to understanding the functional significance of NREM twitches is to examine the brain activity that accompanies them. For example, around the same time that NREM

twitches are emerging, sleep spindles—brief thalamocortical oscillations with peak frequencies of 11-15 Hz[9]—are also emerging along the cortical sensorimotor strip[8]. NREM twitches and spindles are temporally coupled in that the probability of a spindle increases around the onset of a twitch[8]. Given the established association of spindles with learning and heightened neuroplasticity[10], the coupling of NREM twitches with spindles suggested a unique contribution to sensorimotor development. In addition to sleep spindles, high-amplitude delta waves (0.5-4 Hz) are also characteristic of NREM sleep and begin to emerge over the first few postnatal months[11]; the relation between delta and twitching has not been explicitly examined.

After sleep spindles and delta waves emerge, they help to distinguish the three substages of NREM sleep: Specifically, N1 is characterized by the slowing of EEG and marks the transition to sleep without sleep spindles or delta waves; N2 is characterized predominantly by the presence of sleep spindles; and N3 is characterized predominantly by the presence of cortical delta, although spindles can also be present[12]. In our previous study[8], we did not assess whether twitching occurs differentially during NREM stages. Thus, here we characterize the rate and patterning of twitching in N2 and N3 sleep, as well as REM sleep, focusing on infants at 6 months of age when sleep spindles and delta are both clearly expressed. Our findings extend our earlier report in showing that infants twitch during all three sleep stages, but they do so at higher rates during N2 and REM sleep compared to N3. However, sleep spindle characteristics did not differ between N2 and N3 and twitch-spindle coupling occurs in both stages. We also find a negative correlation between twitching and delta power, suggesting that they are incompatible. Altogether, our findings highlight the complex and dynamic relations among the

behavioral and electrophysiological features of sleep in early development. They also set the stage for future delineation of the full developmental trajectory of these fundamental sleep phenomena.

METHODS

Participants

Twenty-four full-term infants, 5.35-6.99 months of age, participated in the study. Three infants were excluded from analysis because either a majority of the EEG for their session was not useable due to movement artifact, or their total sleep time was less than 5 min. Thus, the final sample comprised 21 infants (7F, 14M, 6.19 ± 0.10 months). Of this sample, 10 were included in a previous study[8] and 11 were newly collected. All data collection and scoring procedures are identical for all infants unless otherwise indicated. Infants and caregivers were recruited from the broader Iowa City area via email and print advertisements. All procedures were approved by the Institutional Review Board at the University of Iowa.

Experimental design and procedure

Daytime sleep was recorded during the infant's usual nap time. Upon arrival, infants were changed into a plain black onesie and the experimenters measured the child's head circumference. A 128-channel HydroCel geodesic sensor net (Electrical Geodesics, Inc., Eugene, OR), sized to the child's head, was presoaked in a body temperature potassium chloride and baby shampoo solution. After the cap was applied, electrode impedances were checked, and electrodes were adjusted until the vast majority of electrodes had impedances below 100 KΩ. Impedance values were saved at the start and the end of

each sleep session. The EEG cap was connected to a Net Amps 200 Amplifier (Electrical Geodesics, Inc.). Infants slept in a supine, semi-reclined position in a baby lounger.

EEG and respiratory data were acquired at 1000 samples/s to a computer running Net Station software. A high-definition video camera (Model Q6055, Axis Communications, Lund, Sweden) provided a frontal view of the child to record movements of the eyes, face, head, and limbs. The video recording, acquired at 30 frames/s, was integrated with the EEG recording system to ensure synchrony between the data streams. When video data was exported out of Net Station software timestamps were used to synchronize physiological and video data. Recordings continued for as long as the child remained asleep. While infants slept, caregivers sat with their child in the testing room or waited in a nearby room where they could monitor the child via video.

Behavioral coding and interobserver reliability

Twitches were visually scored by two independent raters using methods similar to those described previously[7,8]. Briefly, two raters first reviewed videos for wake periods and the occurrence of startles and arousals. Only periods of sleep were scored for twitches. Using Datavyu (datavyu.org) or Net Station (Electrical Geodesics, Inc.), coders scored the video data in multiple passes to identify twitches of the face, left and right legs, and left and right arms. In addition, for the 11 participants comprising the newly collected sample, twitches were scored with more precision using Datavyu to measure twitch durations and to separately identify twitches of the toes, feet, and legs (combining knee and hip), and twitches of the fingers, hands, and arms (combining shoulder and elbow). Eye movements were also coded and designated as rapid eye movements (REMs) when they

had durations less than 1 s. Coders did not score movements during sleep that were incidental to movements elsewhere on the body (e.g., respiration; movements of other limbs). Finally, coders were always blind to sleep stage when scoring twitches. A primary scorer was designated to score 100% of a video and a secondary scorer was designated to score at least 25% of the same video; only periods scored by both raters were analyzed for inter-observer reliability.

Inter-observer reliability was assessed as described previously[7,8]. Briefly, onsets and durations of twitches were determined by the two coders across all movement categories. When coders disagreed, they independently reviewed the videos around the periods of interest and, when necessary, rescored the video. After this second pass through the data, for all twitches for which each coder agreed (i.e., twitch onsets within 1 s), the onsets and durations were aligned to the primary scorer. Cohen's kappa ranged from 0.67 to 0.99 (median: 0.87). Finally, the primary and secondary coders made a final pass together through the data record and all remaining discrepancies were resolved by mutual agreement.

Identification of sleep stages

EEG, respiration, and REMs were used to determine sleep stages. Two trained raters, blind to behavioral scoring, identified sleep stages in 30-s epochs together using AASM criteria[12]. REMs were occasionally visible from frontal EEG signals but could not be consistently identified (EOG was not recorded). Therefore, muscle atonia, REMs, and respiratory variability[13] helped to confirm REM sleep.

Data analysis

EEG signals. Preprocessing of EEG signals was performed in MATLAB (MathWorks, Natick, MA) using EEGLab[14]. Data were band-pass filtered (0.5-70 Hz) and a 60-Hz notch filter was applied. Bad channels were visually identified and interpolated with spherical interpolation. After interpolation, data were referenced to the average across all electrode sites. Data were down sampled from 1000 to 250 Hz. Periods with movement artifact were visually identified and excluded from further analysis.

Sleep spindles were detected using custom MATLAB scripts. The methods for spindle detection were described previously[8]; however, here the frequency range was expanded from 12-14 Hz to 11-15 Hz to increase sensitivity to slower frontal spindles that emerge at older ages[15]. Using a Hilbert transform of the filtered data, the amplitude of the signal was generated for artifact-free periods. The onset of a sleep spindle was marked when signal amplitude exceeded a threshold of 2x the median amplitude for at least 1 s. After this 1-s minimum criterion was met, the offset of the spindle was marked when the signal decreased below the threshold for at least 1 s. We visually reviewed all automatically detected spindles to confirm that they corresponded well with visually detected spindles in raw and filtered EEG records. Spindles were identified from the C3 electrode because sigma power is maximal in central electrodes at these ages[8]. The C3 electrode had good data quality across all participants.

Delta power was also analyzed using custom MATLAB scripts. A Hilbert transform was applied to artifact-free EEG data from the C3 electrode which was band-pass filtered in

the delta frequency range (0.5-4 Hz). Delta power was calculated as the amplitude squared (μV^2) of the transformed data at each timepoint.

Twitching. To obtain measures of twitch rate, the number of twitches in each body part was summed across periods of REM and NREM and divided by the total duration of each sleep stage. Twitch rates in N2 and N3 were also calculated. A total of 17 participants had REM, N2, and N3 (3 participants did not have any REM and 1 participant did not have N2). Therefore, for analysis of REM vs. NREM twitching, 18 participants were used; for the analysis of N2 vs. N3 twitching, 20 participants were used. Because twitch rates were not normally distributed (Shapiro-Wilks test, $p<.05$), the non-parametric Wilcoxon matched-pairs signed-ranks test was used.

Twitch duration was calculated using twitch onset and offset times as described above. For each stage of sleep, we analyzed the duration of twitches and the proportion of twitches in each body part; for the latter analysis, we divided the number of twitches in each body part by the total number of twitches for that individual. For these analyses, only newly collected data were used because these were the only data for which we had accurate twitch durations. Of the 11 participants, one did not exhibit REM sleep and was excluded from these analyses. Additionally, one participant had fewer than five twitches during N3 and was excluded from the body-part analysis. Paired t tests were used to compare twitch durations between sleep stages, and a two-way ANOVA was used to compare the proportion of twitching across body parts and sleep stages.

Inter-twitch intervals (ITIs) were calculated as the time between successive twitches. REMs were not included. ITIs were not included if there was an intervening wake period

or a transition between different stages of sleep. ITI data within each sleep stage were pooled across participants to generate log-survivor plots.

We divided twitches into those occurring as part of a burst or as single events. Bursts were defined as a series of at least two twitches with <0.5 s between successive twitch onsets. A twitch burst ended when the onset of the next twitch occurred at an interval >0.5 s. Therefore, single twitch events occurred >0.5 s after the preceding twitch and >0.5 s before the subsequent twitch. Burst rate was calculated by dividing the total number of bursts during each sleep stage by the total time spent in each sleep stage. Burst rates had non-normal distributions (Shapiro-Wilks test, $p<.05$); therefore, the non-parametric Friedman test was used, and a Conover test was used for pairwise comparisons using PMCMRplus package in R[16]. We also analyzed the proportion of twitches occurring in bursts in each sleep stage, calculated by dividing the number of twitches occurring in bursts by the total number of twitches. Only participants with at least 10 twitches across all stages of sleep were used in these analyses; 12 participants met this criterion.

Sleep spindles. Spindle rate was calculated by dividing the total number of spindles detected at the C3 electrode by the total number of minutes spent in artifact-free N2 or N3. Spindle amplitude was considered as the maximum voltage (μV) of the Hilbert-transformed signal for each spindle waveform; sleep spindle frequency (Hz) was also determined. Mean spindle amplitude and mean spindle frequency during N2 and N3 were computed for each participant. One participant did not have N2 and was excluded from this analysis. Two additional participants were excluded from the spindle analyses because they had fewer than 2 min of artifact-free N2 or N3. Thus, the final spindle

analyses included 17 participants. For comparing spindle rate, amplitude, and frequency, paired t tests were used.

Inter-spindle intervals (ISIs) were calculated as the time between successive sleep spindles at the C3 electrode. ISIs were excluded if there was an intervening wake period or a transition between stages of sleep. ISI data within each sleep stage were pooled across participants to generate log-survivor plots.

Association between delta power and twitching. We analyzed the relations between delta power and twitches during NREM sleep. To conduct these analyses, mean delta power and number of twitches were calculated for each 1-min window of artifact-free NREM sleep. First, for each infant, we conducted a linear regression relating delta power to the number of twitches. Next, we used a multilevel linear mixed-effects analysis to evaluate whether delta power predicted variations in twitches across infants. Models were estimated using the lmer function within the lme4 package in R[17]. Intraclass correlations (ICCs) indicated that 57% of the variance in delta power was due to between-infant differences. Therefore, delta power was separated into two predictors: (1) a between-infant measure representing an infant's mean delta power across all NREM windows ($MeanDelta_i$ for each infant *i*), and (2) a within-infant measure representing an infant's delta power deviation in that window from their individual mean ($WithinDelta_{ti}$ for each timepoint *t* for each infant *i*). This approach allows us to examine whether twitching was related to differences in average delta power across infants (between-infant effect) versus fluctuations across time in an individual infant's mean delta power (within-infant effect)[18]. All delta measures were divided by 100 such that a one unit increase in $MeanDelta_i$ or

$WithinDelta_{ti}$ indicates a 100 μV^2 increase in delta. The $MeanDelta_i$ is centered around the grand mean of delta such that when $MeanDelta_i$ is zero, the infant mean delta power is 1100 μV^2. The $WithinDelta_{ti}$ was centered around the individual mean: a higher or lower value represents a higher or lower amount of delta in that window relative to the infant's mean.

The equation for the model is as follows:

Level 1: $\text{Twitches}_{ti} = \beta_{0i} + \beta_{1i}\,(\text{WithinDelta}_{\text{ti}}) + e_{ti}$

Level 2: $\text{Intercept: } \beta_{0i} = \gamma_{00} + \gamma_{01}(\text{MeanDelta}_{\text{i}}) + U_{0i}$

$\text{Within} - \text{Person Delta: } \beta_{1i} = \gamma_{10}$

In the Level 1 model, the number of twitches across time ($Twitches_{ti}$) is a function of an individual intercept (β_{0i}), the within-person effect of delta power (β_{1i}), and the residual number of twitches (e_{ti}) at timepoint *t* for infant *i*. In the Level 2 model, the individual intercept (β_{0i}) is a function of a fixed intercept (γ_{00}), the main effect of between-infant delta power (γ_{01}), and an individual-specific random intercept (U_{0i}). The individual effects of within-infant delta power (β_{1i}) are equivalent to the fixed effect (γ_{10}). Multilevel models were estimated using restricted maximum likelihood (REML) estimation[19,20].

Coupling between twitches and sleep spindles. For each participant, we identified the total number of spindles that co-occurred with a twitch. For each spindle, if at least one twitch occurred within the onset and offset of the spindle, it was classified as co-occurring with a twitch. We then determined the probability of a twitch given that a sleep spindle occurred (i.e., P(Twitch | Spindle)) by dividing the number of spindles co-occurring with twitches by the total number of spindles. Next, we calculated the inverse, that is, the

probability of a spindle given a twitch (i.e., P(Spindle | Twitch)), by dividing the number of twitches co-occurring with a spindle by the total number of twitches. These calculations were performed separately for N2 and N3. Nine participants were excluded from these analyses because they did not have at least 10 spindles and 10 twitches in each stage (N2 & N3), thus leaving 12 participants for analysis.

We plotted perievent histograms to assess the probability of a sleep spindle occurring in the 40-s period around twitch onset (i.e., ± 20 s). Similarly, perievent histograms were used to assess the probability of a twitch occurring in the 40-s period around the midpoint of a spindle. These analyses were performed separately for N2 and N3. Only participants with at least 10 spindles and 10 twitches in each state were included in the analysis, thus leaving 14 participants in N2 and 16 participants in N3. To confirm that the observed probabilities were greater than expected by chance, data were compared to shuffled twitch and spindle probabilities. The shuffled data were used to compute a 95% confidence interval ($p < 0.05$) at each time point.

Finally, to examine if the P(Twitch | Spindle) and P(Spindle | Twitch) occurred at above-chance levels, onset times for the trigger events were shuffled using a Monte Carlo procedure. For instance, for P(Twitch | Spindle), the onset times of the spindles (i.e., the triggers) were shuffled 1000 times in a manner that retained the original distribution of inter-spindle intervals; only the order of the intervals was randomized (a parallel procedure was used when the triggers were twitches). For each of the 1000 permutations of randomized data, the method described above for calculating mean probabilities was repeated and a grand mean for each plot was calculated. The probabilities from the shuffled data were considered the expected probabilities. To test for differences in the

observed and expected probabilities during N2 and N3, a two-way repeated-measures ANOVA was performed.

Statistical analyses

All statical analyses were conducted in SPSS Statistics 29.0 (IBM, Armonk, NY) unless otherwise specified. Before conducting t tests or ANOVAs, data were tested for normality using the Shapiro-Wilks tests. When normality assumptions were violated, non-parametric tests were used. Sphericity was tested using Mauchly's test. When sphericity was violated, a Huynh-Feldt correction was applied to the degrees of freedom. Multiple pairwise comparisons were adjusted using the Dunn-Šidák correction. The central tendency of the data is reported as the mean, and dispersion as SEM. The measure of effect size for ANOVAs is represented as partial eta-squared.

RESULTS

We recorded electrophysiological and behavioral data during daytime sleep in 6-month-old infants (n=21). Approximately half (n=10) of the data were published previously[8] and the remainder were newly collected for this study. Across all sessions, infants slept an average of 26.73 ± 2.76 min, comprising 5.44 ± 1.15 min of REM sleep, 4.87 ± 0.64 min of N2 sleep, and 16.25 ± 2.40 min of N3 sleep.

As expected from our previous report, twitches occurred during NREM sleep, that is, in the presence of sleep spindles and delta oscillations when REMs were absent. We also observed twitches in the presence of REMs, indicative of REM sleep. The rate of twitching during NREM (9.13 ± 1.16 twitches/min) was not significantly different than the rate during REM (13.70 ± 2.68 twitches/min; Z = -1.24; **Figure 2A$_1$**). The duration of individual

twitches also did not differ between NREM (0.44 ± 0.04 s) and REM (0.51 ± 0.05 s; $t(9) = -1.74$; **Figure 2A$_2$**). As described previously [8], a higher proportion of twitches occurred in some body parts (e.g., fingers and toes) than other body parts (e.g., arms and legs; **Figure 2A$_3$**); ANOVA revealed a significant main effect of body part ($F(2.45, 22.04) = 12.72$, $p < .001$, $\hat{\eta}_p^2 = 0.59$), but neither the main effect of sleep stage ($F(1, 9) = .51$) nor the body part x sleep stage interaction was significant ($F(2.61, 23.46) = 1.95$), indicating that twitch patterning across the body was similar in REM and NREM. Thus, at 6 months of age, there are no discernible differences in the characteristics of twitches during NREM and REM sleep.

Twitch characteristics differ between N2 and N3

Although the rate of twitching did not differ between NREM and REM sleep, it did differ significantly within NREM between N2 (13.45 ± 1.95 twitches/min) and N3 (5.68 ± 0.77 twitches/min; $Z = -3.21$, $p = 0.001$; **Figure 2A$_1$**). However, the duration of individual twitches did not differ significantly between N2 (0.45 ± 0.04 s) and N3 (0.43 ± 0.04 s; $t(10) = 0.50$; **Figure 2B$_2$**). Also, although the distribution of twitches across the body was not uniform, it was similar in N2 and N3; specifically, there was a significant main effect of body part ($F(2.57, 23.12) = 16.20$, $p < .001$, $\hat{\eta}_p^2 = 0.64$), but neither the main effect of sleep stage ($F(1, 9) = 1.00$) nor the body part x sleep stage interaction was significant ($F(3.59, 32.34) = 2.75$; **Figure 2B$_3$**).

To further illustrate differences in the rate of twitching across sleep stages, we created log-survivor plots of inter-twitch intervals, pooled across infants, during REM, N2, and N3 (**Figure 2C**). For log-survivor plots, the slopes of the distributions are proportional to the

rate of twitching, with steeper slopes indicating higher rates. Similar to previous reports of the temporal organization of twitching in human infants[7] and infant rats[21], the slopes of the distributions in all three states were steepest at the shortest intervals, indicating that many twitches occurred in rapid succession (i.e., in bursts). Also, it is evident from the slopes of the log-survivor plots that the rates of twitching were very similar during REM and N2, and both were higher than N3.

The log-survivor plots supported our visual assessment of the raw data (see **Figure 1B**) that twitching in all three stages, particularly in REM and N2, often occurs in bursts. To further analyze this phenomenon, we defined a burst as a series of two or more twitches in which each inter-twitch interval was less than 0.5 s. The rate of bursts was significantly different among the three sleep stages (χ^2 (2) = 12.17, p = .002; **Figure 2D$_1$**), with bursts occurring at more than twice the rate during REM (3.33 ± 0.56 bursts/min) and N2 (2.58 ± 0.62 bursts/min) than during N3 (1.22 ± 0.19 bursts/min). Additionally, a higher proportion of twitches was part of a burst during REM (0.65 ± 0.05) and N2 (0.63 ± 0.05) than during N3 (0.48 ± 0.03; *F*(2, 22) = 8.768, p= .002, $\hat{\eta}_p^2$ = .443; **Figure 2D$_2$**). In summary, the differences in the rate and burstiness of twitching lead us to conclude that twitching was more intense during N2 and REM than during N3.

Sleep-spindle characteristics do not differ between N2 and N3

To account for the differences in twitching between N2 and N3, we first considered the possibility that they are related to a lower rate of sleep spindles in N3, as is characteristic in adults[9,22,23]. However, there was no significant difference in the rate of spindles between N2 (3.50 ± 0.26 spindles/min) and N3 (3.38 ± 0.25 spindles/min; t(16) = 0.52;

Figure 3A). Also, there were no significant differences in peak spindle frequency (N2: 13.15 ± 0.09 Hz; N3: 13.19 ± 0.09 Hz; $t(16)= -1.53$; **Figure 3B**), or spindle amplitude (N2: 20.08 ± 0.85 μV; N3: 19.38 ± 0.84 μV; $t(16) = 1.10$, **Figure 3C**). Finally, log-survivor plots of pooled inter-spindle intervals show nearly overlapping distributions for N2 and N3 (**Figure 3D**). Thus, the similar rates, frequencies, amplitudes, and temporal distributions of spindles argue against the possibility that spindles are related in any way to the lower rate of twitching in N3.

Inverse relations between twitching and delta power

We next considered whether the presence of cortical delta during N3 is associated with its lower rate of twitching. For each infant, we calculated linear regressions relating mean delta power to the number of twitches across all 1-min windows of artifact-free NREM sleep. We found inverse relations between delta power and twitches, such that windows with higher delta power were associated with fewer twitches (**Figure 4A**).

Next, a multilevel linear mixed-effect model was used to evaluate whether delta power predicted variation in the rate of twitching across infants (**Figure 4B**). There was a significant within-infant effect of delta, such that for every 500 μV^2 increase/decrease in delta power during a 1-min window, the number of twitches in that window was expected to be 4.6 twitches higher/lower than that infant's average ($t(288.61) = -10.04$, $p < 0.001$; **Table 1**). In contrast, between-infant variation in delta power did not significantly predict the rate of twitching: Infants with higher average delta power did not exhibit lower rates of twitching. In summary, these findings suggest that within periods of NREM, higher delta power, as occurs during N3, is incompatible with higher rates of twitching.

Sleep spindles are temporally coupled with twitches in both N2 and N3

We previously reported that the probability of sleep spindles during NREM sleep increases significantly around the time of a twitch[8]. Here, we analyzed their temporal coupling separately during N2 and N3.

We first assessed the probability of a sleep spindle given the occurrence of a twitch (**Figure 5A$_1$**). During N2 and N3, the probability of a spindle exceeded the 95% confidence intervals in the period surrounding a twitch (**Figure 5A$_2$**). Overall, the probabilities were similar during N2 (29.49% ± 2.44%) and N3 (25.99% ± 3.48%; **Figure 5A$_3$**). The observed probability of a spindle given a twitch was significantly greater than expected by chance across both sleep stages ($F(1, 11) = 77.72$, $p < 0.001$, $\hat{\eta}_p^2 = 0.88$). In contrast, neither the main effect of sleep stage ($F(1, 11) = 1.93$) nor the sleep stage x observed-expected interaction ($F(1, 11) = 0.11$) was significant. Thus, given that a twitch occurred, a sleep spindle was equally likely to co-occur during N2 and N3.

Next, we inverted the analysis and measured the probability of a twitch given the occurrence of a sleep spindle (**Figure 5B$_1$**). During N2 and N3, the probability of a twitch exceeded the 95% confidence intervals in the period surrounding a sleep spindle, although the peak during N3 was lower (**Figure 5B$_2$)**. Overall, the probability was 51.28% ± 3.75% during N2, compared to only 26.34% ± 3.03% during N3 (**Figure 5B$_3$)**, consistent with the significantly lower rate of twitching in N3 (see **Figure 2B$_1$**). Given a sleep spindle, ANOVA revealed that a twitch was significantly more likely to co-occur during N2 than N3, as evidenced by the significant sleep stage x observed-expected interaction ($F(1, 11) = 13.483$, $p = .004$, $\hat{\eta}_p^2 = 0.55$, **Figure 5B$_3$**). Post hoc tests revealed

that for both N2 and N3, the observed probabilities were significantly greater than expected by chance (N2: $t(11) = 6.609$, $p < 0.001$; N3: $t(11) = 4.640$, $p < 0.001$). Thus, although the lower rate of twitching during N3 reduced opportunities for spindles to co-occur with twitches, twitch-spindle coupling was still greater than expected by chance.

DISCUSSION

Building on the surprising discovery that twitches emerge postnatally during NREM sleep and are temporally coupled with sleep spindles[8], here we delved deeper to determine whether these phenomena are expressed differentially during N2 and N3. The first main finding is that twitching during N2 and REM is more intense than during N3, reflecting its significantly higher rate and tendency to occur in bursts. Second, the reduced rate of twitching during N3 was inversely related to cortical delta power, suggesting an incompatibility between delta and twitching. Finally, despite differences in twitching between N2 and N3, twitch-spindle coupling occurred in both stages. Overall, these findings highlight the changing organization of sleep over development and suggest that twitch-spindle coupling, which is not expected to persist into adulthood, makes a transient functional contribution to early sensorimotor development.

The main limitations of this study are the relatively small sample size and the focus on a single age. We chose 6 months because it is the oldest age investigated in our prior study[8], and the infants at that age exhibit distinct N2 and N3. Thus, it was a good choice for extending our previous study to examine the issues of differential twitching and twitch-spindle coupling during N2 and N3. Moreover, in addition to reanalyzing the data from the infant participants in that previous study, we also collected new data to double the size of

the prior dataset. Next, we are building on this detailed assessment at 6 months of age to delineate the developmental trajectories of twitching and twitch-spindle coupling over subsequent months and years.

Why is the rate of twitching lower in N3?

Twitches occurred at half the rate during N3 compared to N2 (and REM). Why? Based on known differences in spindle rates between N2 and N3 in adults[9,22,23], and the twitch-spindle coupling during NREM in infants[8], we considered the possibility that, at least during NREM, differences in spindle rate are associated with differences in twitching. However, we found that spindle rates in N2 and N3 did not differ. This finding is consistent with an earlier study in infants at 4 and 6 months of age[24]. Indeed, one recent study in 6-8-month-olds reported a higher spindle rate during N3[25]. Therefore, spindles cannot explain the differential rate of twitching in N2 and N3.

Given that the cortical delta rhythm distinguishes N3 from both N2 and REM, perhaps the presence of delta is incompatible with twitching (**Figure 6**). Consistent with this idea, we found that within-infant fluctuations in delta power were negatively associated with NREM twitch rate. Assuming this association has a causal basis, through what mechanism might cortical delta influence twitching? One possibility is that cortical delta directly interferes with the production of twitches; however, based on the current understanding of twitch production during REM sleep in infant[3,26] and adult[27,28] animals, twitches are produced in the brainstem, not the cortex. Of course, it is possible that the production of NREM twitches in human infants occurs via a different mechanism, but the many overlapping features of twitches during NREM and REM sleep (see **Figure 2**) point to their having a

similar neural basis. Still another argument against a cortical contribution to twitching, especially in early infancy, concerns the absent or weak motor outflow arising from primary motor cortex[29].

Even if cortical delta is not causally related to twitching, it is still possible that it serves as a proxy for other related processes in the brainstem that are causally related to twitching. For example, it was recently reported in 12-day-old rats, the age when cortical delta is first emerging, that delta oscillations also occur within a medullary structure, the parafacial zone[30], that is implicated in the regulation of NREM sleep[31]. Not only was spiking activity within the parafacial zone phase-locked to the locally produced delta, but the delta rhythms in the cortex and parafacial zone were coherent. Thus, it may be that the presence of cortical delta signifies a brain state that is incompatible with the production of twitching. Testing this idea in human infants may not currently be feasible, but future assessments of the developmental trajectory of twitching in relation to cortical activity during N2 and N3 may help to inform our understanding of possible contributing mechanisms.

Twitch-spindle coupling occurs during N2 and N3

Twitch-spindle coupling is a robust phenomenon; for example, at least half of the sleep spindles during N2 co-occurred with a twitch. Thus, given the established association between sleep spindles and neuroplasticity[9,10,32], twitch-spindle coupling may make a unique functional contribution to typical sensorimotor development. Moreover, twitch-spindle coupling may open new avenues for understanding atypical development, including neurodevelopmental disorders that are marked by deficits in sensorimotor

function and sleep spindles. Sleep-spindle deficits have been well documented in individuals with schizophrenia[33–35], autism[36–38], cerebral palsy[39], and other neurodevelopmental disorders[40]. Sensorimotor problems are also common features of many of the same neurodevelopmental disorders[41–46]. Therefore, the investigation of twitch-spindle coupling in early infancy may help to advance our understanding of the origins of neurodevelopmental disorders and aid in their early detection.

Why have NREM twitches in infants not been reported previously?

There are a variety of methodological and conceptual explanations for why previous investigators appear to have overlooked the presence of NREM twitches in infants. First, although twitching has long been identified as a core component of REM sleep, there have been few systematic investigations of the spatiotemporal properties of twitching in humans at any age. Instead, twitching has typically been assessed in a binary fashion—as either being present or absent[47–51]. Moreover, in our experience, the careful assessment of twitches from video is still the most accurate way to detect twitching across the body; accelerometry and other similar methods fail to detect the vast majority of twitches, and time-lapse video misses twitching altogether. Thus, one can easily understand why investigators who are not interested in twitching per se might fail to notice its occurrence outside of REM sleep, especially given the strong association of twitching with REM sleep. Without systematic behavioral assessments of twitching in relation to EEG and other electrophysiological signals, periods of twitching with NREM-related components would either be ignored, misclassified as REM sleep, or designated as indeterminate sleep.

The considerations above are only magnified by the likelihood that twitching during NREM sleep, at least at rates similar to those during REM sleep, may only exist within a narrow developmental window. In adults, there are reports of twitching—particularly finger twitching—during NREM sleep, but it occurs at much lower rates than during REM sleep[52–54], and twitch-spindle coupling is only a recently discovered phenomenon[8]. Thus, the present findings—made possible by real-time, coordinated behavioral and electrophysiological assessments of sleep—lay the foundation for a full explication of NREM twitching and twitch-spindle coupling over the infant and toddler years.

Conclusions

By detailing the spatiotemporal structure of twitching in relation to other sleep components at 6 months of age, this study reveals new aspects of the dynamic organization of infant sleep. The dynamic concept of sleep organization was first introduced to account for the dramatic changes in the sleep of premature infants[55]. But it is clear that equally dramatic changes continue throughout the postnatal period, including the emergence of NREM twitching and twitch-spindle coupling around 3 months of age[8]. We extended our earlier findings here by showing that twitching is differentially expressed during N2 and N3 at 6 months of age. Also, although twitching occurs across all stages of sleep at this age, some sleep components (e.g., REMs, sleep spindles) are compatible with high-intensity twitching, while other components (e.g., delta) are not (**Figure 6**). Looked at another way, N2 shares characteristics with both REM (i.e., high-intensity twitching) and N3 (i.e., sleep spindles). Such findings defy conventional adult-centric assumptions of sleep organization and underscore the difficulty of cleanly differentiating sleep stages in infancy when what we describe as “sleep” is itself changing over time.

Research in adults tells us where sleep organization is headed, but not how it gets there. Notably, we do not expect the high rates of NREM twitching or twitch-spindle coupling to persist into adulthood. Thus, it is likely that both of these aspects of infant sleep are transient, not unlike many other transient developmental features[56]. Thus, looking beyond 6 months of age, we expect that a full description of the developmental trajectory of these seemingly transient NREM phenomena will provide critical clues regarding their neural substrates and functional significance for the developing infant.

Acknowledgments. This research was supported by grants from the National Institute of Child Health and Human Development (R01-HD104616; R21-HD095153) to M.S.B. We thank Jimmy Dooley for help with MATLAB scripts, and Becky Yu, Hayley Chappel, and Daylan Carney for help with behavioral scoring.

Disclosure Statement
Financial disclosure: none.
Non-financial disclosure: none.

Data Availability. By the time of publication, coded data and a subset of videos will be shared on Databrary. Custom MATLAB scripts will be shared on Github.

REFERENCES

1. Roffwarg HP, Muzio JN, Dement WC. Ontogenetic development of the human sleep-dream cycle. *Science*. 1966;152:604-619. doi:10.1126/science.152.3722.604

2. Blumberg MS, Dooley JC, Sokoloff G. The developing brain revealed during sleep. *Curr Opin Physiol*. 2020;15:14-22. doi:10.1016/j.cophys.2019.11.002

3. Del Rio-Bermudez C, Sokoloff G, Blumberg MS. Sensorimotor processing in the newborn rat red nucleus during active sleep. *J Neurosci*. 2015;35(21):8322-8332. doi:10.1523/JNEUROSCI.0564-15.2015

4. Dooley JC, Sokoloff G, Blumberg MS. Movements during sleep reveal the developmental emergence of a cerebellar-dependent internal model in motor thalamus. *Curr Biol*. 2021;31:5501-5511. doi:10.1016/j.cub.2021.10.014

5. Mukherjee D, Sokoloff G, Blumberg MS. Corollary discharge in precerebellar nuclei of sleeping infant rats. *eLife*. 2018;7:e38213. doi:10.7554/elife.38213

6. Richardson AM, Sokoloff G, Blumberg MS. Developmentally unique cerebellar processing prioritizes self- over other-generated movements. *J Neurosci*. 2024;44(19):e2345232024. doi:10.1523/JNEUROSCI.2345-23.2024

7. Sokoloff G, Hickerson MM, Wen RY, Tobias ME, McMurray B, Blumberg MS. Spatiotemporal organization of myoclonic twitching in sleeping human infants. *Dev Psychobiol*. 2020;62(6):697-710. doi:10.1002/dev.21954

8. Sokoloff G, Dooley JC, Glanz RM, et al. Twitches emerge postnatally during quiet sleep in human infants and are synchronized with sleep spindles. *Curr Biol*. 2021;31(15):3426-3432.e4. doi:10.1016/j.cub.2021.05.038

9. Fernandez LMJ, Lüthi A. Sleep spindles: Mechanisms and functions. *Physiol Rev*. 2020;100(2):805-868. doi:10.1152/physrev.00042.2018

10. Rasch B, Born J. About sleep's role in memory. *Physiol Rev*. 2013;93(2):681-766. doi:10.1152/physrev.00032.2012

11. Jenni OG, Borbély AA, Achermann P. Development of the nocturnal sleep electroencephalogram in human infants. *Am J Physiol Regul Integr Comp Physiol*. 2004;286(3):R528-38. doi:10.1152/ajpregu.00503.2003

12. Berry R, Albertario C, Harding S, et al.; for the American Academy of Sleep Medicine. *The AASM Manual for the Scoring and Associated Events: Rules, Terminology and Technical Specifications*. Version 2.5. Darien, IL: American Academy of Sleep Medicine; 2018.

13. Isler JR, Thai T, Myers MM, Fifer WP. An automated method for coding sleep states in human infants based on respiratory rate variability. *Dev Psychobiol*. 2016;58(8):1108-1115. doi:10.1002/dev.21482

14. Delorme A, Makeig S. EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *J Neurosci Methods*. 2004;134(1):9-21. doi:10.1016/j.jneumeth.2003.10.009

15. McClain IJ, Lustenberger C, Achermann P, Lassonde JM, Kurth S, LeBourgeois MK. Developmental changes in sleep spindle characteristics and sigma power across early childhood. *Neural Plast*. 2016;2016:3670951. doi:10.1155/2016/3670951

16. Pohlert T. PMCMplus: Calculate pairwise multiple comparisons of mean rank sums extended. Published online 2024. https://CRAN.R-project.org/package=PMCMRplus

17. Bates D, Mächler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. *J Stat Softw*. 2015;67(1):1-48. doi:10.18637/jss.v067.i01

18. Hoffman L, Walters RW. Catching up on multilevel modeling. *Annu Rev Psychol*. 2022;73:659-689. doi:10.1146/annurev-psych-020821-103525

19. Patterson HD, Thompson R. Recovery of inter-block information when block sizes are unequal. *Biometrika*. 1971;58(3):545-554. doi:10.1093/biomet/58.3.545

20. Verbyla AP. A conditional derivation of residual maximum likelihood. *Aust J Stat*. 1990;32(2):227-230. doi:10.1111/j.1467-842X.1990.tb01015.x

21. Blumberg MS, Marques HG, Iida F. Twitching in sensorimotor development from sleeping rats to robots. *Curr Biol*. 2013;23(12):R532-R537. doi:10.1016/j.cub.2013.04.075

22. Cox R, Schapiro AC, Manoach DS, Stickgold R. Individual differences in frequency and topography of slow and fast sleep spindles. *Front Hum Neurosci*. 2017;11. doi:10.3389/fnhum.2017.00433

23. Gais S, Mölle M, Helms K, Born J. Learning-dependent increases in sleep spindle density. *J Neurosci*. 2002;22(15):6830-6834. doi:10.1523/JNEUROSCI.22-15-06830.2002

24. Louis J, Zhang JX, Revol M, Debilly G, Challamel MJ. Ontogenesis of nocturnal organization of sleep spindles: a longitudinal study during the first 6 months of life. *Electroencephalogr Clin Neurophysiol*. 1992;83(5):289-296. doi:10.1016/0013-4694(92)90088-y

25. Friedrich M, Wilhelm I, Mölle M, Born J, Friederici AD. The sleeping infant brain anticipates development. *Curr Biol*. 2017;27(15):2374-2380.e3. doi:10.1016/j.cub.2017.06.070

26. Kreider JC, Blumberg MS. Mesopontine contribution to the expression of active “twitch” sleep in decerebrate week-old rats. *Brain Res*. 2000;872(1-2):149-159. doi:10.1016/s0006-8993(00)02518-x

27. Gassel MM, Marchiafava PL, Pompeiano O. Phasic changes in muscular activity during desynchronized sleep in unrestrained cats. An analysis of the pattern and organization of myoclonic twitches. *Arch Ital Biol*. 1964;102:449-470.

28. Gassel MM, Marchiafava PL, Pompeiano O. Activity of the red nucleus during deep desynchronized sleep in unrestrained cats. *Arch Ital Biol*. 1965;103(3):369-396.

29. Blumberg MS, Adolph KE. Protracted development of motor cortex constrains rich interpretations of infant cognition. *Trends Cogn Sci*. 2023;27(3):233-245. doi:10.1016/j.tics.2022.12.014

30. Ahmad M, Kim J, Dwyer B, Sokoloff G, Blumberg MS. Coincident development and synchronization of sleep-dependent delta in the cortex and medulla. *Curr Biol*. 2024;34(12):2570-2579.e5. doi:10.1016/j.cub.2024.04.064

31. Anaclet C, Ferrari L, Arrigoni E, et al. The GABAergic parafacial zone is a medullary slow wave sleep–promoting center. *Nat Neurosci*. 2014;17(9):1217-1224. doi:10.1038/nn.3789

32. Manoach DS, Stickgold R. Abnormal Sleep Spindles, Memory Consolidation, and Schizophrenia. *Annu Rev Clin Psychol*. 2019;15:451-479. doi:10.1146/annurev-clinpsy-050718-095754

33. Ferrarelli F. Sleep spindles as neurophysiological biomarkers of schizophrenia. *Eur J Neurosci*. 2024;59(8):1907-1917. doi:10.1111/ejn.16178

34. Ferrarelli F, Huber R, Peterson MJ, et al. Reduced sleep spindle activity in schizophrenia patients. *Am J Psychiatry*. 2007;164(3):483-492. doi:10.1176/ajp.2007.164.3.483

35. Lai M, Hegde R, Kelly S, et al. Investigating sleep spindle density and schizophrenia: A meta-analysis. *Psychiatry Res*. 2022;307:114265. doi:10.1016/j.psychres.2021.114265

36. Farmer CA, Chilakamarri P, Thurm AE, Swedo SE, Holmes GL, Buckley AW. Spindle activity in young children with autism, developmental delay, or typical development. *Neurology*. 2018;91(2):e112-e122. doi:10.1212/WNL.0000000000005759

37. Mylonas D, Machado S, Larson O, et al. Dyscoordination of non-rapid eye movement sleep oscillations in autism spectrum disorder. *Sleep*. 2022;45(3):zsac010. doi:10.1093/sleep/zsac010

38. Page J, Lustenberger C, Fröhlich F. Nonrapid eye movement sleep and risk for autism spectrum disorder in early development: A topographical electroencephalogram pilot study. *Brain Behav*. 2020;10(3):e01557. doi:10.1002/brb3.1557

39. Berja ED, Kwon H, Walsh KG, Bates SV, Kramer MA, Chu CJ. Infant sleep spindle measures from EEG improve prediction of cerebral palsy. *Clin Neurophysiol*. 2024;167:51-60. doi:10.1016/j.clinph.2024.08.017

40. Herrera CG, Tarokh L. A thalamocortical perspective on sleep spindle alterations in neurodevelopmental disorders. *Curr Sleep Med Rep*. 2024;10(2):103-118. doi:10.1007/s40675-024-00284-x

41. Abboud R, Noronha C, Diwadkar VA. Motor system dysfunction in the schizophrenia diathesis: Neural systems to neurotransmitters. *Eur Psychiatry*. 2017;44:125-133. doi:10.1016/j.eurpsy.2017.04.004

42. Fournier KA, Hass CJ, Naik SK, Lodha N, Cauraugh JH. Motor coordination in autism spectrum disorders: a synthesis and meta-analysis. *J Autism Dev Disord*. 2010;40(10):1227-1240. doi:10.1007/s10803-010-0981-3

43. Green D, Charman T, Pickles A, et al. Impairment in movement skills of children with autistic spectrum disorders. *Dev Med Child Neurol*. 2009;51(4):311-316. doi:10.1111/j.1469-8749.2008.03242.x

44. Picard H, Amado I, Mouchet-Mages S, Olié JP, Krebs MO. The role of the cerebellum in schizophrenia: An update of clinical, cognitive, and functional evidences. *Schizophr Bull*. 2008;34(1):155-172. doi:10.1093/schbul/sbm049

45. Verbecque E, Johnson C, Scaccabarozzi G, Molteni M, Klingels K, Crippa A. Motor difficulties in children with neurodevelopmental conditions: A report from a cross-national study in Belgian and Italian children. *Eur J Pediatr*. 2025;184(2):174. doi:10.1007/s00431-025-06009-8

46. Walther S, Strik W. Motor symptoms and schizophrenia. *Neuropsychobiology*. 2012;66(2):77-92. doi:10.1159/000339456

47. Anders TF, Emde RN, Parmelee AH. *A Manual of Standardized Terminology, Techniques and Criteria for Scoring of States of Sleep and Wakefulness in Newborn Infants*. UCLA Brain Information Service/BRI Publications Office, NINDS Neurological Information Network; 1971.

48. Anders TF, Sostek AM. The use of time lapse video recording of sleep-wake behavior in human infants. *Psychophysiology*. 1976;13(2):155-158. doi:10.1111/j.1469-8986.1976.tb00092.x

49. Fukumoto M, Mochizuki N, Takeishi M, Nomura Y, Segawa M. Studies of body movements during night sleep in infancy. *Brain Dev*. 1981;3(1):37-43. doi:10.1016/S0387-7604(81)80004-6

50. Grigg-Damberger M, Gozal D, Marcus CL, et al. The visual scoring of sleep and arousal in infants and children. *J Clin Sleep Med*. 2007;03(02):201-240. doi:10.5664/jcsm.26819

51. Thoman EB. Sleeping and waking states in infants: A functional perspective. *Neurosci Biobehav Rev*. 1990;14(1):93-107. doi:10.1016/S0149-7634(05)80165-4

52. Baldridge BJ, Whitman RM, Kramer M. The concurrence of fine muscle activity and rapid eye movements during sleep. *Psychosom Med*. 1965;27(1):19-26. doi:10.1097/00006842-196501000-00003

53. Reiter AM, Roach GD, Sargent C, Lack L. Finger twitches are more frequent in REM sleep than in non-REM sleep. *Nat Sci Sleep*. 2020;12:49-56. doi:10.2147/nss.s233439

54. Stoyva JM. Finger electromyographic activity during sleep: Its relation to dreaming in deaf and normal subjects. *J Abnorm Psychol*. 1965;70(5):343-349. doi:10.1037/h0022511

55. Dreyfus-Brisac C. Ontogenesis of sleep in human prematures after 32 weeks of conceptional age. *Dev Psychobiol*. 1970;3(2):91-121. doi:10.1002/dev.420030203

56. Hall WG, Oppenheim RW. Developmental psychobiology: prenatal, perinatal, and early postnatal aspects of behavioral development. *Annu Rev Psychol*. 1987;38:91-128. doi:10.1146/annurev.ps.38.020187.000515

FIGURE LEGENDS

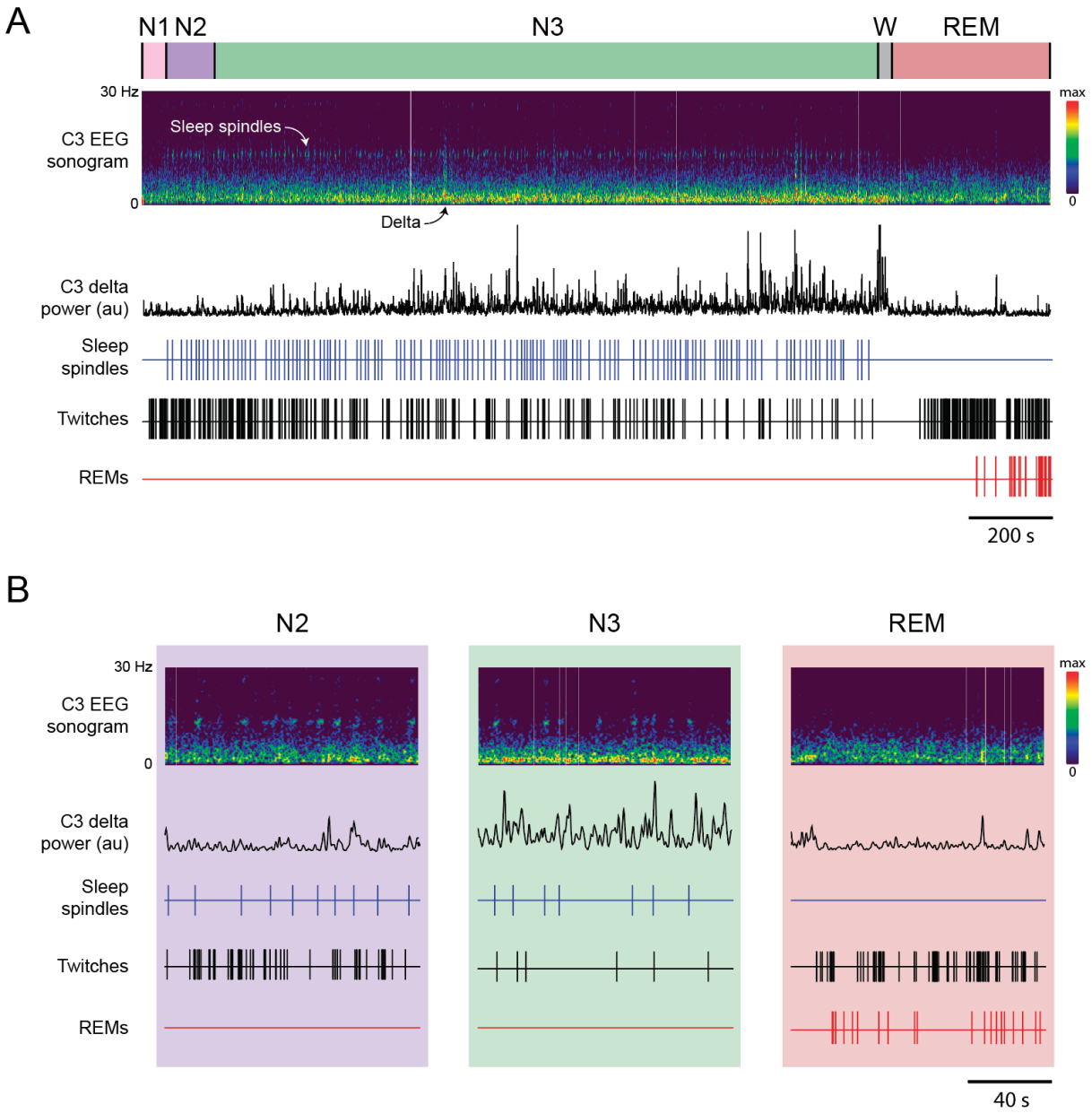

Figure 1. Representative behavioral and EEG data in a 6-month-old human infant. (A) Continuous 37.5-min record of N1 (pink), N2 (purple), N3 (green), wake (W, grey) and REM (red). EEG data are from the C3 electrode site and are represented as a sonogram (0-30 Hz) and delta power (0.5-4 Hz, black trace). The onset of spindles (blue ticks), limb twitches (black ticks), and REMs (red ticks) are also shown. **(B)** Same infant and data presentation as in (A) but for 2-minute periods of N2, N3, and REM sleep.

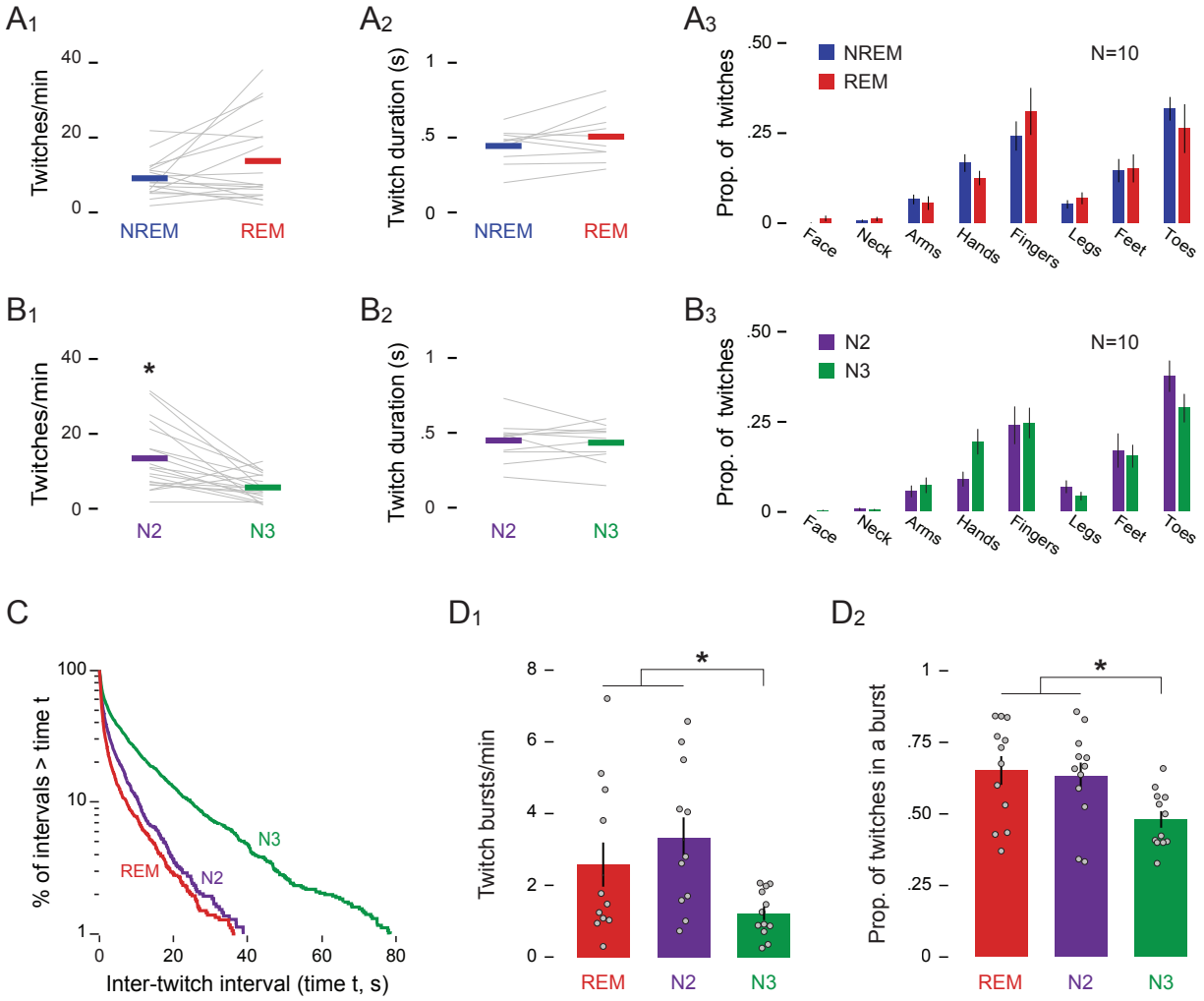

Figure 2. Characteristics of twitching during REM and NREM sleep. (A_1) Mean rate of twitching (twitches/min), **(A_2)** mean duration of twitches (s), and **(A_3)** mean (±SEM) proportion of twitching across various body parts during NREM (blue) and REM (red) sleep. Grey lines show data for individual infants. **($B_{1\text{-}3}$)** Same as in ($A_{1\text{-}3}$) but for N2 (green) and N3 (purple) sleep. **(C)** Log-survivor plot of inter-twitch intervals during periods of REM, N2, and N3 sleep. ITIs were pooled across sleep sessions: REM = 1795 twitches; N2 = 1239 twitches; N3 = 2060 twitches. **(D_1)** Mean (±SEM) twitch-burst rate (bursts/min) and **(D_2)** mean (±SEM) proportion of twitches occurring in a burst during REM, N2, and N3 sleep. Grey dots show data for individual infants. * $p < .05$

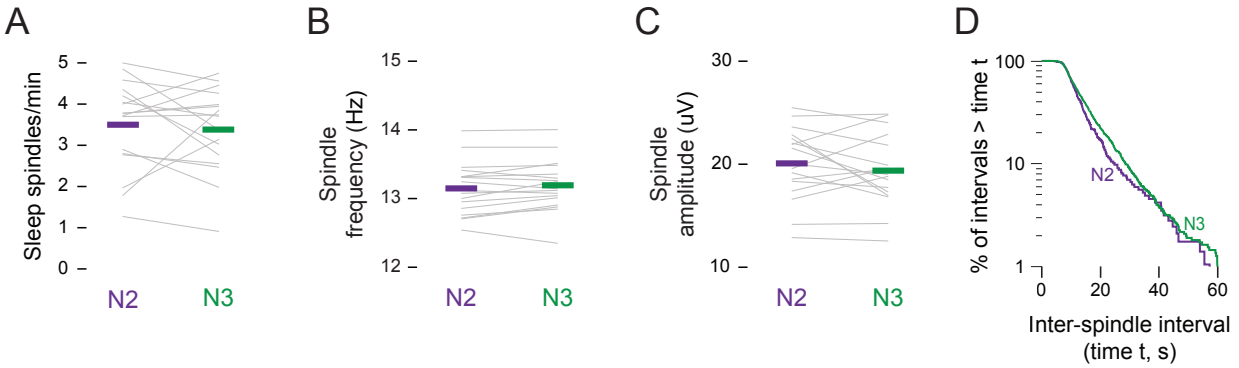

Figure 3. Characteristics of spindles do not differ between N2 and N3 sleep. (A) Mean spindle rate (spindles/min), **(B)** mean spindle frequency (Hz), and **(C)** mean spindle amplitude (μV) during N2 (green) and N3 (purple) sleep. Grey lines show data for individual infants. **(D)** Log-survivor plot of inter-spindle intervals (ISIs) during N2 (green) and N3 (purple) sleep. ISIs were pooled across sleep sessions: N2 = 286 spindles; N3 = 1104 spindles.

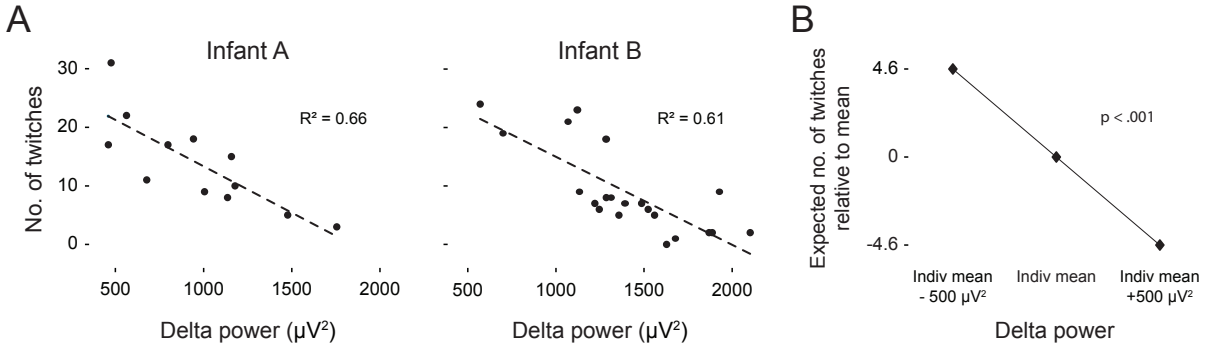

Figure 4. Twitches are inversely related to delta power. (A) Representative linear regressions from two infants showing the number of twitches in relation to mean delta power (μV^2) across 1-min windows of artifact-free NREM sleep. **(B)** Multilevel linear mixed-effects analysis of the effect of within-infant changes in delta power on the expected number of twitches within each 1-min window.

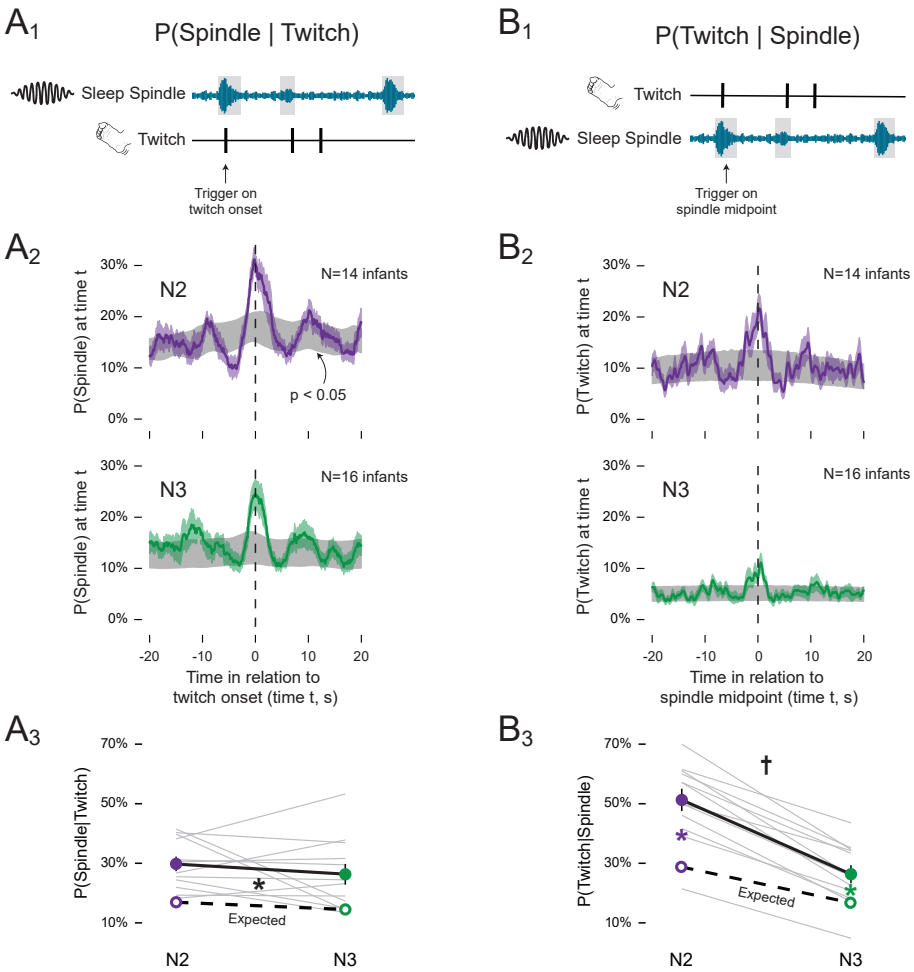

Figure 5. Spindles and twitches are coupled in N2 and N3 sleep. (A_1) Illustration of our method for assessing the probability of a sleep spindle given a twitch: P(Spindle | Twitch). For every twitch, we determined whether a spindle (from onset to offset) overlapped with that twitch. **(A_2)** Perievent histograms showing the P(Spindle | Twitch) during N2 (top; purple) and N3 (bottom; green) sleep. Each plot shows mean probabilities with SEM (shaded values). For each plot, 95% confidence intervals based on shuffled data are shown in grey. **(A_3)** Mean (±SEM) P(Spindle | Twitch) during N2 (purple solid circle) and N3 (green solid circle). Grey lines show observed data for individual infants. Expected mean probabilities (unfilled circles) based on shuffled data are also shown (expected probabilities for individual infants are not shown). **($B_{1\text{-}3}$)** Same as in ($A_{1\text{-}3}$) but for the probability of a twitch given a sleep spindle: P(Twitch | Spindle). Black * significant main effect (p<.05) between observed and expected values. Colored * significant observed and expected probability differences in N2(purple) and N3(green) (p<0.05). † Significant interaction (p<.05) between sleep state and observed-expected probability differences.

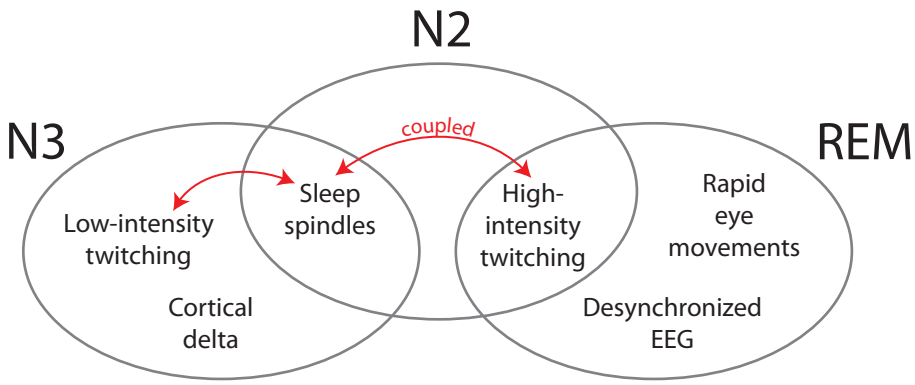

Figure 6. Organization of sleep components of REM, N2, and N3 at 6 months of age. Venn diagram to illustrate the sleep-stage distribution of twitching (high and low intensity), sleep spindles, rapid eye movements, and cortical activity (delta, desynchronized). The red double arrows indicate the coupling of twitches and spindles that occurs in N2 and N3.

Tables

Table 1. Results of mixed-effect model of the association of within- and between-infant fluctuations in delta power with the rate of twitching during NREM sleep.

Effect	Estimate	Std Err	DF	t Value	p Value
Intercept	6.07	0.83	15.43	7.29	<.001
Within-Infant Delta	-0.93	0.09	288.61	-10.04	<.001
Between-Infant Delta	-0.01	0.25	16.28	-0.04	.971