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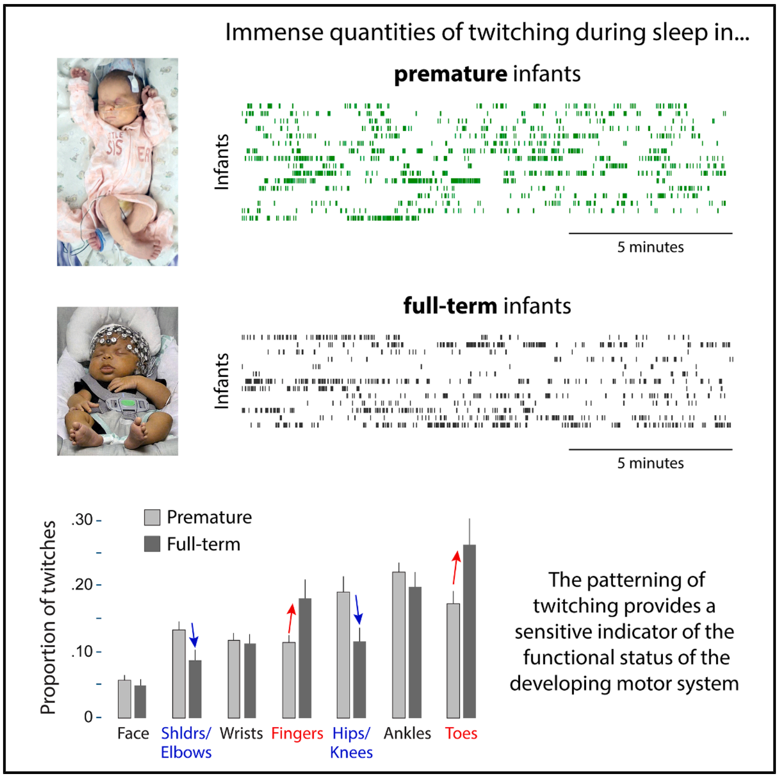
Graphical abstract



Highlights

- In human infants, myoclonic twitches are a key component of active sleep
- Premature infants exhibit an immense quantity of twitching across the body
- With age, finger and toe twitching increases relative to the rest of the body
- Surprisingly, twitching occurs selectively in the legs during tracé alternant

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In brief

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Article

Twitching in sleeping premature infants provides a sensitive behavioral assay of early motor control

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SUMMARY

Limb twitching is among the earliest observable behaviors in human development and a hallmark of active (rapid eye movement, REM) sleep. Systematic assessments in full-term infants reveal a spatiotemporal organization of twitching that informal observation cannot. Because preterm infants spend even more time asleep and are at heightened risk for neurodevelopmental disorders, we provide the first systematic characterization of twitching at 34–35 weeks postmenstrual age. Preterm infants exhibit an immense quantity of twitching across the body, underscoring its potential functional significance. The spatiotemporal structure of twitching also changes with age, including a selective increase in finger and toe twitching. Unexpectedly, during periods of tracé alternant, a precursor to quiet sleep, twitching appears in brief bouts that are almost exclusively restricted to the legs. These findings show how this abundant but overlooked sleep behavior provides a sensitive assay of the developing neural control of movement, with implications for understanding typical and atypical development.

INTRODUCTION

Two facts about active (rapid eye movement, REM) sleep capture its mystery: it predominates in early life, and comprises a suite of components that emerge and coalesce over development.[1] One such component is myoclonic twitching: spontaneous, discrete movements of the limbs, face, and eyes. In rats, twitching begins before birth and continues into postnatal life.[2] The abundance of twitching and its diverse and unique contributions to early brain activity give rise to varied contributions to sensorimotor development.[3] Because twitching in human infants has received little formal attention among basic and clinical sleep scientists, we initiated an effort to describe its expression in full-term (FT) infants over the first six postnatal months. That effort documented many similarities and some distinct differences in the quantity and patterning of twitching between human infants and newborn rats.[4,5]

Here, we turn our attention to preterm infants, for several reasons. First, the immediate postnatal period of rats corresponds most closely to the third trimester of humans,[6] when active sleep (AS) is even more prevalent than it is in term newborns.[7–10] With such high rates of AS, it follows that movements in fetuses[11–13] and preterm infants[8,14,15] also occur at high rates. Also, similar to newborn rats,[16,17] twitches in preterm infants trigger brief cortical oscillations[18,19] that are implicated in activity-dependent brain development.[3,20–23] Thus, precise analysis of twitching and its neural correlates holds promise for understanding both typical and atypical development in human infants.[24,25]

Second, regarding atypical development, preterm infants are at increased risk for deficits in motor skills,[26] sensory processing,[27] and functional connectivity.[28,29] They are also at increased risk for cognitive delays[30] and neurodevelopmental disorders such as cerebral palsy and autism spectrum disorder.[31–34] A deeper grasp of twitching within its developmental context may accelerate progress toward understanding the links between neurodevelopmental disorders and sensorimotor dysfunction,[35–39] and the mediating role of sleep.[40–42]

Finally, twitching in preterm infants can inform lingering issues surrounding the relative contributions of cortical and subcortical structures to early motor control. These issues linger in part due to evidence that cortical motor outflow in human infants only begins to influence behavior 3–6 months after birth.[43,44] Although brainstem motor structures, including the red nucleus (RN), are implicated in the production of twitching in infant rats and adult cats,[45–47] the part of the RN that would produce movement in human adults is often designated as rudimentary or vestigial.[48–50] These and other factors reveal an apparent paradox at the heart of early motor control that an assessment of twitching in premature infants may help to resolve.

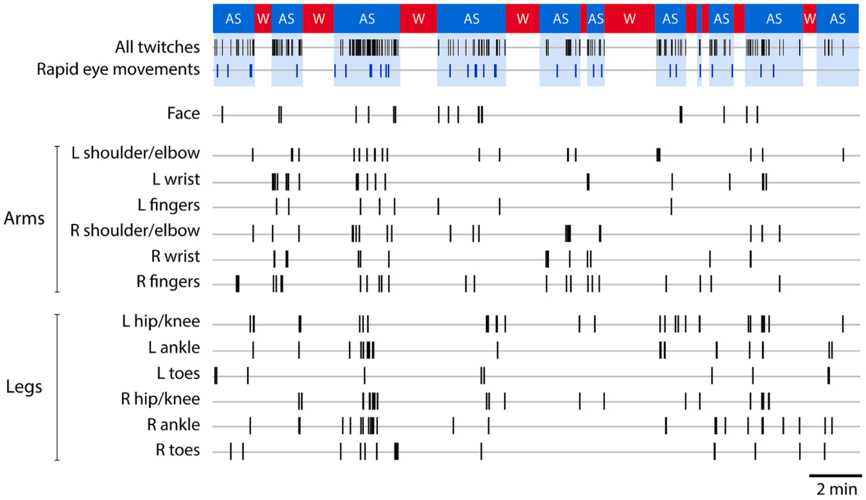

Figure 1. Representative data showing twitching across all body parts during active sleep in a moderately premature infant

The infant is a male, born at 34.4 weeks PMA, and recorded at 35.1 weeks PMA. From top to bottom, designation of behavioral state as active sleep (AS, blue) and wakefulness (W, red). Next, all face, arm, and leg twitches are denoted as vertical ticks, as are REMs (blue ticks). Finally, twitching in all individual body parts, including left (L) and right (R) parts of the arms and legs, are shown. See also Figure S1 and Video S1.

The above considerations motivate our effort here to provide the first systematic analysis of the spatiotemporal structure of twitching in extremely and moderately premature human infants. We focus on infants at 34–35 weeks postmenstrual age (PMA) when cortical motor outflow is almost certainly absent.[43,44] We show that the rate and spatiotemporal patterning of twitching is similar in the extremely preterm (EPT) and moderately preterm (MPT) infants, suggesting that twitching at this age is unaffected by chronological age (i.e., time since birth). We show that twitching in preterm infants is abundant and dispersed across the body, and that changes in the spatiotemporal structure of twitching are developmentally continuous across preterm and FT infants. Further, our approach demonstrates how attention to a previously overlooked sleep behavior can refine current models of brainstem involvement in early motor behavior.

RESULTS

Twitching is abundant in premature infants

We recorded sleep-wake behavior during the day in EPT ($n = 16$; 8 female), MPT ($n = 13$; 5 female), and FT ($n = 12$; 6 female) infants. Premature infants were recorded at 34–35 weeks PMA: Because the preterm infants were recorded at the same postmenstrual age, the chronological ages (i.e., time since birth) of the EPT infants (mean: 5.7 weeks; range, 2.9–11.7 weeks) were longer than those for the MPT infants (mean, 1.1 wks; range, 0.4–2.7 weeks). The FT infants were recorded at 6.0 weeks mean chronological age (range, 3.0–8.4 weeks).

Recording sessions lasted an average of 106.9 min (range, 34.0–138.2 min). The EPT infants slept an average of 40% (range, 11%–82%) during the recording session compared with 57% (range, 15%–85%) for the MPT infants ($t[27] = 2.3$, $p < 0\ .05$). FT infants slept an average of 64% (range, 28%–98%). See Table S1 for additional participant details.

A representative 13-min recording from one MPT infant, born at 34.4 weeks and recorded at 35.1 wks PMA, is shown in Figure 1. During AS, twitches of the arms (shoulders/elbows, wrists, and fingers), legs (hips/knees, ankles, and toes), and face occur often—though not always—alongside rapid eye movements (REMs). Video S1 presents a 4-min segment of twitching in the same infant, accompanied by the irregular breathing that is also a hallmark of AS, followed by a transition to wakefulness (W) as evidenced by high-amplitude, coordinated movements. The immensity of twitching over representative 15-min sleep-wake periods across all body parts for each infant is illustrated in Figure S1.

Figure 2A shows the number of scored twitches in relation to sleep duration for each recording session for the EPT, MPT, and FT infants; the right-hand axis shows the same data as twitch rate. Sleep duration is the sum of multiple bouts, and we illustrate the statistical structure of those bouts in Figure 2B using log-survivor plots. Those plots show that the sleep bouts of the EPT and MPT infants are similar in being highly fragmented (see Figure S2), with median bout durations ranging from 54 to 82 s (Figure 2B, inset). The linear distributions of the bout durations, which mirror those reported in infant rats,[51] signify that they occur randomly. Although the sleep bouts of the FT infants are somewhat more consolidated than those of the preterm infants, due to a subset of relatively long sleep bouts ($\chi^2[2] = 31.0$, $p < 0.0001$), the median sleep-bout durations do not differ significantly among the three groups ($\chi^2[2] = 2.5$). Finally, it should be stressed that the sleep records were scored continuously; had we instead used epochs (of 30 s or more) to score the records, an approach that is widely used but that obscures the occurrence of short sleep bouts, the sleep-bout durations reported here would have been substantially longer.

Log-survivor plots of inter-twitch intervals (ITIs) during sleep also allow us to assess their temporal structure. As shown in Figure 2C, approximately two-thirds of twitches in the EPT, MPT, and FT infants occur in bouts with ITIs less than 2 s, similar to the structure observed at later ages.[4] Also, although the ITIs distribute similarly for the EPT and MPT infants, the distribution for the FT infants separates from the other two at ITIs greater than 2 s, indicating a slowing of the rate of twitching. Although median ITIs are not significantly different for the three groups ($\chi^2[2] = 4.2$; Figure 2C, inset), the log-survivor distributions are different ($\chi^2[2] = 66.9$, $p < 0.0001$).

A developmental shift in the distribution of twitching across the body

We next assessed changes in the distribution of twitching across the body in the EPT, MPT, and FT infants (Figure 2D). A repeated-measures ANOVA revealed a significant body

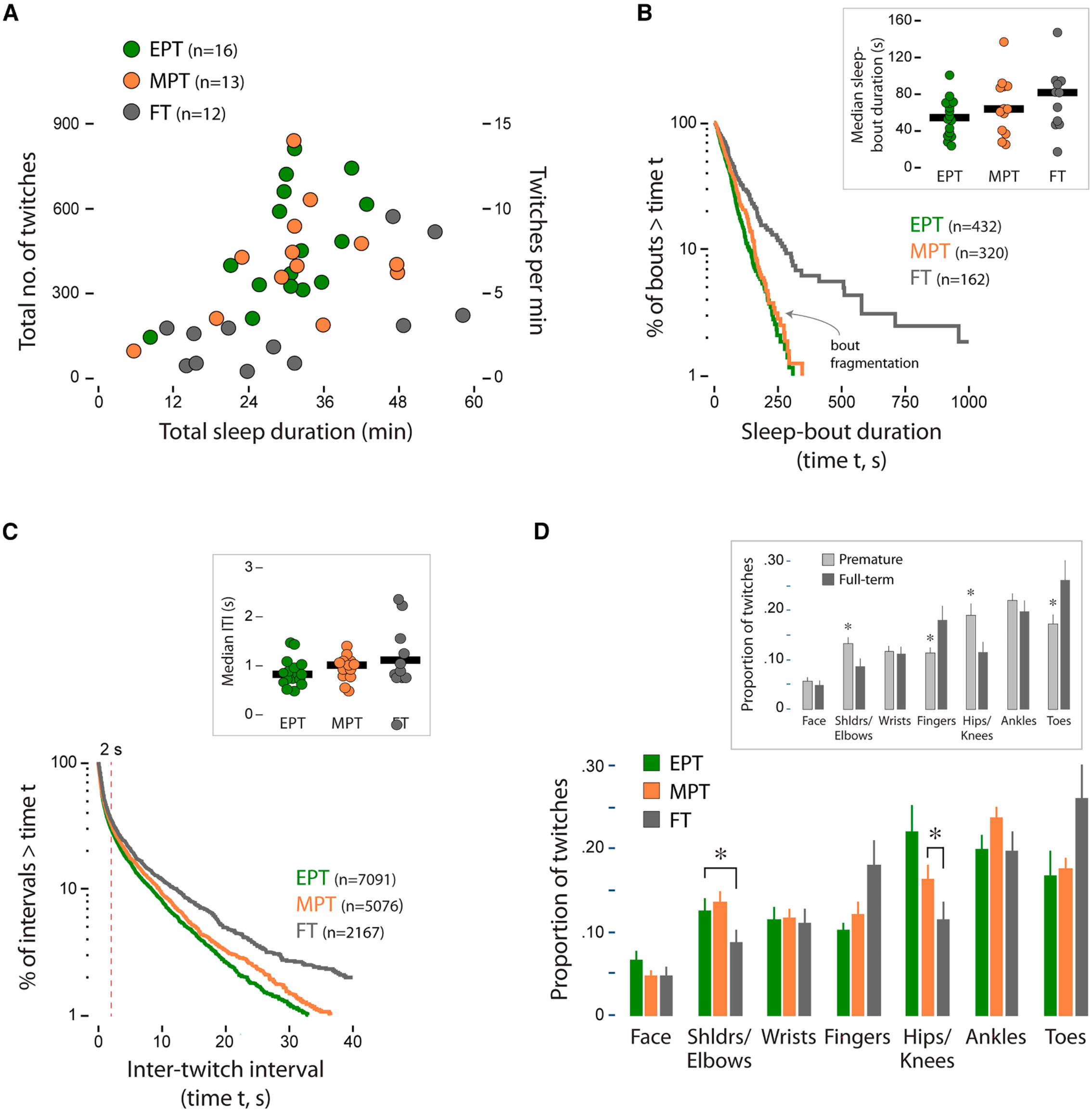

Figure 2. Spatiotemporal properties of sleep durations and twitching in premature and FT infants

(A) Scatterplot showing the total number of twitches (left axis) against total sleep duration for all the recording sessions in this study. Data are shown for EPT (green), MPT (orange), and FT (gray) infants. The right axis shows the rate of twitching (twitches/min).

(B) Log-survivor distributions of sleep-bout durations for EPT, MPT, and FT infants. Bout durations are pooled within each group. Inset, median bout durations for each individual infant within each group, and the horizontal black bars indicate group medians.

(C) Log-survivor distributions of ITIs for EPT, MPT, and FT infants during sleep. Intervals are pooled for each group. The vertical dashed red line shows that approximately two-thirds of ITIs are less than 2 s. Inset, median ITI durations for each individual infant for each group, and the horizontal black bars indicate group medians.

(D) Mean proportion of twitches during sleep across body parts for EPT, MPT, and FT infants (sample sizes are the same as in A). * Significant difference between designated groups. Inset: similar plot except that the data from the extremely and moderately premature infants are combined into a single group. Data are represented as mean ± SEM. * Significant group difference, $p < 0.05$. Outlier data from one FT infant were removed from the insets in (B) and (C). See also Figure S2.

part × group interaction ($F[8.4, 160.0] = 3.1$, $p < 0.003$, adj. $\eta^2_p = 0.10$; both main effects are also significant, $ps < 0.02$). The interaction appears to be driven largely by a shift in twitching from the shoulders and elbows to the fingers, and from the hips and knees to the toes, although only two of the posthoc comparisons are significant (Figure 2D).

One notable pattern in Figure 2D is the dominance of leg twitching in all three groups: the mean percentage of leg twitching relative to arm twitching is 60.6% in the EPT infants (range, 42.3%–78.3%; 14/16 infants, $W = 1$, $p < 0.001$); 61.0% in the MPT infants (range, 47.9%–78.3%; 12/13 infants, $W = 7$, $p < 0.001$), and 60.1% in the FT infants (range, 41.3%–79.2%; 10/12 infants: $W = 13$, $p < 0.05$).

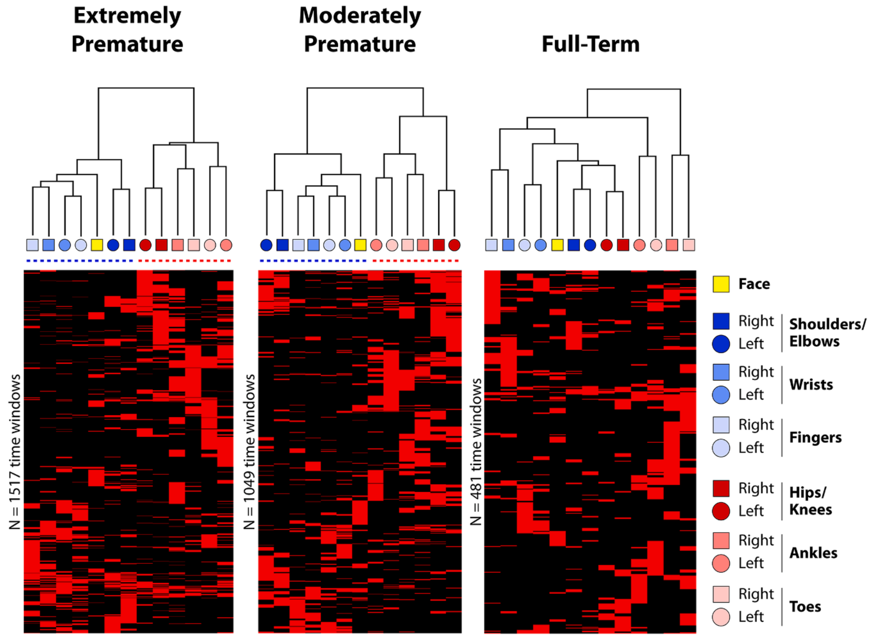

Figure 3. Hierarchical cluster analyses of twitching across body parts in preterm and FT infants

Cluster analyses were performed on pooled data for extremely premature (left), moderately premature (middle), and FT (right) infants. The key at right shows each of the body parts scored in each infant. The horizontal dashed lines beneath the dendrograms for the preterm infants highlight the separate clusters for the arms (blue) and legs (red), with facial twitching clustering with the arms. Analyses were performed on twitch data based on the presence or absence of a twitch during sequential 10-s windows during sleep. Each window is represented as a row in the cluster diagram below, with red denoting the presence of a twitch and black denoting its absence. The total number of rows is shown to the left of each cluster diagram; seriation was used to reorder the rows such that windows with similar patterns are closer to each other.

Because there were no significant differences in the EPT and MPT groups, we collapsed them into a single preterm group and repeated the analysis (Figure 2D, inset); again, a repeated-measures ANOVA revealed a significant body part × group interaction ($F[4.3, 166.7] = 5.0$, $p < 0$ .001, adj. $\eta^2_p = 0.09$; both main effects were again significant, $ps < 0.003$). Now, in the preterm infants compared with the FT infants, we find a higher proportion of finger and toe twitching and a lower proportion of shoulders/elbows and hips/knees twitching, with no differences for the wrists and ankles.

In summary, we see a developmental shift toward finger and toe twitching between 34-35 weeks PMA and 6 weeks after FT birth. This shift progresses further in FT infants through at least six months of age.[5,52] Finally, this shift does not appear to result from extrauterine experience as the chronological age of the EPT infants was ∼4 weeks greater than that of the MPT infants, similar to the FT infants (Table S1).

The temporal structure of twitching reflects an emerging focus on the distal limbs

To better understand the temporal structure of twitching across the body, we performed hierarchical cluster analysis with seriation.[53] Cluster analysis enables us to assess the degree to which the twitching of body parts group (i.e., cluster) together in time. This analysis was performed on 10-s windows localized to periods of sleep, as described previously for FT infants over the first six postnatal months.[4]

The hierarchical relations among the body parts are revealed by the dendrograms at the top of Figure 3. In these dendrograms, shorter vertical lines indicate stronger clusters. The EPT and MPT groups exhibit similar primary structures highlighted by two clusters, one for the arms and one for the legs (the face clusters with the arms in the preterm and FT infants). Within the arms, a secondary structure emerges characterized by clustering of wrist and finger twitching within the right and left hands, and clustering of shoulder/elbow twitching across the right and left arms. Similarly, within the legs, the structure is characterized by clustering of ankle and toe twitching within the right and left feet, and clustering of twitching in the hips/knees across the left and right legs.

Whereas the clusters in the EPT and MPT groups clearly distinguish the arms from the legs, the clusters for the FT infants are no longer segregated in that way; instead, the dendrograms suggest greater refinement of the control of parts within each limb, particularly the wrist/fingers and ankle/toes on both sides of the body. Again, the distribution of twitching across the body in the preterm infants appears unaffected by extrauterine experience, and the shift in patterning toward the distal limbs progresses further in FT infants over the next several months.[4]

Twitching occurs unexpectedly during periods of tracé alternant

Although 50%–60% of sleep in premature infants at 34–35 weeks PMA is composed of AS[8], the remainder is quiet sleep that is primarily composed of bursts of high-amplitude electroencephalographic (EEG) activity separated by inter-burst periods of relative quiescence.[54,55] Such periods—called tracé alternant (TA)—begin with a discontinuous pattern of EEG bursts that subsequently transitions to a periodic pattern. Representative data from an EPT infant, born at 26.6 weeks PMA and recorded at 34.4 weeks, show several TA periods (Figure 4A). The high-amplitude, mixed-frequency EEG bursts (Figure 4B) resemble those reported previously.[55,56]

Overall, 11 of the EPT infants and 9 of the MPT infants exhibited clear TA periods, with a mean total duration of 9.3 min (range, 2.4–31.3 min; Figure 4C). These TA periods occupied a mean of 14.6% of total sleep time (range, 4.9%–44.9%). The EEG bursts occurred rhythmically at median intervals of ∼13 s (Figure 4D); although the log-survivor distributions of inter-TA intervals indicated a small but significant difference between the EPT and MPT infants driven by deviations at the longer intervals

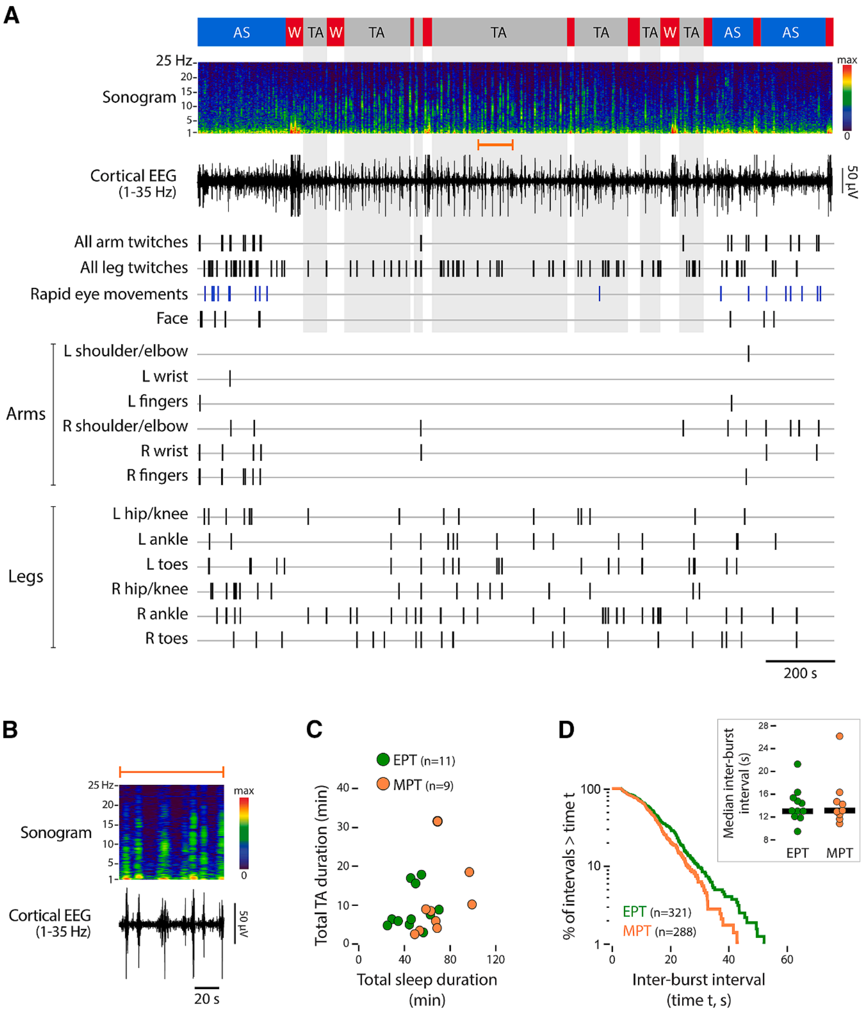

Figure 4. Twitching occurs during periods of tracé alternant

(A) Representative data from an extremely premature male infant born at 26.6 weeks PMA and recorded at 34.4 weeks PMA. From top to bottom: designation of behavioral state as AS (blue), TA (gray), and W (red), followed by cortical EEG (1–35 Hz) and associated sonogram. Next, all arm twitches and leg twitches are denoted as vertical ticks, as are REMs (blue ticks). Finally, twitching in all individual body parts, including left (L) and right (R) parts of the arms and legs, are shown.

(B) Enlarged section of cortical EEG and associated sonogram for the orange-bracketed segment in (A).

(C) Scatterplot showing the total duration of TA periods against total sleep duration for the EPT (green) and MPT (orange) infants.

(D) Log-survivor distributions of the intervals between high-amplitude EEG bursts during TA periods for EPT and MPT infants. Intervals are pooled within each group. Inset, median inter-burst intervals for each individual infant within each group, and the horizontal black bars indicate group medians. See also Video S2.

($\chi^2[2] = 5.78$, $p < 0.05$), the median intervals are not significantly different ($U = 47$).

TA is conventionally defined as a quiet-sleep-like period characterized by regular respiration and an absence of REMs and twitching. Although we did not observe REMs during these periods, we were surprised to observe limb twitching, as illustrated in the representative data from Figure 4A (see also Video S2). Careful visual inspection of the movements indicated that they are indistinguishable from twitches during AS, though they represent only ~10% of all twitches produced in those 20 premature infants that exhibited periods of both AS and TA. The temporal distributions of ITIs during AS and TA (collapsed across EPT and MPT infants) are identical up to ITIs of ~800 ms, after which the curves diverge significantly (Figure 5A; $\chi^2[1] = 91.8$, $p < 0.0001$). The sharp inflection at ~800 ms (Figure 5A, inset) indicates that most twitching during TA occurs in more concentrated bouts than does twitching during AS.

We did not find evidence during TA of a temporal association between EEG bursts and twitching. For example, the rate of twitching during the periods encompassing EEG bursts (4.5 ± 1.0 twitches/min) and those between EEG bursts (5.6 ± 0.8 twitches/min) did not differ significantly ($t[19] = 0.9$), nor were there differences between the EPT and MPT infants ($t[18] = 0.0$). Therefore, unlike the association between twitches and sleep spindles during quiet sleep in FT infants beginning around 3 months of age,[5,52] it appears that there is no association between twitches and EEG bursts during TA.

The bodily distribution of twitches during TA exhibits a particularly striking pattern, namely, an extreme bias toward the legs (Figure 5B). Although twitching in the premature infants is significantly biased toward the legs during both TA (legs > arms in 20/20 infants, $W = 0.0$, $p < 0.001$) and AS (legs > arms in 26/29 infants, $W = 26.0$, $p < 0.001$), leg twitching during TA accounts for 91% of all limb twitches compared with 61% during AS. Looking across body parts, the leg bias during TA was expressed in the ankles and toes, not the hips/knees (Figure 5C). Overall, a repeated-measures ANOVA (necessarily restricted to only those 20 infants that exhibited periods of both AS and TA) revealed a significant body part × state interaction ($F[4.7, 89.4] = 25.5$, $p < 0.001$, adj. $\eta^2_p = 0.55$; the main effects were also significant). Finally, whereas 20/20 infants exhibited higher rates of twitching in the arms during AS than during TA ($W = 253$, $p < 0.001$), for the legs that was true for only 13/20 infants ($W = 156$, not significant; Figure 5D). Altogether, the unique spatiotemporal structure and extreme leg bias of twitching during TA suggests that this state is phenomenologically distinct from AS on this dimension.

DISCUSSION

In common parlance, “to sleep like a baby” is to sleep deeply and well. Ironically, the sleep of actual babies looks very

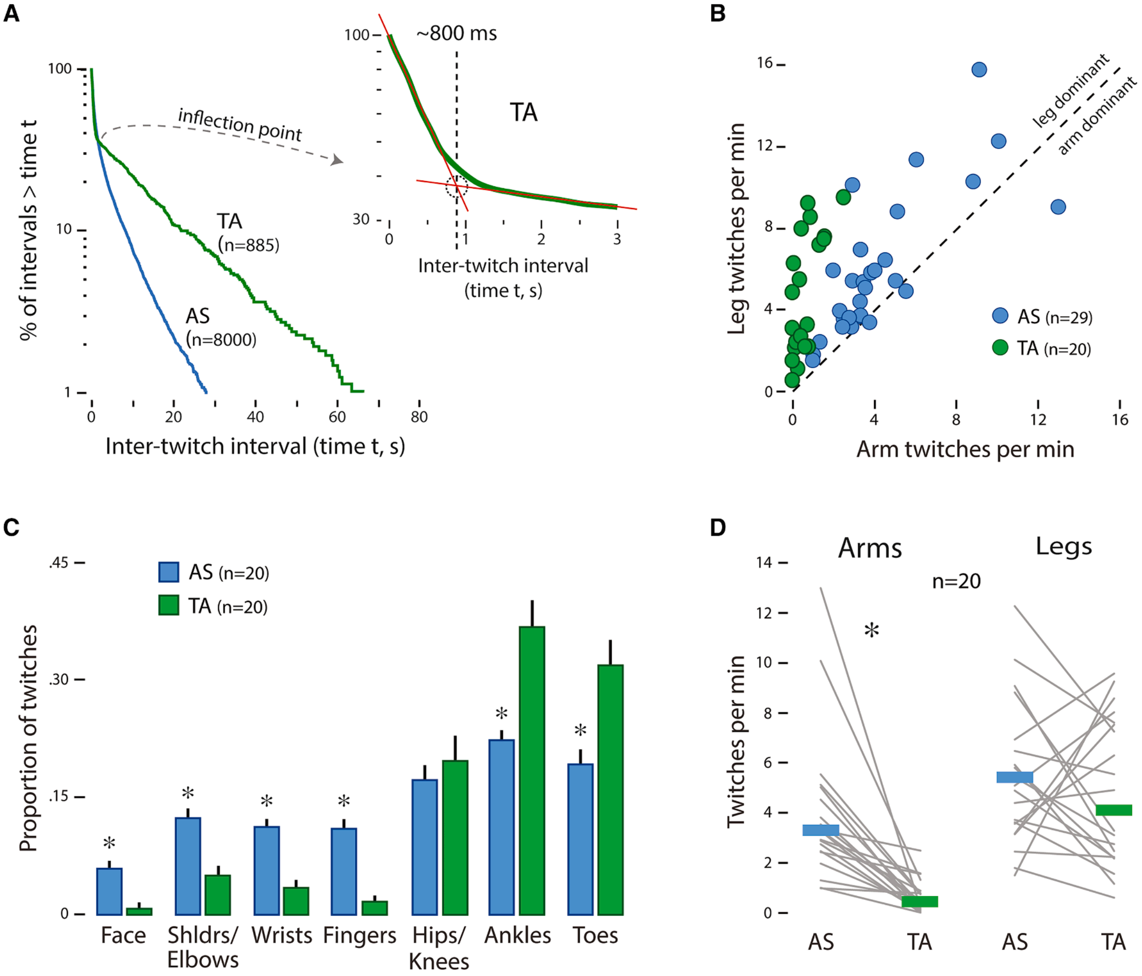

Figure 5. Spatiotemporal features of twitching during periods of tracé alternant in premature infants

(A) Log-survivor distributions of ITIs for premature infants periods of AS (blue) and TA (green). Intervals are pooled for each group. Inset, magnification of the TA plot at left to show where the TA and AS plots diverge; the inflection point indicates that bouts of twitching during TA periods do not last longer than ∼800 ms.

(B) Scatterplot showing the rate of leg twitching versus the rate of arm twitching for all infants with periods of AS and/or TA. Points above the line of isometry (dashed diagonal line) indicate infants with higher rates of leg twitching.

(C) Proportion of twitches during sleep across body parts for infants exhibiting periods of both AS and TA. Data are represented as mean ± SEM. * Significant difference from the other group, $p < 0.05$.

(D) Median twitch rate for the arms (left) and legs (right) during periods of AS (blue bars) and TA (green bars) in infants that exhibited both states. Paired data for individual infants are shown as gray lines. * Significant group difference, $p < 0.05$. See also Video S2.

different, featuring many short bouts of sleep interspersed with equally short awakenings. In addition, infant sleep often appears agitated due to the presence of limb and facial twitches, REMs, and irregular breathing, which are characteristics of AS, the predominant behavioral state of early life.[1,57,58] Although previous studies documented the presence of spontaneous movements in fetuses and preterm infants, this is the first study to explore the spatiotemporal properties of twitching in premature infants. Our findings parallel those in infant rats[59] by showing that twitching is abundant in human infants and its structure becomes increasingly more organized with age. Moreover, our findings suggest that twitching provides a unique and sensitive behavioral assay of the developing motor system.

Our findings also provide evidence for developmental continuity regarding some aspects of the expression of twitching. The notion of developmental continuity is complex,[60–62] but here we use the concept to refer to similarities in several core features of twitching in premature and FT infants. Those core features include the discrete, jerky nature of the movements, their occurrence during sleep, their marked contrast with wake movements, and several spatiotemporal features. Combined with our earlier assessments of twitching in FT infants over the first six postnatal months,[4,5] the addition of data from premature infants provides compelling evidence of developmental continuity in the early expression of key aspects of the behavior—including gradual changes in the distributions of ITIs (Figure 2C) and in the proportion (Figure 2D) and temporal clustering (Figure 3) of twitching across body parts. We stress, however, that this assertion of continuity does not necessarily imply continuity in all aspects of twitching across these ages. Indeed, in addition to those features of twitching that appear continuous, there are others that appear unique to specific ages, including the expression of twitching during periods of TA in preterm infants (as found here) and during quiet sleep in FT infants beginning around three months of age.[4,55]

Psychologists have long noted how large quantities of experiential input—for example, how many words children hear[63] or how many steps children take[64]—play formative roles in cognitive and motor development. Similarly, we previously estimated that FT infants produce approximately 600 twitches/h; with 8 h of AS each day, that rate yields a daily output of ~4,800 twitches.[4] With preterm infants twitching at a similar rate but with a daily quota of AS that is roughly double that of FT infants,[65] the daily quantity of twitches approaches 10,000. By providing discrete opportunities for relating self-generated motor output with sensory input, twitching contributes in multiple ways to sensorimotor plasticity.[3] The immense quantity of twitching in preterm infants suggests that its developmental significance begins very early (Figure S1).

It is notable that we did not detect group differences between the EPT and MPT infants on the dimensions of twitching or sleep-bout organization analyzed here. Even though the sample sizes were relatively small, we might have expected to detect some differences given that the EPT infants were, on average, 5 weeks older chronologically than the MPT infants. We might also have expected to see group differences because EPT infants are more likely to exhibit motor impairments.[66] However, clinical outcomes are highly variable and may not show up as group differences, especially at early ages. Instead, it may be more informative to assess sleep and twitching in premature infants with low body weight at birth and with evidence of early neurological insult. Thus, we continue to follow these and other infants in this project to assess possible relations among twitching, motor development, and the emergence of neurodevelopmental disorders.

Although we cannot directly assess the neural mechanisms that produce twitching in human infants, research in non-human animals provides valuable guidance. Specifically, in newborn rats[67] and adult cats,[68] the cortex has little or no role to play in the production of twitches. Instead, brainstem motor structures, including the RN, are implicated in the production of twitching in these animals.[45–47] The RN consists of two parts: the magnocellular part—the source of the rubrospinal tract—generates motor commands, and the parvocellular part modulates cerebellar circuits but plays no direct role in motor behavior.[49,50,69] In human adults, the relative prominence of the corticospinal tract contrasted with the small volume and limited spinal connectivity of the magnocellular RN justifies the latter's designation as a rudimentary or vestigial structure.[48–50] From that adult perspective, the present observations raise an apparent paradox: *If the magnocellular RN is vestigial in humans and cortical motor outflow is developmentally delayed*, *what accounts for the prominence of twitching in premature infants?*

One possible resolution of this paradox is to consider that the reticulospinal and vestibulospinal tracts, which are also implicated in the production of twitching,[68,70] compensate for the absence of a functioning magnocellular RN. However, a role for the magnocellular RN cannot be ruled out because studies in human fetuses reveal that the magnocellular RN is actually larger than the parvocellular RN and continues to enlarge before birth.[71,72] The authors of one of these studies[72] suggested that the magnocellular RN in early human development contributes to "a specific transitory pattern of motor behavior"[70] (p. 95). Based on the present findings, we hypothesize that the production of twitching is a major, if not primary, function of the magnocellular RN in preterm infants. Accordingly, we predict that the reduced rates of twitching in FT infants across age will mirror the diminution of the magnocellular RN's size and contribution to behavior.

Thus, humans may be similar to rats in relying on the magnocellular RN and other brainstem structures for the production of twitching, at least in early development.[47,67] If so, then the developmental changes found here in the spatiotemporal properties of twitching—including the increased focus on twitches of the fingers and toes—suggest developmental tuning of motor maps in the brainstem.[73]

The present findings raise additional questions. For example, it is not clear what accounts for the 60:40 leg:arm bias in twitching in the preterm infants and the 3–8 week-old FT neonates. This bias, which is not evident by 5–6 months postnatal age,[5,52] may reflect transient anatomical biases in motor projections to the cervical and lumbosacral spinal cord. The more extreme 90:10 leg:arm bias during TA is even more difficult to explain, especially given that twitching has not been previously reported during this state. The absence of previous reports may be attributable to the widespread use of swaddling in the neonatal intensive care unit (NICU), a practice that obstructs visual observation of the legs. Regardless, at this time we can only speculate that the extreme leg:arm bias reflects selective activation of descending motor tracts, such as the lateral vestibulospinal tract whose axons project directly to the lumbar spinal cord.[74,75]

Similarly, it is difficult to account for the selective increase in finger twitching that emerges between 34 and 35 weeks PMA and 3–8 weeks postnatal age. This developmental increase in independence need not reflect the emergence of corticospinal circuits as both the rubrospinal and reticulospinal tracts of primates are also implicated in finger movement.[76–79] Systematic assessment of finger twitching in human infants may prove useful for understanding the developmental origins of independent finger use, which emerges during wake after 6 months of age.[80] Such assessments should also include the toes as they twitch at similarly high rates as the fingers in preterm infants, as well as FT infants through at least 5–6 months of age.[5,52] Although such high rates of toe twitching may seem surprising, it should be noted that human infants reach for objects with their feet at earlier ages than they reach with their hands,[81] a possible remnant of our primate heritage.[82]

Given the large amount of sleep in fetuses and infants,[45] one might assume that protecting sleep is a central concern of pediatric facilities—especially NICUs. However, only recently have neonatologists, noting basic sleep research, reassessed the often brightly lit, noisy, and disruptive NICU environments that preterm infants traditionally experience.[83–87] In addition to improving the external environment within the NICU, efforts are being made to implement safe sleep practices that focus on the environment within the incubator.[88,89] These efforts complement a growing appreciation for the importance of sleep, including AS,[40,90] to short- and long-term development. Nonetheless, as a prominent spontaneous behavior and core component of AS, twitching has been variously dismissed in adults as a by-product of dreams[91] or, in fetuses and premature infants, as a sign of distress.[11,92] Instead, we encourage neonatologists and developmental scientists to reconsider this behavior in light of accumulating evidence of its functional and potentially clinical significance.

Limitations of the study

This study has several limitations. First, we chose to focus here on sleep at one preterm age in relation to FT newborns. Consequently, we were unable to assess developmental changes across the preterm period, including determining whether TA-related twitching is specific to that one age. Second, the NICU is a very different environment than the sleep laboratory; assessing developmental changes in sleep and twitching within the NICU will better control for such factors as light, noise, medical interventions, and recording methods. Finally, data collection for this project is ongoing and larger samples sizes will be needed to assess individual differences in twitching in relation to clinical outcomes. These limitations will be mitigated in subsequent studies that will report larger sample sizes across the preterm period in the NICU.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to, and will be fulfilled by, the lead contact, Greta Sokoloff (greta-sokoloff@uiowa.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- Summary data are shared publicly on GitHub (https://github.com/gsokoloff/Sokoloff-et-al-iScience-2026). Individual data and videos are shared on Databrary (https://nyu.databrary.org/volume/1920) for registered users on that site; only data for which consent was obtained from guardians are available.
- This study does not report original code.
- Any additional information required to reanalyze the data reported in this study is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

G.S. and M.S.B. designed the study with input from the other authors. B.J.C., T.T.C., and D.R.R. facilitated recruitment of and data collection from premature infants. G.S., T.G.C., H.C.L., L.K.K., O.K.K., D.N.K., A.C.S., W.L., and I.K.J. collected data from full-term infants and analyzed data from all infants. G.S., T.G.C., and M.S.B. wrote the manuscript. All authors provided feedback and approved the final manuscript for publication.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and algorithms		
MATLAB, version 2023b	Mathworks	RRID: SCR_001622
Spike2 Software, version 8	Cambridge Electronic Design	RRID: SCR_000903
Adobe Illustrator 2024	Adobe	RRID:SCR_010279
Adobe Premiere Pro	Adobe	RRID:SCR_021315
PermutMatrix	ATGC Montpellier Bioinformatics	RRID:SCR_026979
Handbrake	Handbrake	https://handbrake.fr/
Net Station EEG software, version 5.5	Magstim	RRID:SCR_002453
SPSS 30	IBM	RRID: SCR_002865
JMP	JMP Statistical Discovery, LLC	RRID: SCR_014242
Datavyu	Datavyu	RRID:SCR_003587
Databrary	Databrary	RRID: SCR_010471
GitHub	GitHub	https://github.com/gsokoloff/Sokoloff-et-al-iScience-2026
Other		
Net Amps 400 EEG amplifier with advanced PTZ video package	Magstim	https://www.egi.com/research-division/net-amps-eeg-amplifier
HydroCel 128-channel geodesic sensor nets	Magstim	https://www.egi.com/research-division/geodesic-sensor-net
Pico movement sensor	Unimed Electrode Supplies Ltd.	http://www.unimed-electrodes.co.uk/Pico-Movement-Sensor-100cm-lead-TP-Connector/141
Olympic Brainz Monitor (OBM)	Natus Medical	https://natus.com/neuro/cfm-olympic-brainz-monitor/
OBM neonatal hydrogel sensors	Natus Medical	https://www.natusmedicalstore.com/product/obm00042/01t5w00000FViGPAA1
iPhone 13 Pro Max	Apple	https://www.apple.com/my/iphone/compare/?modelList=iphone-13,iphone-13-pro-max

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

A total of 29 preterm infants participated in this study. Premature infants were divided into two groups: extremely preterm (EPT; <32 weeks postmenstrual age at birth; n=16; 8 female) and moderately preterm (MPT; 32-37 weeks postmenstrual age at birth; n=13; 5 female). An additional cohort of full-term infants (FT; 3.0-8.4 weeks of age at testing; n=12, 6 female) are also included.

Preterm infants were drawn from a pool of infants recruited and consented into the discovery cohort of a larger, ongoing project conducted in the Neonatal Intensive Care Unit (NICU) at the Stead Family Children's Hospital at the University of Iowa Hospital and Clinics. Preterm infants for this study were selected based on age (i.e., 34-35 wks PMA) and the ability to visualize the arms, legs, and face in the video recordings. Full-term infants were recruited from the community using email and print advertisements and were similarly drawn from a pool of infants for the larger study. Table S1 provides demographic, age, weight, and sleep-session information for all 41 infants included in this study. Guardians identified the race of the infants as 65.9% White, 14.6% two or more races, 4.9% African American/Black, and 2.4% Asian; 12.2% preferred not to specify. Guardians also identified the ethnicity of the infants as 19.5% Hispanic or Latino; 7.3% preferred not to specify. All study procedures were approved by the Institutional Review Board at the University of Iowa (IRB #202205044).

METHOD DETAILS

Recording of sleep sessions

Preterm infants consented into the study participated in multiple sessions, at approximately 2-week intervals, while in the NICU. All NICU recording sessions were conducted by clinical research nurses and took place between 0800 and 1600, preferably after a feeding. While in an incubator or crib, in a supine position, infants were fitted with two pairs of Neonatal Hydrogel Sensors connected to an Olympic Brainz Monitor (Natus Medical Incorporated, Middleton, WI). After preparing the scalp with Nuprep (Weaver and Company, Aurora, CO) and coating the electrode with conductive paste (Ten20, Weaver and Company), electrodes were secured at standard EEG sites (C3/C4, P3/P4) using neonatal positioning strips (Natus) and a surgical skin marker; an additional ground electrode was secured to the shoulder blade. Vital signs of all infants in the NICU, including respiration, heart rate, and blood-oxygen levels, were measured and stored continuously using Sickbay (Medical Informatics Corporation, Houston, TX). An iPhone was placed above the infant on the surface of the incubator or attached to an adjustable stand next to the crib to acquire video during sleep sessions. At the start of each session, the video captured the time on the Olympic Brainz Monitor, thus allowing us to synchronize the EEG and video data offline.

Full-term infants were recorded in a supine, semi-reclined position in the sleep laboratory, while wearing a lab-supplied onesie, as described previously.[5] Participants were prepared for the recording session by fitting them with a high-density (124-site) EEG electrode cap (Geodesic Sensor Net; Magstim EGI, Roseville, MN). Before application, the cap was soaked in an electrolyte solution composed of potassium chloride and baby shampoo (Johnson & Johnson, New Brunswick, New Jersey) dissolved in warm water. To record respiration, a piezoelectric sensor (Unimed Electrode Supplies, Surrey, UK) was taped to the onesie on the abdomen just below the xyphoid process. The infant was recorded from a frontal view using a high-resolution video camera. EEG, respiration, and video data were synchronized during acquisition.

Sleep staging

Videos of sleep sessions were scored using Datavyu.[93] For all videos, two experienced raters visually scored 100% of the video for sleep, wake, startles, and arousals. All videos were scored continuously, that is, epochs were not used. Scorers also noted periods when the infant's face or limbs were obscured. Sleep and wake were scored as described previously.[4,5] Briefly, we used established behavioral criteria to define sleep and wake.[7] During wake, infants' eyes may be open for sustained periods, eye movements are horizontal and focused, postural tone is high, and the infant exhibits high-amplitude and often synchronous limb movements.[94] During sleep, the eyes are typically closed (although they may be open) and postural tone is low; during AS, myoclonic twitches, REMs, and irregular breathing are also evident.[8,94] Importantly, twitch movements differ from wake movements in that they are relatively brief, low in amplitude, and discrete.

Periods of tracé alternant (TA) were defined as consisting of a background of low-amplitude EEG interrupted at regular intervals by high-amplitude bursts ($\sim$100 μV). All other sleep periods were designated as AS. Finally, all periods in which nurses or clinicians were providing bedside care or a guardian was interacting with the infant were noted and excluded from analysis.

Twitch coding and reliability

Experienced behavioral coders reviewed sleep periods for myoclonic twitches using methods similar to those described previously.[4,5] Briefly, in Datavyu, coders—blind to the EEG record—marked the onset and offset times for twitches of the face, arms, legs, hands, feet, fingers, and toes. REMs were also scored. Limb twitches were not scored if they were artifacts of other movements, such as breathing, stretching, or twitching in another limb. The average twitch durations of $\sim$630 ms were uniform across the infants in all three groups, consistent with our finding in rats that twitches are highly stereotyped across age.[95]

A primary coder was assigned to each session to score twitches during designated sleep periods. For sessions with fewer than 30 min of sleep, all sleep periods were scored; for sessions with more than 30 min of sleep, sleep periods with optimal limb visibility were selected for scoring. For each session, a secondary coder was assigned to score at least 25% of the scored periods to assess inter-rater reliability (IRR). IRR was calculated for each period scored by both coders to quantify their level of agreement regarding the onset times and durations of all twitch events (5). Periods of disagreement between coders were identified and reviewed independently by both coders. The median value of Cohen's kappa was 0.848 (range: 0.617-0.968). A final pass with both coders resolved any remaining discrepancies by mutual agreement. Finally, only twitch-related data scored by the primary coder were used for analysis.

Exclusion criteria

Premature infants in the NICU were not approached for consent into the study if they were on high-frequency ventilation, tested positive for COVID-19, and/or were administered opioids or benzodiazepines as part of their care plan. In addition, one FT infant had only one very long sleep bout during the session, which resulted in anomalous median values for sleep-bout duration and inter-twitch interval; those data were excluded from those two analyses.

Data analysis

Total sleep time and sleep-bout durations

The duration of each sleep bout was determined. Sleep bouts were pooled across infants within groups and JMP Pro 18 (JMP Statistical Discovery, Cary, NC) was used to plot log-survivor distributions.

Counts and rates for twitching

Counts for twitches (of the face, arms, and legs) and REMs were summed across periods of sleep and divided by the total time asleep. For some analyses, rates of twitching were computed separately during AS and TA.

Inter-twitch intervals

The time between successive twitches within a sleep period was defined as an inter-twitch interval (ITI). ITIs were excluded when there was an intervening startle, microarousal, or transition between states. ITI data were pooled across infants within groups (EPT, MPT, FT), and JMP Pro 18 was used to plot log-survivor distributions.

Proportion of twitching in individual body parts

To assess the distribution of twitching across the face, arms (shoulders/elbows, wrists, fingers), and legs (hips/knees, ankles, toes) for each sleep session, the number of twitches in each part was divided by the total number of twitches during the session. Proportions were arc-sine transformed before statistical analysis.

Cluster analysis

To assess the spatiotemporal patterning of twitching, hierarchical cluster analysis with seriation was performed using PermutMatrix software.[53] The settings were Manhattan distance and Ward's minimum variance; for seriation, we used the multiple-fragment heuristic. Using an approach similar to that described previously,[4] sequential 10-s windows were created within each sleep session; each window began at the onset of the first twitch. Twitches of the face, arms, and legs were included. To ensure that clustering was not biased by the occurrence of more than one twitch of a single body part, the maximum value for each body part within a window was 1. For the cluster analysis, data were pooled within groups. In addition to producing dendrograms showing clustering among body parts, the windows (i.e., rows) were reordered using seriation to reveal their structure.

Cortical EEG bursts during tracé alternant (TA), TA-period durations, and inter-burst intervals

TA periods began when an alternating EEG trace was observed with bursts exceeding 50 μV and typically a 25-50% reduction in EEG amplitude during the inter-burst intervals (see Figure 4A). TA periods ended with the cessation of bursts and an increase in baseline EEG amplitude. To capture the EEG bursts associated with TA, EEG data were downsampled to 100 Hz and low-pass filtered at 2 Hz. Filtered data were transformed by taking the root mean square (RMS, 150-ms time constant). Over entire TA periods, the average RMS amplitude of the filtered signal was calculated and then multiplied by 3.5 to create a threshold for detecting EEG bursts. The threshold was used to determine the onset of a burst, and a 3-s step was added to minimize double counting. Inter-burst intervals were calculated as the time between successive bursts within the TA period. Inter-burst intervals that spanned a transition from TA to another state (e.g., wake, AS) were removed.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analyses were conducted using JMP Pro 18 or jamovi.[96] Before conducting ANOVAs, data were tested for normality using the Shapiro-Wilks test and for sphericity using Mauchly's test. When sphericity was violated, a Huynh-Feldt correction was applied to the degrees of freedom. The measure of effect size for ANOVAs is represented as partial eta-squared (η^2_p), adjusted for positive bias.[97] Group differences in log-survivor distributions were tested using the Mantel-Cox log-rank test, which gives equal weight to all time points; because data were pooled across infants within each group, these tests should be interpreted with caution. Group differences in median sleep-bout duration, median ITI, and median inter-TA interval were tested using the non-parametric Kruskal-Wallis χ^2 test or Mann-Whitney U test, as appropriate. The Wilcoxon signed-ranks test was used to assess leg dominance within the AS and TA groups, and the Wilcoxon matched-pairs signed-ranks test was used to assess within-infant differences in the rate of arm and leg twitching. Unless otherwise noted, means are presented with their standard errors, and alpha was set at 0.05.

For Figures 2B and 2C, the sample sizes (i.e., pooled sleep-bout and inter-twitch intervals, respectively) are shown in each plot. For Figure 2D, for the main plot and inset, mean proportions (+/- SEM) are presented and the sample sizes are the same number of infants as shown in Figure 2A. Statistical analyses for this figure are presented in the results.

For Figure 3, the sample sizes (i.e., the number of time windows) are shown to the left of each cluster. Hierarchical cluster analysis with seriation was performed using PermutMatrix software.[53] Analytical details for this figure are presented in the results.

For Figures 4C and 4D, the sample sizes (i.e., number of infants in each group and the number of inter-burst intervals, respectively) are shown for each plot. Statistical analyses for this figure are presented in the results.

For Figure 5A, the sample sizes (i.e., pooled inter-twitch intervals) are shown in the figure. For Figure 5B, the sample sizes are the number of infants contributing data to either the AS and TA groups. For Figure 5C, mean proportions (+/- SEM) are shown and the sample sizes are the number of infants contributing data to both the AS and TA groups. For Figure 5D, median twitch rates are presented and the sample sizes (i.e., number of infants) are shown in the figure. Statistical analyses for this figure are presented in the results.