Emergence of resting state networks in the preterm human brain

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The functions of the resting state networks (RSNs) revealed by functional MRI remain unclear, but it has seemed possible that networks emerge in parallel with the development of related cognitive functions. We tested the alternative hypothesis: that the full repertoire of resting state dynamics emerges during the period of rapid neural growth before the normal time of birth at term (around 40 wk of gestation). We used a series of independent analytical techniques to map in detail the development of different networks in 70 infants born between 29 and 43 wk of postmenstrual age (PMA). We characterized and charted the development of RSNs from recognizable but often fragmentary elements at 30 wk of PMA to full facsimiles of adult patterns at term. Visual, auditory, somatosensory, motor, default mode, frontoparietal, and executive control networks developed at different rates; however, by term, complete networks were present, several of which were integrated with thalamic activity. These results place the emergence of RSNs largely during the period of rapid neural growth in the third trimester of gestation, suggesting that they are formed before the acquisition of cognitive competencies in later childhood.

blood oxygen level-dependent | functional MRI | neurodevelopment | intrinsic brain activity | newborn

The detection of spontaneous spatially coherent fluctuations of the blood oxygen level-dependent (BOLD) signal by functional MRI (fMRI) (1) offers potential insights into the large-scale organization of neural function at the system levels (comprehensive review presented in 2). These resting state networks (RSNs) replicate the set of functional networks exhibited by the brain over its range of possible tasks (3), encompassing various spatially distinct neural systems, including the medial visual and lateral visual, auditory, somatosensory, motor, cerebellum, executive control, and frontoparietal or dorsal visual stream networks as well as the default mode network (DMN) (2–4). However, although the configurations and consistency of these networks are established, their functions are still not fully understood.

Elucidating the ontogeny of RSNs could clarify the role of this large-scale neural organization. Previous studies at the time of normal birth [term, around 40 wk of postmenstrual age (PMA)] have detected RSNs in the primary visual areas, somatosensory and motor cortices, temporal cortex, cerebellum, prefrontal cortex (5, 6), and incomplete DMN (7) but did not find the complete DMN, executive control network, or dorsal visual network. This led to the suggestion that the full architecture emerges during later childhood in parallel with the development of corresponding cognitive functions.

Here, we test the alternative hypothesis: that the full adult repertoire of resting state dynamics emerges during the period of rapid neural growth before the normal time of birth at 38–43 postconceptional weeks (term).

We used a series of independent analytical techniques to map in detail the development of different networks in 70 infants born

between 29 and 43 wk of PMA. We used optimized techniques, including improved registration and a unique spatiotemporal atlas of the developing brain with consistent thresholding, applying both data-driven exploratory analysis and hypothesis-based approaches to show that synchronous BOLD activity develops from fragmentary RSNs in preterm infants into facsimiles of adult patterns at term, including involvement of relevant thalamic nuclei, and to quantify the growing network coherence during this period.

Results

Identification of RSNs During the Third Trimester. First, we used probabilistic independent component analysis (ICA) for hypothesis-free detection of RSNs (Fig. 1). We analyzed the BOLD signals from resting state fMRI in early preterm, preterm, and term infants (full details are presented in *Methods* and *SI Methods*). This analysis revealed correlated low-frequency (0.01–0.1 Hz) spatially distributed BOLD signals that characterized recognizable neuroanatomical systems comparable to adult networks, including visual, auditory, somatosensory, motor, frontoparietal, and executive control networks as well as DMNs.

Some activation patterns were not found in the most preterm group, although all the major RSNs characterized in the adult brain (e.g., 4) could be detected at term. As development progressed, cortical activity became more coherent across recognized neuroanatomical systems with a general increase in interhemispheric correlation.

In older infants, complex networks representing the DMN (Fig. 1H), frontoparietal network (Fig. 1 I and J), and executive control network (Fig. 1K) were detected. Before term, the DMN was incomplete: In the preterm group, the inferior parietal lobules were not involved, whereas in the most preterm infants, the parietal opercular cortex but not the precuneus was included. Infants scanned at term-equivalent age showed a facsimile of the consensus adult network structure, with coherent activity encompassing the medial prefrontal cortex, posterior cingulate/precuneus, and inferior parietal lobules, with additional coherent activity in the parietal opercular cortex and cerebellum.

The frontoparietal network was present at term and in the preterm group as two left-right mirrored networks: Fig. 1I dis-
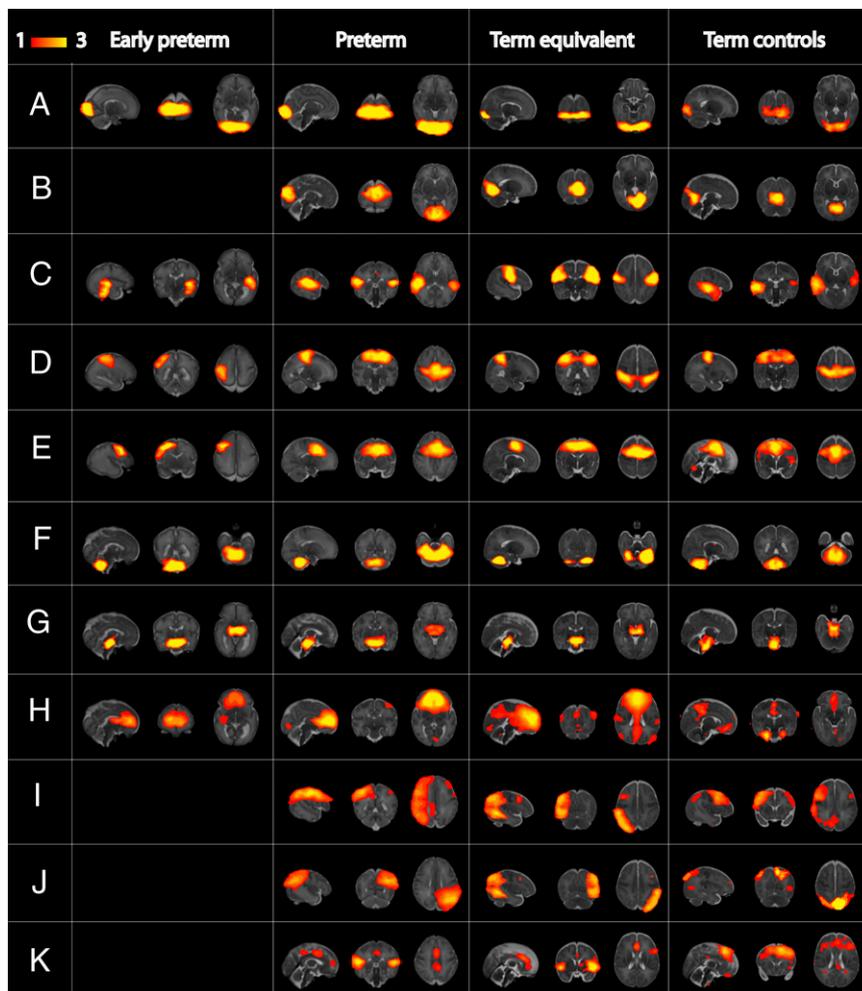
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Fig. 1. Probabilistic ICA, group analysis. Spontaneous activity patterns in the three age groups of infants born prematurely and in the group of control babies born at term. Example sagittal, coronal, and axial slices for meaningful spatial patterns (corresponding to independent components from probabilistic ICA) are overlaid onto the age-specific templates. Images were thresholded controlling the false discovery rate at $P < 0.05$ and are shown as z-statistics in radiological convention. The following networks have been detected: medial visual (A), lateral visual (B), auditory (C), somatosensory (D), motor (E), cerebellum (F), brainstem and thalami (G), default mode (H), dorsal visual stream (left and right components) (I and J), and executive control (K).

plays the right hemisphere map, including the right lateral occipital complex and right inferior parietal cortex. Fig. 1*J* displays the mirror component. The executive control network (Fig.1*K*) detected in term-equivalent and preterm infants covered medial-frontal areas, including the anterior cingulate, paracingulate, and superior frontal gyrus bilaterally. Prominent activations encompassing the whole thalamus and brainstem were seen at all ages.

Specificity of RSNs Defined by Seed-Based Whole-Brain Correlation Analysis. Next, using anatomically defined seed masks, we performed whole-brain correlation analysis, which independently confirmed increasing convergence on adult-pattern networks at term. The primary advantage of such seed-based analysis approaches over ICA is the ability to assess directly the degree of specificity in whole-brain correlations relative to a priori-selected seed regions of interest (ROIs; i.e., it permits a hypothesis-led analysis of functional connectivity). Fig. 2 summarizes the seed correlation findings for visual, auditory, somatosensory, motor, frontoparietal, and executive control networks as well as DMNs.

At term, several networks, including the somatosensory, auditory, and motor networks, also showed correlated activity in relevant portions of the thalamus. To confirm this, we placed seeds in the region of the left ventrolateral nucleus of the thalamus. In the

preterm and very preterm infants, the correlations were fragmented and widely distributed, whereas at term, there is a clear correlation with the motor cortex (Fig. S1).

Development of Network Architecture. Both data-driven and hypothesis-led analyses showed that a complete set of RSNs emerged before term. We next used two approaches to characterize the development of these networks in more detail. First, we applied partial correlation analysis to quantify the change in overall connectivity within networks over time, and, second, we evaluated the spatial extent of each network using a unique 4D (three spatial dimensions plus time) brain atlas and consistent thresholding (details are presented in *SI Text*).

Partial correlation analysis (details presented in *SI Text*) allowed us to quantify the change in connectivity by evaluating the partial correlation of the BOLD time series over time. Motor and somatosensory networks (Fig. 3 *B* and *C*) became less variable and showed a statistically significant increase in inter-hemispheric connectivity with PMA ($r = 0.4$, $P = 0.00004$; $r = 0.3$, $P = 0.002$, respectively). The DMN (Fig. 3*E*) also increased and became less variable with age ($r = 0.1$, $P = 0.029$), with the coherence of interhemispheric connectivity between left and right inferior parietal lobules ($r = 0.2$, $P = 0.001$) being more
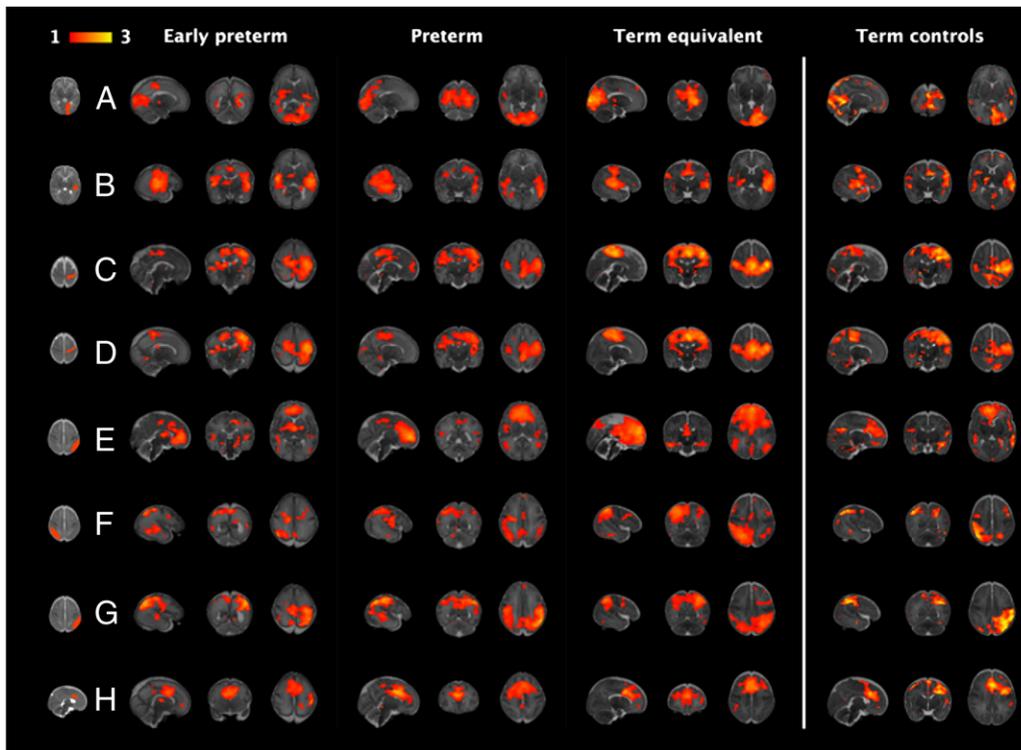
Doria et al.

Fig. 2. Whole-brain regression analyses. Seed regions are shown in the first column on the left. Example sagittal, coronal, and axial slices for meaningful spatial patterns are overlaid onto the age-specific templates. Images were thresholded using a false discovery rate correction at $P < 0.05$ and are shown as z-statistics in radiological convention. The following networks have been detected: visual (*A*), auditory (*B*), somatosensory (*C*), motor (*D*), default mode (*E*), dorsal visual stream (right component) (*F*), dorsal visual stream (left component) (*G*), and executive control (*H*).

prominent than anteroposterior coherence between the medial parietal frontal cortex and the precuneus ($r = 0.07$, $P = 0.345$) (Fig. S2). The executive control network (Fig. 3*F*) showed an

increase in connectivity ($r = 0.324$, $P = 0.003$), whereas the dorsal visual stream or frontoparietal network (Fig. 3 *G* and *H*) appeared stable. The auditory network (Fig. 3*A*) demonstrated
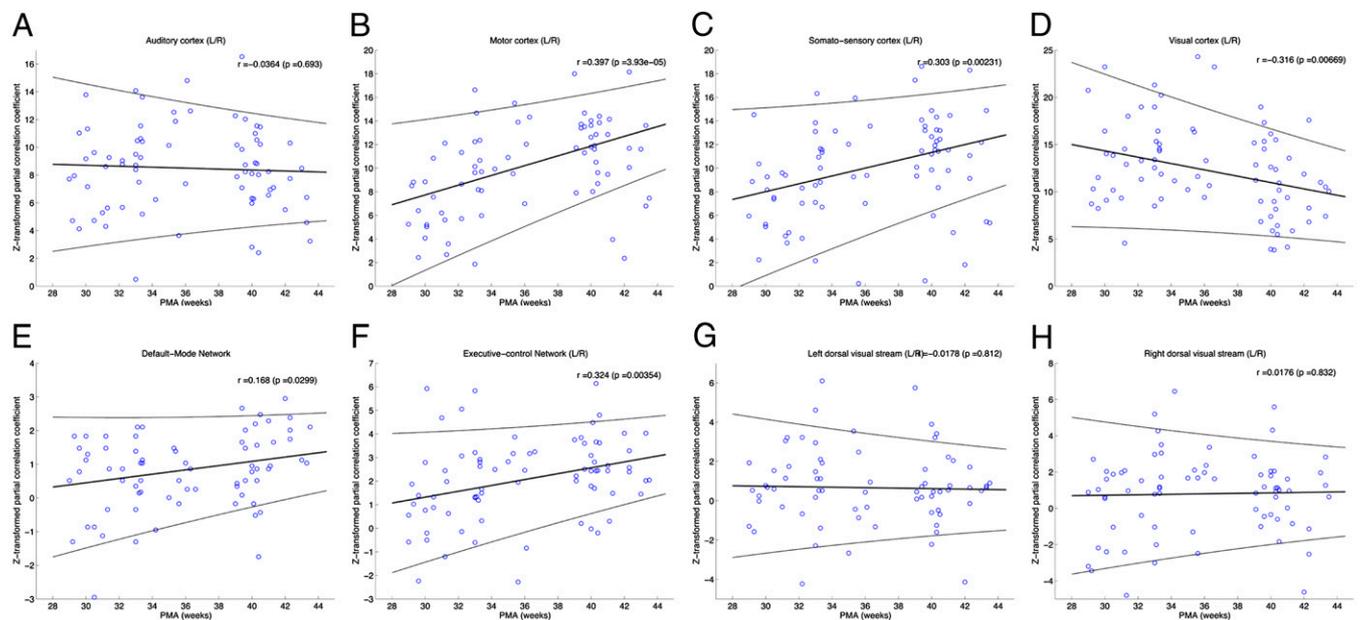

Fig. 3. Quantification of developmental changes of connectivity in the different networks. For the auditory (*A*), motor (*B*), somatosensory (*C*), and visual (*D*) networks, the connectivity is expressed as the z-transformed partial correlation coefficient between the right and left cortices. For the complex networks [DMN (*E*), executive control (*F*), and left and right components of the dorsal visual stream (*G* and *H*)], the connectivity is expressed as the average of the partial correlation coefficients computed between the different pair of nodes within each network and z-transformed. The line plots show the robust regression line with prediction error bounds.

MEDICAL SCIENCES

significant interhemispheric connectivity from the earliest age, which changed little ($r = -0.036$, $P = 0.693$); this was consistent with the whole-brain correlation analysis, which showed interhemispheric coherence in the auditory cortices of the early preterm group (Fig. 2*B*). The visual network (Fig. 2*D*) also exhibited strong interhemispheric connectivity at early ages with a slight trend to greater independence at term ($r = -0.317$, $P = 0.007$). Fig. S2 shows results for each single pair of nodes, and Fig. S3 displays the manually drawn seed regions.

The age-specific templates provided by the spatiotemporal atlas combined with consistent thresholding allowed accurate and detailed evaluation of the changes in the spatial extent of synchronous BOLD activation. Fig. 4 shows an example of this approach, where correlations maps generated from a seed in the left motor cortex were registered to the age-specific templates. This and the other networks are best visualized in the movies provided in *SI Text*. They confirm the group analyses, showing increasing within-network coherence and bilateral correlation over time (Fig. 4 and Movies S1, S2, S3, S4, and S5). This detailed mapping of the development of RSNs showed a rich pattern of growth from fragmentary activations at 29 wk to facsimiles of the adult network at term.

Potential Bias. We examined the data to see if these results could be explained by clinical factors in the population. Demographic and clinical data on the subjects are given in Table S1. Table S2 gives results of the linear regression for sedated and nonsedated

infants, together with the differences in the estimated correlation between the two groups, converted to *z*-statistics (details presented in *SI Text*). There was no significant difference between correlations in sedated and nonsedated infants in any network. We also compared two different groups that were both studied at term age: term-born infants and preterm infants who had been born some weeks earlier. Infants born at term showed qualitatively similar results to the term-equivalent preterm infants, both with probabilistic ICA and the whole-brain regression approach. We compared each RSN between term controls and ex-preterm infants at term, using a two-sample *t* test on the correlation maps, which was *z*-transformed using a second level random effects analysis implemented in SPM5 (http://www.fil.ion.ucl.ac.uk/spm). Age at scan, postnatal age at scan, gender, use of sedation, presence of white matter disease on T2-weighted MRI, and motion parameters were included as covariates of no interest (*SI Text*); no statistically significant differences between term-equivalent preterm infants and term-born subjects were detected in any network when taking these into account after correcting for multiple comparisons, although uncorrected results show some differences between term controls and term-equivalent preterm infants (*SI Text*).

Discussion

This study combined a series of independent analytical approaches to study the development of resting state dynamics in the brain before the normal time of birth. Model-free ICA and
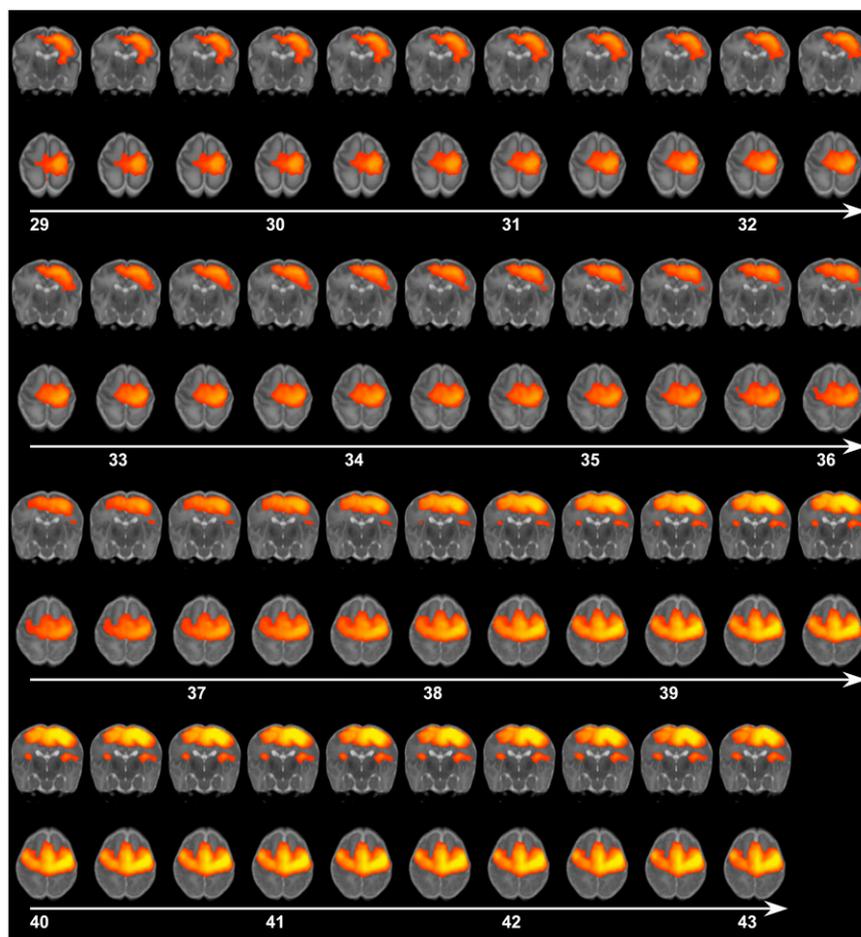

Fig. 4. Frames at given weeks illustrate the motor network through development using the regression of connectivity strength projected onto the 4D spatiotemporal template for visualization. Images were thresholded using a consistent thresholding across different ages, controlling the false discovery rate, and using an empirical null distribution at each time point at $P < 0.01$. Images are shown as *z*-statistics in radiological convention.

seed voxel correlation produced similar results that were confirmed and quantified using the spatiotemporal brain atlas and by partial correlation analysis. These optimized methods showed the development of RSNs from recognizable but often fragmentary elements at 30 wk of PMA to facsimiles of adult networks at term, with term-born infants indistinguishable from infants born preterm but studied at term. Although previous studies (5–7) have detected several, sometimes partial, RSNs in newborns, we present here evidence that complex networks previously described only in adults develop before term.

The differences between our results and previous studies in the reported number and spatial extent of RSNs across gestation are likely attributable to methodological differences and technical considerations. A groundbreaking analysis of a small sample of 12 preterm infants has previously demonstrated the existence of a variety of these networks (5, 6) in the preterm brain but failed to demonstrate the existence of a DMN in this population. This analysis used probabilistic ICA for signal identification but did not have available a precise time-resolved anatomical template brain, which permits accurate coregistration before the ICA. Given that the computational step is performed after registration in the probabilistic ICA group, the availability of an age-specific template for coregistration allows more accurate spatial normalization and more sensitive detection of activated areas.

More recently, Smyser et al. (7) demonstrated longitudinal changes in resting functional connectivity in the infant brain using a seed-based analysis approach. The findings presented here are consistent with results reported by Smyser et al. (7) and go beyond, testing for the presence of more complex networks, such as the executive network and frontoparietal network, and using high-dimensional coregistration and manually drawn anatomically precise masks, which help to explain the added sensitivity.

The presence of an adult-like repertoire of RSNs at around term age does not preclude further development during childhood and adolescence or changes with aging and disease. The precise spatial structure of some networks is still being characterized, and we applied only the most basic anatomical definitions in this study. It is hoped that subtle changes in the spatial network structure will give insights into abnormal brain function, although, at present, it appears that normal aging has more effect on signal amplitude than spatial structure (8).

Although the preterm infants in this study were selected as being without obvious clinical problems, prematurity engenders an increased risk for cerebral palsy and cognitive deficits (9). No subjects had the MRI abnormalities typical of cerebral palsy, although a proportion did show diffuse excessive high signal intensity on T2-weighted images, which is common in preterm infants. However, MRI is insensitive (10, 11) and neurodevelopmental impairment, although unlikely, cannot be completely excluded without a long-term follow-up even in the apparently healthy preterm infants in this study. For this reason, we compared preterm infants at term age with normal term-born controls. No differences were detected, suggesting that any undetected problems caused by preterm birth did not override the normal RSN development. We note that Smyser et al. (7) reported a difference between term and preterm infants at term before correcting for multiple comparisons, and we observed a similar effect. It is possible that future work may confirm these differences or show that infants with unequivocal evidence of cerebral damage have abnormal RSN development.

We studied sedated and nonsedated infants and found no differences between these groups with either qualitative or quantitative measures. Previous studies have included results obtained from sedated (6) and nonsedated infants (5, 12–14). The effect of sedation on RSNs is still not thoroughly understood in adults, although the effects of sleep and anesthesia have been investigated (15–19), and in adult populations, RSNs are detectable regardless of the level of consciousness, although there is

a suggestion that sedation might modulate the correlation within RSNs (16, 18, 20).

The hypothesis that resting state architecture develops during childhood in parallel with specific cognitive abilities was supported in particular by previous studies of the DMN. Activity in this network is proposed to support internally focused tasks, such as autobiographical memory retrieval, envisioning the future, and "mentalizing" or taking the perspectives of others in the waking state (comprehensive review presented in 21). Previous studies detected an incomplete version of the DMN at normal term age (7) and at 2 wk (12), whereas patterns similar to those in the adult DMN were seen in children aged 7–9 y (22, 23). This led to the concept that the network develops in parallel with the cognitive competencies associated with stimulus-independent thought (22, 23).

The present study questions this interpretation, however; instead, it suggests that all RSNs develop during the period of rapid neural growth before term, although not entirely synchronously, because the auditory and visual networks appear mature in the preterm infant. This is consistent with current concepts of brain development. Spontaneous activity is a fundamental characteristic of the developing nervous system (24–26), and periodic activity on the order of minutes is seen in networks with highly interconnected excitatory synapses (26, 27).

Spontaneous neural activity (28, 29) and sensory inputs (30, 31) are both required for the establishment of cortical networks, and during late gestation, they coexist in the cortical subplate, a large transient cerebral structure maximal at about 33 wk of PMA, which regresses as intercortical connection and intracortical circuitry and synaptogenesis predominate (32). Thalamic axons destined to become thalamocortical projections form temporary synapses in the subplate and are essential for normal cortical development. We observed coherent activity involving the whole thalamus, which, by term, had developed so that visual, auditory, and motor networks integrated portions of the thalamus, replicating the correlated intrinsic thalamic activity seen in adults and older children (33–35). The emergence of RSNs at the time when thalamocortical projections are developing suggests that it may be fruitful to address the hypothesis that the spatially distributed correlations that form RSNs are linked to thalamocortical connectivity.

Conclusion

This study shows that RSNs develop with different trajectories but that the repertoire of resting state dynamics emerges during the period of rapid neural growth in the last trimester of gestation, before the acquisition of cognitive competences.

Methods

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