Rich-club organization of the newborn human brain

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Combining diffusion magnetic resonance imaging and network analysis in the adult human brain has identified a set of highly connected cortical hubs that form a “rich club”—a high-cost, high-capacity backbone thought to enable efficient network communication. Rich-club architecture appears to be a persistent feature of the mature mammalian brain, but it is not known when this structure emerges during human development. In this longitudinal study we chart the emergence of structural organization in mid to late gestation. We demonstrate that a rich club of interconnected cortical hubs is already present by 30 wk gestation. Subsequently, until the time of normal birth, the principal development is a proliferation of connections between core hubs and the rest of the brain. We also consider the impact of environmental factors on early network development, and compare term-born neonates to preterm infants at term-equivalent age. Though rich-club organization remains intact following premature birth, we reveal significant disruptions in both cortical–subcortical connectivity and short-distance corticocortical connections. Rich club organization is present well before the normal time of birth and may provide the fundamental structural architecture for the subsequent emergence of complex neurological functions. Premature exposure to the extrauterine environment is associated with altered network architecture and reduced network capacity, which may in part account for the high prevalence of cognitive problems in preterm infants.

To understand the functional properties of a complex network it is necessary to examine its structural organization and topological properties. In the human brain this can be achieved at a macroscale by tracing white matter connections between brain regions with diffusion MRI; this enables the interrogation of structural network topology in vivo with millimeter-scale spatial resolution, providing complementary evidence to experimental studies (1, 2).

Network analysis of the adult human structural connectome has revealed a set of highly connected cortical “hubs” predominantly located in heteromodal association cortex, that provide a foundation for coherent neuronal activation across distal cortical regions (3–5). Further, some hub regions tend to be densely connected to each other, forming a “rich club” comprised of frontal and parietal cortex, precuneus, cingulate and the insula, as well as the hippocampus, thalamus, and putamen (6). Rich-club organization has been identified in a number of complex networks (7) and represents an attractive feature for investigation in the brain because rich-club connections tend to dominate network topology (8). Rich-club architecture appears to be a fundamental feature of the mature mammalian brain with similar organization identified in animal models (9, 10).

It has been suggested that the emergence of complex neurological function is associated with the integration of major hubs across the cortex (11, 12), and that the neural connectivity underlying this undergoes substantial remodeling after birth (13–14). Initial studies of neonatal structural networks have reported only dense local connectivity within segregated modules and few long-distance connections (12, 15). In contrast, functional MRI reveals large-scale dynamic functional networks analogous to those seen in adults (16, 17) and compatible with more advanced cerebral maturation. To address the possibility that the newborn brain may be structurally more developed than previously thought, and to understand better the role of structural network architecture in emergent neurological functions, we have developed an approach to assess the topological development of structural connectivity in the human brain up to the normal time of birth.

We used this approach to define network topology at ~30 and 40 wk of gestation and, in a group of infants studied at both time points, charted the emergence of structural organization. We also explored the specific relations of cortical and deep gray matter hubs in the network. To determine whether network development was independent of environmental factors, we compared healthy term-born subjects with infants prematurely born and exposed to the extrauterine environment. We report that highly ordered cerebral structural connectivity with rich club topology is established by 30 wk gestation; additionally, we identify aspects of network organization that develop during this period and specific features that are disturbed by premature extrauterine life.

Results

Rich-Club Organization of the Newborn Brain. As an initial examination of connectivity in the developing brain, datasets were grouped according to age at scan: early (median age at scan = 31+4 weeks; 28 preterm infants) and term (median age at scan = 41+2 weeks; 46 preterm infants and 17 term-born controls).

At the early, 30-wk time point, average node degree was lower, but both time points had similar asymmetric and heavy-tailed degree distributions (Fig. 1 A and B) that follow an exponential truncated power law \( p(k) = k^{a-1}e^{-bk}; \) Fig. 1 B, Inset.

Significance

To investigate the organizational principles of human brain development, we analyzed cerebral structural connectivity in the period leading up to the time of normal birth. We found that a “rich club” of interconnected cortical hubs previously reported in adults is present by 30 wk gestation. From mid to late gestation, connections between core hubs and the rest of the brain increased significantly. To determine the influence of environmental factors on network development, we also compared term-born infants to those born prematurely. Alterations in cortical–subcortical connectivity and short-distance connections outside the core network were associated with prematurity. Rich-club organization in the human brain precedes the emergence of complex neurological function, and alterations during this time may impact negatively on subsequent neurodevelopment.


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Longitudinal Development of the Rich Club. Network topology and connectivity within and between core rich-club nodes and peripheral, low-degree nodes were examined in a longitudinal dataset of 28 infants scanned twice during the preterm period at 30 and 40 wk.

Network topology was summarized by averaging density, mean clustering coefficient (C) and characteristic path length (L) over 100 networks for each subject (Fig. S1D). At 30 wk, density = 0.22 ± 0.04 (mean ± SD), C = 0.64 ± 0.03, and L = 1.88 ± 0.10. By 40 wk, density = 0.29 ± 0.04, C = 0.66 ± 0.02, and L = 1.77 ± 0.06. After comparison with a set of randomized networks, normalized C and L metrics, Cnorm and Lnorm were found to significantly decrease over the preterm period (n = 28; paired t test: P < 0.001 both).

Network connectivity was examined by distinguishing different types of connections: local connections between peripheral nodes; feeder connections between peripheral and core nodes; and core connections between core nodes only (21). The degree for an individual node can be separated into components according to its connections (core, feeder, or local). Fig. 2 shows the average degree of core nodes in these components. Degree based on all connections is shown in Fig. 2A. Fig. 2B restricts the degree count to feeder connections, and Fig. 2C considers only core connections. In each case, we vary the definition of the core by adjusting the threshold for core membership (as a percentile of maximum degree), and the effect of this variation on the average core node’s degree is plotted. At low percentiles, the network core contains most of the nodes, whereas at higher levels, only the most connected, topologically dominant nodes (i.e., the rich club) form the core.

By 40 wk, average degree of core nodes was significantly higher [repeated-measures ANOVA: F(1,99) = 67.0, P < 0.001]. There was a significant increase in both the average number of feeder connections (Fig. 2B) and the number of local connections between excluded peripheral nodes (F = 57.8, 69.1, P < 0.001 both). Average within-core node degree (core connections only), normalized to the number of nodes in the core network, increased over time (F = 42.2, P < 0.001), but a significant group × core-threshold interaction (F = 57.2, P < 0.001) and post hoc t tests revealed that there was no significant increase in core network degree within the peak rich-club domain. In

Figure 1. Structural organization of the developing human brain. Mean degree distribution, normalized by the number of nodes in each network, at 30 wk (A, red solid line) and 40 wk (B, blue line), overlaid on individual distributions (dashed lines; n = 28 and 63, respectively). (A, Inset) Distributions at both time points. (B, Inset) Cumulative distribution plots (solid lines) and power law fits (dashed lines). (C and D) Mean normalized rich-club curves (ϕnorm) and individual curves (30 wk, C; 40 wk, D). The red dashed line indicates the RC90 rich-club threshold. (E) Maps at both time points showing the membership probabilities of regions belonging to the RC90 network.

Figure 2. Rich-club organization during the preterm period. Average node degree of core nodes by connection type: (A) all connections, (B) feeder connections, and (C) core connections (normalized by the total number of nodes, number of peripheral nodes, and number of core nodes, respectively). Data show group mean at the 30-wk (red) and 40-wk (blue) time points (n = 28; shading indicates SD). Significant differences are shown with a black bar (post hoc paired t tests: P < 0.001). Gray shading represents the peak rich-club domain. Schematics show connections of interest in each plot. (D) Mean percent increase in node degree due to feeder connections (Upper) and core connections (Lower) in RC90 nodes are compared in E and F. Feeder connectivity of RC90 nodes is related to global network metrics L (characteristic path length) and C (clustering coefficient) at both time points (G and H). Developmental changes in rich-club connectivity are illustrated in I (filled circles are core nodes); adding feeder connections decreases L and increases C.
the $RC_{90}$ network, a significant increase in the number of feeder connections was evident over the preterm period (Fig. 2E), but there was no significant increase in the number of core connections (Fig. 2F). Feeder connectivity within the $RC_{90}$ network was significantly associated with decreasing $L$ at both time points (Fig. 2G; 30 wk: $R^2 = 0.90$; 40 wk: $R^2 = 0.91$, $P < 0.001$ both), whereas $C$ was found to be significantly associated with feeder connectivity at the 30-wk time point only (Fig. 2H; 30 wk: $R^2 = 0.37$, $P < 0.001$; 40 wk: $R^2 = 0.04$, $P = 0.29$).

Fig. 2I illustrates these changes using a simple representative network. The addition of four feeder connections (dashed red lines), representing an increase in connectivity between high-degree core nodes and low-degree peripheral nodes, decreases path length by 14% while the clustering coefficient rises; reflected in the absolute (nonnormalized) values of $C$ observed at the early and term time points (30 wk: mean $\pm$ SD = 0.64 $\pm$ 0.03; 40 wk: 0.66 $\pm$ 0.02; $P < 0.001$).

**Rich-Club Organization and Prematurity.** Structural connectivity and network topology was compared at term in all infants born preterm ($n = 46$) and a cohort of term-born controls ($n = 17$). No significant differences were found in mean network density (preterm: 0.28 $\pm$ 0.04; term: 0.28 $\pm$ 0.03, $P = 0.80$) or path length $L$ (Fig. 3D; preterm: $L = 1.78$ $\pm$ 0.07; term: $1.76$ $\pm$ 0.05, $P = 0.25$). After normalization, a small increase in $L_{norm}$ was apparent in the preterm infants (Fig. 3D; $P < 0.05$). In contrast, $C$ was significantly greater in the preterm cohort both before (Fig. 3D; preterm: $C = 0.66$ $\pm$ 0.02; term: $C = 0.64$ $\pm$ 0.02, $P < 0.01$) and after normalization (Fig. 3D; $P < 0.001$). Voxel-wise comparison revealed significantly higher clustering in preterm infants in the lateral parietal cortex, ventral and lateral frontal cortex, and around the Sylvian fissure (Fig. 3E; $P < 0.01$, family-wise error corrected after threshold-free cluster enhancement) (22).

No significant differences were found between groups in core network node degree when considering all connections [Fig. 3A; $F_{1,99} = 0.77, P = 0.38$, feeder connections (Fig. 3B; $P = 0.25$, $P = 0.62$), or core connections (Fig. 3C; $F = 1.76, P = 0.19$). However, a significant group $\times$ core threshold interaction ($F = 2.87, P < 0.001$) and post hoc $t$ tests revealed a significant increase in local connections between peripheral nodes at lower core network thresholds in the preterm cohort (Fig. 3F; $P < 0.01$). Exploring the influence of peripheral connectivity on network topology, linear regression also revealed that the number of local connections between the lowest ranked 10% of nodes was significantly associated with $C$ ($R^2 = 0.46, P < 0.001$). Fig. 3G illustrates an increase in peripheral connectivity in a simple network. The addition of a small number of local connections significantly increases $C$ but has little effect on $L$.

**Exploring Connectivity Within the Rich Club.** In the adult, connections linking rich-club nodes are physically longer than those connecting non–rich-club nodes, forming a high-cost backbone (21). By calculating the Euclidean distance between pairs of connected nodes we confirmed that, in all three groups, core connections of the $RC_{90}$ networks were significantly longer than both feeder and local connections ($P < 0.01$ all; Fig. 3D).

The adult rich club consists of several cortical hubs alongside deep gray-matter structures, including the thalamus and putamen (6). To explore the influence of deep gray-matter pathways on rich-club organization, whole-brain tractography was repeated excluding all streamlines passing through deep gray matter. Retaining only edges that connected two cortical regions of interest (ROI) both before and after removal of deep gray-matter connections allowed us to distinguish direct corticocortical connections from those that pass through deep gray matter.

Direct corticocortical connectivity accounted for the majority of connections in dorsal medial cortical regions, and superior parietal cortex at both time points, and additionally in the insula by term (Fig. 4). Difference maps show node degree exclusive of direct corticocortical connections, representing an indirect measure of cortex to deep gray matter to cortex connectivity. Although these connections represent a lower proportion of total connectivity, at 40 wk, ~20% of all connections from the lateral frontal and medial frontal cortices connect to other cortical regions through the deep gray matter.

The proportion of all core and feeder connections that were exclusively corticocortical did not significantly alter during the preterm period [$F_{1,99} = 0.42, 0.001, P = 0.52, 0.98$, respectively]. However, at the 30-wk time point, despite fewer connections in total, a significantly greater proportion of local connections were exclusively corticocortical [$F_{1,99} = 18.07, P < 0.001$]. By 40 wk, although there was no significant difference in the total number of core or feeder connections between groups, the proportion of direct corticocortical connections was significantly greater in preterm infants [$F_{1,99} = 7.40, 8.02$, respectively, $P < 0.01$ both].

**Discussion**

A rich-club network of densely connected cortical hubs is established before the time of normal birth. Rich-club regions include dorsal, medial frontal, and parietal cortex, precuneus, hippocampus, and insula, emulating the highly ordered organization previously described in adults (6). During the third trimester, the number of connections between rich-club regions and the rest of the cortex increases significantly, compatible with the development of the rich club as a communications spine for information transfer across the cerebral network. Network organization is also significantly influenced by the environmental stress of premature extrauterine life, with notable alterations in deep gray matter connectivity compatible with previous studies of thalamocortical connections in preterm populations (23, 24) that result in a greater proportion of direct corticocortical connections compared with healthy term-born infants.
Rich-club organization is a property common to complex networks across many domains (7) and is hypothesized as a basis for efficient global information transfer in the brain (21). Rich clubs are found in the mammalian brain and in the neural circuitry of Caenorhabditis elegans, where rich-club neurons are among the first to develop, suggesting that rich-club organization reflects a common and scale-invariant property of neuronal networks 

Although the clinical significance of these disruptions is not yet known, prematurity is associated with higher rates of neurodevelopmental outcome disorders, such as attention deficit hyperactivity disorder and autism, which have both been recently characterized as disorders of connectivity (55, 56). A consensus has not yet been reached on the nature of the network disruptions underlying these disorders, but we speculate that structural disruptions arising from changes secondary to the early emergence
of rich-club organization, such as the increases in local–cortical connectivity observed in the present study, may represent possible candidate features. The presence and state of rich-club organization in these clinical populations therefore warrants further investigation.

Anatomical dissections have revealed that a substantial proportion of frontoparietal connections are formed by short-assocation U fibers (57), whereas both the frontal and parietal cortices exhibit extensive, direct, high-captureation to the striatum and thalamus (58, 59). Given that the roles of parallel and distributed fronto–striatal–thalamic circuits in higher-order cognitive functions are well establised (60), it is also of note that connectivity of the lateral frontal cortex appeared to be highly dependent on connections passing through the deep gray matter by term-equivalent age; this suggests that alterations to fronto–subcortical connectivity may limit communication efficiency between hubs within the rich club with a potential impact on subsequent neurocognitive development. Whether the signifi-

cant increase in corticocortical connectivity between peripheral nodes reflects exuberant corticocortical connectivity via short U fibers in preterm neonates remains unclear, and further in-

vestigation is needed. The combination of advanced diffusion model-
in techniques (i.e., spherical deconvolution) (61, 62) and high-resolution sampling of corticocortical fibers will enable the targeted exploration of corticocortical connectivity in this pop-

ulation, and appropriate acquisition protocols for neonates are currently under active research (63).

Our observations are limited by the resolution available to whole-brain diffusion MRI, and by the relatively coarse nature of tractography-based connectomics, but it is proposed that postnatal development of neurocognitive functions is dependent on the fine-tuning of synapses and short-range fibers with lim-

ited remodeling of the fundamental structural architecture. With advances in microstructural modeling of the developing cortex (40) and the development of methods to combine func-

tional and structural connectivity infor-
mation (44, 46), these data will provide insights into human brain development and dysfunction.

Materials and Methods

Ethical permission for this study was granted by the local Research Ethics Committees (the City and East London Research Ethics Committee; the Hammersmith, Queen Charlotte’s and Chelsea Research Ethics Commit-
te). Written parenteral consent was obtained for each infant. Subjects. Forty-six preterm-born infants (22 male; median (range) gestational age at birth 27.4 (24.4–34.5) weeks; median birth weight 0.97 (0.62–2.02) kg) were scanned at term-equivalent age [median postmenstrual age 41.4 (38.5–44.1) weeks]. A subset of 28 (14 males) were also scanned at an earlier time point during the preterm period [median age 31.7 (25.4–33.5) weeks]. See SI Materials and Methods and Table S2 for details.

Seventeen healthy term-born control infants were also examined (six male; median (range) gestational age at birth 38.6 (36.0–41.1) weeks; median postmenstrual age at scan 41.4 (39.5–44.0) weeks; median birth weight 3.12 (2.68–4.20) kg).

Imaging. Each infant successfully underwent T2-weighted MRI and 32-
direction diffusion MRI acquisition. MRI was performed on a Philips 3-Tesla system (Philips Medical Systems) within the neonatal intensive care unit using an eight-channel phased-array head coil (see SI Materials and Methods for details).

Whole-Brain Connectivity. For each infant, a cortical mask was derived from tissue segmentation driven by age-specific priors (64). Cortical masks were inspected slice-by-slice and manually edited to remove any remaining noncortical voxels. Poisson disk sampling was used to parcellate each cortical mask into ~500 ROIs (mean ± SD = 499.5 ± 6.1) as described previously (23). This process produces a set of randomly distributed and similarly sized cortical ROI and does not rely upon atlas-based anatomical borders or landmarks but results in different cortical ROI across individuals, precluding direct comparison; to address this, we adopted a sampling procedure in which 100 sets of random cortical ROI were produced for each infant, and whole-brain tractography was repeated for each set to generate 100 networks per subject. Topological metrics and nodal connectivity maps derived from each network were then combined to provide summary measures and to generate voxel-wise con-

nectivity maps that allow concatenation and comparison across subjects (Fig. S1).

All sets of cortical ROI were transformed from T2 space into diffusion space using nonrigid registration as implemented in the IRTK software package (65). Before processing, all datasets were visually assessed for motion arti-
facts (SI Materials and Methods). Diffusion data were preprocessed using FSL’s Diffusion Toolkit (www.fmrib.ox.ac.uk/fsl). For each set of cortical target regions, 1,000 streamlines were propagated per seed voxel using a modified version of ProtrackX (66, 67). Tracking stopped when stream-
ines reached a target region, left the brain mask, or entered voxels con-
taining cerebrospinal fluid. Although deep gray-matter structures were not included as targets during tractography, streamlines passing through them to connect two cortical targets were included in the initial network analysis.

Network Construction and Analysis. A connection matrix was constructed from each of the 100 sets of cortical ROI for each subject (SI Materials and Methods and Fig. S1). The Brain Connectivity Toolbox (68), implemented in Matlab (MathWorks, Inc.), was used for network analysis. Edge density, clustering coefficient, C, and characteristic path length, L, were averaged over all nodes in each network and the mean over 100 networks taken as a sum-

nary measure per subject (Fig. S1D). Normalized L and C metrics were estimated by comparing each network to a set of randomized networks (n = 100 per graph, n = 10,000 per subject) with equivalent size and degree distribution (69).

Rich-Club Organization. For a N × N matrix M, where N equals the number of ROI in a parcellation, a measure for rich-club organization can be calculated over a range of degrees k:

$$\phi(k) = \frac{2E_k}{N(N-1)}$$

where, after removing all nodes N with degree less than k, the rich-club coefficient \(\phi(k)\) equals the ratio of connections present between the remaining nodes \(E_k\) and the total number of possible connections that would be present if the remaining nodes were fully connected (6, 7). For comparison across individuals, \(\phi(k)\) is typically normalized by the average coefficient calculated over a set of random networks \(\phi_{random}(k)\). A normal-
ized rich-club coefficient \(\phi_{norm}\) of >1 across a range of k indicates rich-club organization in the network (Fig. S1B). Because \(\phi\) varies as a function of degree k, a threshold is often applied to designate a discrete set of high-

degree nodes for which \(\phi\) and \(\phi_{norm}\) are calculated (6). In this study, to allow for comparison across networks with varying node number and degree, nodes were labeled as belonging to a network core or periphery based on their respective degree. All nodes were ranked according to degree, and nodes with the lowest number of connections removed in one-percentile increments (approximately five nodes at a time); excluded nodes were des-

ignated as peripheral, with the remaining nodes forming the network core. \(\phi\) and \(\phi_{norm}\) were then calculated for each network core, as lower degree nodes were incrementally removed; this allows for comparison between core networks of equal size in each group.

Subject Comparison. To visualize and compare nodal metrics, a recently de-
volved technique—gray matter-based spatial statistics—was used to pro-
ject connectivity data onto a skeletonized representation of group mean cortical anatomy to mitigate the effects of misalignment from registration, intersubject variability, and partial volume contamination (40).

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