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Marion I. van den Heuvel[a,b,d,*], Elise Turk[e], Janessa H. Manning[a,b], Jasmine Hect[a], Edgar Hernandez-Andrade[b,f], Sonia S. Hassan[b,f], Roberto Romero[b,c,g,h,i], Martijn P. van den Heuvel[j], Moriah E. Thomason[a,b,k]

[a] Merrill Palmer Skillman Institute for Child and Family Development, Wayne State University, Detroit, MI 48202, USA
[b] Perinatology Research Branch, NICHD/NIH/DHHS, Detroit, MI, USA
[c] Perinatology Research Branch, NICHD/NIH/DHHS, Bethesda, MD, USA
[d] Department of Cognitive Neuropsychology, Tilburg University, Tilburg, The Netherlands
[e] Brain Center Rudolf Magnus, Department of Neonatology, University Medical Center, Utrecht, The Netherlands
[f] Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI 48202, USA
[g] Department of Obstetrics and Gynecology, University of Michigan School of Medicine, Ann Arbor, MI 48104, USA
[h] Department of Epidemiology, Michigan State University, East Lansing, MI 48825, USA
[i] Center for Molecular Medicine, Wayne State University, Detroit, MI 48202, USA
[j] Brain Center Rudolf Magnus, Department of Psychiatry, University Medical Center, Utrecht, The Netherlands
[k] Department of Pediatrics, Wayne State University School of Medicine, Detroit, MI 48202, USA

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ABSTRACT

Advances in neuroimaging and network analyses have lead to discovery of highly connected regions, or hubs, in the connectional architecture of the human brain. Whether these hubs emerge *in utero*, has yet to be examined. The current study addresses this question and aims to determine the location of neural hubs in human fetuses. Fetal resting-state fMRI data (N = 105) was used to construct connectivity matrices for 197 discrete brain regions. We discovered that within the connectional functional organization of the human fetal brain key hubs are emerging. Consistent with prior reports in infants, visual and motor regions were identified as emerging hub areas, specifically in cerebellar areas. We also found evidence for network hubs in association cortex, including areas remarkably close to the adult fusiform facial and Wernicke areas. Functional significance of hub structure was confirmed by computationally deleting hub *versus* random nodes and observing that global efficiency decreased significantly more when hubs were removed ($p < .001$). Taken together, we conclude that both primary and association brain regions demonstrate centrality in network organization *before* birth. While fetal hubs may be important for facilitating network communication, they may also form potential points of vulnerability in fetal brain development.

1. Introduction

Advances in neuroimaging and network analyses have revealed the existence of sets of highly connected regions, often called “hubs”, that are critically important for enabling efficient neuronal signaling and communication (van den Heuvel and Sporns, 2013; Sporns et al., 2007). In the adult human brain network, functional hubs are consistently found in the ventral and dorsal precuneus, posterior and anterior cingulate gyrus, ventromedial frontal cortex, and interior parietal brain regions (Zuo et al., 2012; Tomasi and Volkow, 2011), brain areas with considerable overlap with sub regions of the default mode network (DMN) (Greicius et al., 2003). Accumulating evidence suggests that, because of their high functional connectedness in the brain, hubs support information integration that forms the foundation for numerous aspects of complex cognitive function. Another consideration is that this high level of centrality in the brain may make hubs highly susceptible to insults and/or disconnection (Crossley et al., 2014; Aerts et al., 2016; Bullmore and Sporns, 2012). In line with this, abnormal hub organization has been implicated in several neurological and psychiatric brain disorders (Bullmore and Sporns, 2012; Bassett and Bullmore, 2009). Given the central role of hubs in brain organization, knowledge about properties of hubs at the beginning of human life is particularly valuable and may offer insight into the origins of developmental and psychiatric disorders.

Developmental studies in the first year of life have shown that functional hubs are already observable in infancy and are, while not

* Corresponding author at: Merrill Palmer Skillman Institute, Wayne State University, 71 E. Ferry Street, Detroit, MI 48009, USA.
E-mail address: m.vdnheuvel@wayne.edu (M.I. van den Heuvel).

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fully mature, transitioning to an adult configuration (De Asis-Cruz et al., 2015; Fransson et al., 2011; Gao et al., 2009, 2011; van den Heuvel et al., 2015). While initial studies reported that functional hubs were largely confined to primary sensory and motor brain regions in the infant brain (Fransson et al., 2011; Gao et al., 2011), a more recent study found functional hubs in other areas of the infant brain, such as subcortical-limbic-paralimbic areas, and reported that functional hub organization may constitute a more mature configuration than previously thought (De Asis-Cruz et al., 2015). Further support for nascent hub structure comes from examination of the default mode network in infancy. An immature and incomplete DMN is present in 2-week-olds and the posterior cingulate cortex (PCC) seems to anchor this configuration as the main functional "hub" within this network (Gao et al., 2009). Together, these studies show that a hub configuration, although immature, already exist in the infant brain. The question remains, however, whether functional hub configuration of the brain emerges *before* birth, as networks already begin to form *in utero* (Thomason et al., 2014, 2013, 2015; Schöpf et al., 2012).

Insight into hub emergence before birth may be of great importance, since the fetal period is a critical time in brain development (Rice and Barone, 2000; Keunen et al., 2017) in which seemingly small disturbances, insults or exposures can produce lifelong changes in neurological and mental functioning. Consistent with this notion, the origins of neurodevelopmental disorders are increasingly attributed to alterations occurring during the prenatal period (Di Martino et al., 2014; Paneth et al., 2005; Rosenbaum et al., 2007) despite onsets in symptomatology between the 3rd and 20th years of life (Paus et al., 2008). Studies involving premature infants have started to examine hub development as a proxy for prenatal brain development, scanning neonates with MRI prior to post conception week 42. These studies have revealed the existence of hubs and demonstrate that hubs are highly connected, an observation suggesting a functional rich-club organization is present in the brain network in very early life (van den Heuvel et al., 2015; Ball et al., 2014). Although these studies provide valuable insights into hub development in the antenatal period, several factors inevitably linked to preterm birth, including premature exposure to the extra uterine world and deprivation from a variety of hormones (*i.e.*, IGF-1, estrogens, progesterone and thyroid hormones) that are normally provided during (late) pregnancy (Berger and Soder, 2015; Elitt and Rosenberg, 2014), make generalizing the results from these studies to normal fetal hub development challenging.

Until now, information about fetal functional hub development *in vivo* has been utterly absent as information about human fetal brain function has been inaccessible. Recently, new advances in MRI have overcome this limitation. It is now possible to examine coordinated action in the human brain before birth (for a recent review, see van den Heuvel and Thomason, 2016). Pioneering cross-sectional studies on fetal resting-state functional connectivity (RSFC) have began to map development of the brain function before birth and have shown that intra-hemispheric, cross-hemispheric, and long-range connectivity become stronger with advancing fetal age (Thomason et al., 2014, 2013, 2015). Additionally, fetal fMRI studies have postulated the importance of PCC connectivity for the fetal brain network (Thomason et al., 2014, 2015). Nevertheless, functional hub development during the prenatal period remains unstudied.

The current study addresses the question whether functional hubs are operational before birth and aims to isolate and describe those neural hubs in a large sample of human fetuses using network analyses. In network analyses, the brain is viewed as a network or "graph" consisting of nodes (brain regions; ROIs) that are connected by edges (connectivity strength between nodes). Hubs will be identified based on graph theoretical measures of nodal importance (van den Heuvel and Sporns, 2013; Sporns et al., 2007; Rubinov and Sporns, 2010). Based on previous reports on hubs in infants (De Asis-Cruz et al., 2015; Fransson et al., 2011), we expect to find functional hubs mostly in primary sensory and motor brain areas in the fetal brain. After isolation of the putative hubs, we will examine whether the identified hubs are critical for efficient functioning of the fetal brain network by testing how the fetal brain network responds to computational attack targeted on hubs as opposed to attack on random non-hub nodes.

2. Materials and methods

2.1. Participants

Healthy pregnant women were recruited in Metro Detroit, USA to participate in a fetal magnetic resonance imaging (MRI) brain study in the second and third trimester. Inclusion criteria for scanning were: maternal age > 18 years, English as first language, singleton pregnancies, and typical fetal brain development (as assessed by ultrasound and anatomical MRI examination prior to functional MRI scanning). From the 138 fetuses available for this study with preprocessed fMRI data, we excluded those that were later born moderately or extremely premature and/or had low birth weight (< 35 weeks of GA, < 2400 g; N = 9), were born to a mother that was older than 40 years of age (N = 1), and/or were diagnosed as high risk pregnancies (*i.e.*, PPROM, IUGR, preeclampsia; N = 15). Additionally, we excluded those fetuses of which fMRI data did not meet quality criteria after removing high movement volumes (< 100 low-motion volumes; N = 7) and/or motion (1.7 mm max excursion, 0.6 mm mean; rotational: > 2.5°; N = 1).

These quality criteria resulted in a final sample of 105 fetuses (64 male; 41 female). Included fetuses had a mean gestational age of 33.49 (range = 20.6–39.6 weeks of gestation; s.d. = 3.97) weeks at scanning and were born, on average, at 39.25 (s.d. = 1.22) weeks of gestation. Gestational age was determined by a study physician (E.H.-A.) by ultrasound examination within 1 week of MRI testing. More detailed characteristics of our sample are provided in Table 1.

2.2. Functional data acquisition and preprocessing

Between 12 and 24 min of fetal brain resting-state fMRI data were collected using a 3-T Siemens Verio 70-cm large bore system and 4-Channel Siemens Flex Abdominal Coil. Images were collected with the following parameters: echo planar imaging (EPI) BOLD, TR/TE 2000/30 ms, 360 frames, axial 4 mm slice thickness (voxel size: 3.4 × 3.4 × 4 mm). This fMRI sequence was repeated a second time within the same scan session to maximize available resting-state fMRI data. Computed SAR values = 0.22 (SD = 0.07). For the final sample included in analyses, frame count, after excluding high motion frames, was range = 100–332, mean = 168 (SD = 57). Time frames corresponding to periods of head motion < 1 mm frame-to-frame displacement and < 1.5° rotation in the fetus were identified using FSL image viewer (FSL, 2018). Brainsuite (Shattuck and Leahy, 2002) was used to manually draw 3D masks for a reference frame from each period of fetal

Table 1
Characteristics of mother and fetus (N = 105).

Outcome	Value
Maternal age, years	24.84 ± 4.46
Race/ethnicity, %	
Caucasian	10.5
African–American	81
Bi-racial	2.9
Not disclosed	5.7
Child sex, %	
Female	39
Male	61
Gestational age at scan, weeks	33.49 ± 3.97
Gestational age at birth, weeks	39.25 ± 1.22
Birth weight, grams	3304.05 ± 438.65

Values presented as mean ± SD where appropriate

movement quiescence. Masks were binarized and applied only to frames corresponding to their select segment, and only those data were retained for further analyses. 56% of data collected were retained after motion censoring. Subsequent within-segment preprocessing steps included reorientation, realignment and normalization to an average 32 week gestational age fetal anatomical template (Serag et al., 2012) using SPM8 (Statistical Parametric Mapping 8 from the Wellcome Trust Centre for Neuroimaging, 2009). To correct for variation in normalization across segments, within-participant normalized images were then concatenated into one time-series, realigned, and smoothed using a 4 mm Gaussian kernel across.

2.3. Regions of interest

A spatially constrained group level clustering approach (Craddock et al., 2012) was used to parcellate the preterm template brain at 32 weeks of gestation (Serag et al., 2012) into spatially contiguous, similarly sized ROIs. Briefly, this method produces functionally homogenous clusters at the individual level by assessing voxel-level time-series similarity in a given data set, using Pearson correlations, then iteratively merging voxels whose within-cluster similarity is maximal and between-cluster similarity is minimal. Next, it identifies the most representative clusters of voxels using a normalized cut algorithm (van den Heuvel et al., 2008) and performs group level clustering. This method produces ROIs that are optimally functionally homogenous and consistent across individuals. Seventy-six concatenated fetal fMRI data sets, processed in the same manner as stated above and occur in the sample analyzed here, were submitted to this procedure. 200 ROIs distributed across the cortex, subcortical structures, and the cerebellum were generated, three of the which were anomalous and excluded from analysis, resulting in a fetal ROI Pycluster atlas of N = 197 regions, available for download at www.brainnexus.com.

2.4. Connectivity matrices and computation of hub measures

The CONN-fMRI Toolbox (Whitfield-Gabrieli and Nieto Castanon, 2012) was used to compute functional connectivity (FC) matrices. The anatomical component correction (aCompCor) method of estimating and removing noise (Behzadi et al., 2007; Chai et al., 2012) was applied. Principal components of signals from white matter and cerebral spinal fluid, as well as translational and rotational movement parameters (with another six parameters representing their first order temporal derivatives), were removed with covariate regression analysis. Pearson's correlation coefficients were then estimated from time series data for each pair of nodes. Fisher's transformation was used to convert coefficients to z-scores to produce FC correlation matrices for each participant.

From these matrices, measures of network centrality, degree and betweenness centrality (BC), were computed for every region by means of graph theoretical analysis, using the brain connectivity toolbox for Matlab (Rubinov and Sporns, 2010). The degree of a node is the number of connections that node has with other nodes, while the BC of a node is the number of times that node is included in the shortest path of each node to every other node (van den Heuvel and Sporns, 2013; Rubinov and Sporns, 2010). We used a threshold of T = 0.25 (Medium-high z-scores) for the computation of our network metrics, to minimize the effect of spurious correlations. To mitigate potential influence of the threshold set for participant functional graphs (T = 0.25) (van den Heuvel et al., 2017), we adjusted the threshold to T = 0 (*i.e.*, no thresholding, negative correlations removed), T = 0.35, and T = 0.45, recomputed resulting graph metrics, and compared our results with the T = 0.25 results (cf. van den Heuvel et al., 2015). A graphical representation of our preprocessing steps is presented in Fig. 1.

2.5. Analytic approach

In line with the work of Fransson et al. (2011), the 10 regions with highest weighted degree and betweenness centrality were identified. Subsequently, putative hubs were classified by hemisphere, by lobe, and by coordinates corresponding to the center of mass for the 32-week GA fetal template brain (Serag et al., 2012). Next, we examined hub network development by using a median split of our data to create two gestational age groups. The top 10 nodes in both groups were identified for both gestational age groups separately and compared. Finally, we examined the effect of computational attack to measure the relative importance of hub nodes in facilitation of efficiency in the overall fetal network as compared to random non-hub nodes. For this simulated attack, we first computed the global efficiency of the network by computing the inverse of the average number of steps needed to travel from every node in the network to every other node in the network, with longer paths being less efficient. We then simulated an attack to random nodes by randomly selecting 12 nodes and deleting all connections of those nodes with others from the group matrix and subsequently re-computing the global efficiency. The same was performed for the 12 nodes that were identified as hubs. To generate an empirical cumulative distribution function amenable to statistical testing, we repeated the random deletion procedure a 1000 times, as has been done in prior works (Hwang et al., 2013; van den Heuvel and Sporns, 2011). We then compared the resulting global efficiency after deleting hub nodes with the group average global efficiency after deleting random nodes with a one-sampled *t*-test. To create a measure of change, we computed the percentage change between the non-attacked and attacked global efficiency for both the randomly and targeted (hub) attack.

3. Results

3.1. Isolation of fetal brain hubs

We computed two measures of nodal importance: weighted degree (*i.e.*, strength) and betweenness centrality (BC). The weighted degree of a node, or strength, is the sum of all connection weights of the nodes that are connected with that node, while the BC of a node is the sum of the connection weights of all shortest paths of each node to every other node that "run through" that node (Rubinov and Sporns, 2010). Consistent with Fransson et al. (2011), we report the 10 strongest degree and BC nodes as hubs. We found that resultant degree and BC hubs have strong overlap, with 8 nodes falling in the top 10 for both degree and BC.

In line with our hypothesis, several hubs were located in primary sensory and motor brain areas, specifically the left and right cerebellum, left precentral gyrus, and right primary visual cortex. In addition, hubs were identified in association cortex, including left and right inferior temporal gyrus, angular gyrus, and medial temporal lobe. Fig. 2 depicts the spatial distribution of top identified hubs as defined by degree and BC. Notably, hubs identified in the left and right inferior temporal lobe are close to the area that will later develop into the fusiform facial area. Further, the area identified as hub in the left angular gyrus seems to overlap with what will develop as Wernicke's area. We also found that hub development follows a pattern of myelination, as 25% of peak hubs reside in the cerebellum, one of the earliest brain regions to myelinate (Deoni et al., 2011). Additionally, all isolated cortical hubs are located in areas marked as earliest to myelinate by Glasser and Van Essen (Glasser and Van Essen, 2011). It is also noteworthy that several hubs were localized in homologous contralateral brain areas, including bilateral cerebellum and medial temporal lobes. Overall, more hubs were observed in the left rather than right hemisphere (7 *versus* 5 hubs), suggesting some asymmetry of hub organization in our findings. Spatial coordinates for characterized hubs are provided in Table 2.

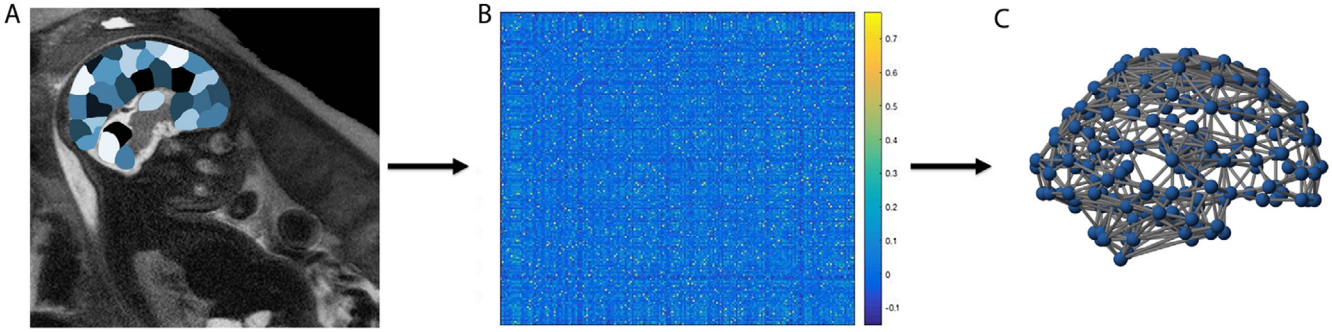

Fig. 1. Graphical representation of preprocessing steps. After acquiring fetal resting-state functional MRI data in N = 105 fetuses, a data-driven functional parcellation strategy was used to divide a 32-week template fetal brain into 197 similarly sized regions of interest (ROIs; panel A). Functional connectivity strength between every pair of ROIs was then computed to construct connectivity matrices for every fetus (panel B). In a final step, graph theoretical metrics, degree and betweenness centrality, were computed from connectivity matrices to identify functional hubs in the fetal network (panel C).

3.2. Influence of thresholding on derived fetal graphs

Since network metrics computed with graph theoretical analyses can vary with different thresholds (Drakesmith et al., 2015), our next step was to test the influence of different thresholds (T) on our results. We computed weighted degree and BC with three different thresholds, T = 0, T = 0.35, and T = 0.45, and re-isolated the top 10 degree and BC hubs (cf. van den Heuvel et al., 2015). The different thresholds yielded very similar results, indicating the robustness of our findings to thresholding. Additionally, binary metrics (*i.e.*, not weighted) resulted in similar results than the weighted measures.

3.3. Age differences in hub development

To examine potential effects of fetal age on hub localization, we performed a secondary analysis, splitting the sample at median age, 35 weeks gestational age, and recomputing degree and BC within older and younger fetal subgroups. The process resulted in N = 49 < 35 weeks, and N = 59 > 35 weeks with very similar hub locations. The most consistent pattern was observed for the cerebellum; all three cerebellar hubs observed in the full group were also observed when splitting the data. Some hubs were only observed at earlier or later

Table 2
Identified hubs in the fetal brain network.

Hub #	Region	Left/Right	Coordinates		
			x	*y*	*z*
Overlapping Hubs					
1	Inferior Temporal Gyrus	Left	−24	0	−26
2	Inferior Temporal Gyrus	Right	30	−6	−22
3	Cerebellum	Right	8	−18	−32
4	Precentral Gyrus	Left	−24	−2	24
5	Cerebellum	Left	−16	−16	−28
6	Medial Temporal Lobe	Left	−20	−12	−20
7	Agular Gyrus	Left	−30	−16	14
8	Medial Temporal Lobe	Right	20	−8	−22
Degree Hubs					
9	Cerebellum	Left	−10	−20	−34
10	Inferior Temporal Gyrus	Left	−32	−8	−20
BC Hubs					
11	Primary Visual Corex (V1)	Right	14	−44	−14
12	Inferior Temporal Gyrus	Right	32	4	−24

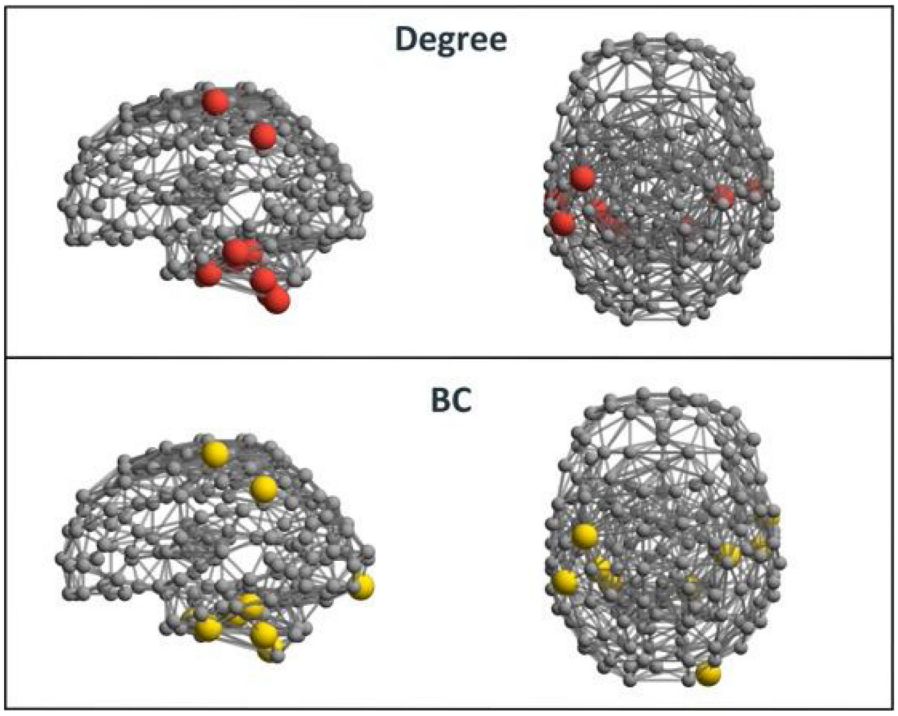

Fig. 2. Location of putative hubs in the fetal brain. Graphs were constructed with connections showing at threshold T = 0.25, separately for weighted degree (upper panel; larger red spheres) and betweenness centrality hubs (lower panel; larger yellow spheres). Grey spheres represented other non-hub nodes in the network. Hubs were observed in areas of the cerebellum, inferior temporal gyrus, precentral gyrus, angular gyrus, medial temporal lobe, and the primary visual cortex. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

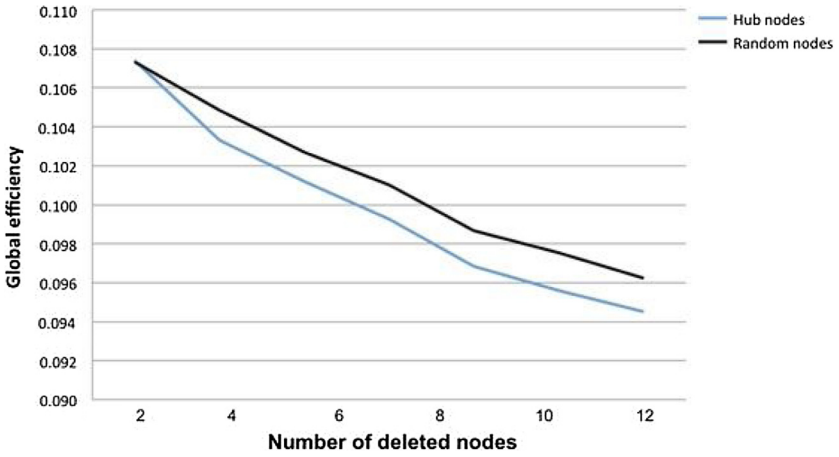

Fig. 3. Computational attack of hub nodes *versus* random nodes. The plot presents changes in global neural efficiency of the fetal brain in response to computational attack of hub nodes (blue) and random nodes (black). The plot shows that global efficiency decreases faster when hub nodes are computationally deleted from the network then when to random nodes are deleted. Attacking the 12 putative hubs decreased global efficiency more than attacking random nodes ($p < .0001$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

gestational age only: The left Medial Temporal Lobe and right Inferior Temporal Gyrus were identified as hub in the later gestational age group only, whereas the Precentral Gyrus and Angular Gyrus were only observed as hubs in earlier gestational age group. Similar effects were obtained for degree and BC hubs.

3.4. Importance of hubs for network efficiency in the fetal brain

To address the functional relevance of derived hub *versus* non-hub nodes, the consequences of node elimination on overall network efficiency was assessed. When simulating random attack by deleting random nodes from the network the global efficiency degraded with a 12.7% change. In contrast, when simulating targeted attack by deleting the hub nodes only, neural global efficiency degraded with a 14.5% change. The change resulting from targeted attack is significantly higher ($t = 46.910$, $p < .001$) as compared to change resulting from random attack, indicating that, as expected, the hubs are more important for global network efficiency than the randomly selected nodes. Change in global neural efficiency when removing hub nodes and random nodes one by one is reported in Fig. 3. Global efficiency decreases more when hub nodes are computationally removed from the network then when to random nodes are removed. These results suggest that hubs may already have a key role in facilitating efficiency in the network before birth.

4. Discussion

This is the first study to examine functional hubs in the human brain prior to birth *in utero*. We discovered that within the connectional functional organization of the human fetal brain there are hubs and that they are already important for neural efficiency in the fetal brain. Both primary (visual cortex, precentral gyrus) and association brain regions (inferior temporal gyrus, medial temporal lobe) demonstrated centrality in network organization *before* birth. Interestingly, 25% of hubs were localized in the cerebellum and all other hubs were located in areas marked to myelinate first according to Glasser and Van Essen (Glasser and Van Essen, 2011), which suggests that hub emergence in the fetal brain may follow early myelination patterns. Additionally, several interesting observations were made: 1) hubs were found in areas close to adult fusiform facial area and Wernicke's area, 2) several hubs were located in homologous areas, and 3) more hubs were located in the left than the right hemisphere. Taken together, these results suggest that hubs emerge *before* birth and that they may serve as important building blocks for early brain development, making them points of vulnerability in the developing network.

4.1. Fetal hubs are located in both primary and association cortices

The location of the identified fetal hubs largely overlaps with hubs located in preterm and term neonates in previous studies (De Asis-Cruz et al., 2015; Fransson et al., 2011; van den Heuvel et al., 2015; Gao et al., 2011; Ball et al., 2014). Consistent with prior reports in infants (Fransson et al., 2011; Gao et al., 2011), we have identified visual and motor regions as functional connectivity hubs. Studies examining structural hubs in the neonatal network also found a large portion of hubs in sensorimotor areas (van den Heuvel et al., 2015; Ball et al., 2014). Interestingly, we also found evidence for network hubs in inferior temporal and medial frontal regions, indicating that functional hubs are also present in association cortex. This latter finding is in line with more recent reports on early hub development in term newborns (De Asis-Cruz et al., 2015). Similar hubs, located in temporal and frontal areas, have been derived from assessment of structural architecture of the preterm and neonatal cortex using diffusion tensor imaging (DTI) (van den Heuvel et al., 2015; Ball et al., 2014). Furthermore, our observation of hubs in the fusiform gyrus was also observed in two other studies examining hubs in health term newborns (De Asis-Cruz et al., 2015) and preterm infants (van den Heuvel et al., 2015). One brain area that was consistently reported in previous postnatal studies, both structural (Ball et al., 2014) and functional (De Asis-Cruz et al., 2015; Fransson et al., 2011; Gao et al., 2011), was not found in the current study: the insula. Gao et al. (2011) even observed the insula as major hub in all three age groups investigated (newborn, 1-year-olds, and 2-year-olds). The lack of hubs identified in the insula in the fetal period could point to a developmental trajectory of centrality in this area, with higher centrality emerging around birth and continuing into the first few years of life. Another notable difference in hub locations observed in our study compared to previous reports is the finding of hubs in the cerebellar area. This is most likely the result of the fact that previous studies have not considered the cerebellum into their analyses. Our results emphasize the importance for future studies to include cerebellar regions into hub analyses.

Taken together, our results show considerable overlap with previous reported hubs in term and preterm neonates and indicate that both primary and association brain regions demonstrate centrality in network organization beginning in fetal life. The fetal brain network may not be wired to solely support tasks that are of a perception–action nature, as has previously been thought, but instead, prepare the brain for higher order cognitive functioning that develops later in life. In this view, fetal network hubs may be important building blocks for later life cognition and emotion development.

4.2. Hubs follow developmental pathways of brain development

The identified fetal hubs seem to follow the characteristics of early brain development. For instance, several hubs were located at homologous areas, such as in the cerebellar and medial temporal regions. This finding is in line with reports of cross-hemispheric connectivity increases with advancing age in the fetal brain (Thomason et al., 2013). Additionally, we observed more hubs in the left hemisphere than in the right. This latter finding fits with prior studies reporting left-hemisphere asymmetry (Dehaene-Lambertz et al., 2002) and more rapid myelination of the left hemisphere in early infancy (Deoni et al., 2011). This, together with the finding of a putative hub in the proto-Wernicke's area, seems to suggest emerging asymmetry of the fetal brain focused around the language system. Furthermore, we found hubs in the left and right inferior temporal gyrus that are close to the fusiform facial area in adults, suggesting the intriguing possibility that the developing brain may prepare for recognizing faces *before* birth. However, more research is necessary to confirm this theory.

Additionally, the observation of multiple hubs in the cerebellum could be related to the finding that myelination starts in this region (Deoni et al., 2011; Yakolev and Lecours, 1967; Gilles and Dooling, 1983; Barkovich et al., 1988). Mature myelin is already detected from 37 to 40 weeks of gestation in the cerebellum (Dubois et al., 2014). Hubs were also identified in the primary visual area and motor area, which are both areas that are reported to myelinate early in development (Deoni et al., 2011; Glasser and Van Essen, 2011; Dubois et al., 2014). One could speculate that the high centrality of hub regions creates high regional functional signaling, which, in turn, may stimulate myelin formation. This possible explanation for the simultaneous emergence of hubs and myelination in the same area is supported by the recent demonstration that neuronal activity regulates changes in myelin-forming cells within an active circuit in the mouse brain (Gibson et al., 2014). Moreover, animal models have shown that spontaneous bursts of synchronized neuronal activity, or spindle bursts, play an instructive role in key developmental processes that set early cortical circuits (Hanganu-Opatz, 2010). Interestingly, a recent study in preterm human infants found that spontaneous bursting neuronal activity was mostly found in the insula and temporal cortices (Arichi et al., 2017), two areas that have been identified as hubs in the developing brain (see discussion in Thomason, 2018). Our observation of several fetal hubs in the temporal cortex could fit with the notion that hubs arise in brain areas with spontaneous bursting activity, or vice versa. Again, the insula may only become a hub in later prenatal and early postnatal stages. The relationship between myelination, spontaneous bursting neuronal activity, and hub topology over development should be investigated more closely in future studies.

4.3. Cerebellar hubs: importance of cerebellum in the fetal period

The cerebellum may be of particular importance for the fetal brain network, as cerebellar growth is known to be exceptionally rapid over the third trimester of pregnancy and unparalleled by any other brain structure during this period (Limperopoulos et al., 2005). Accumulating research has emphasized the importance of this region in the early developing brain (Volpe, 2009). Moreover, alterations in cerebellar structure and function are frequently implicated in major developmental neuropsychological disorders, such as ADHD and autism (Seidman et al., 2005; Castellanos et al., 2002; Wang et al., 2014; Stoodley, 2014), and are assumed to develop prenatally. Recent work has also revealed that the fetal cerebellum may be particularly sensitive to premature birth (Limperopoulos et al., 2005; Pierson and Al Sufiani, 2016) and prenatal exposure to maternal psychological stress (Ulupinar and Yucel, 2005;). The results of the current study demonstrate that there are cerebellar hubs in the fetal brain network, both in the left and right hemisphere, and that the cerebellum is consistent hub in both earlier (< 35 weeks) and later gestation (> 35 weeks), emphasizing the importance of this structure for developing neural architecture. The fact that we consistently identified the cerebellum as hub in earlier gestation, as well as late gestation, shows that this brain region may already become important early in gestation. However, we cannot infer any conclusions from our data about the cerebellum as hub in the first half of pregnancy, as our data starts at 20.6 weeks of gestation. Interestingly, cerebellar hubs have also been identified in late childhood and adolescence (Hwang et al., 2013), suggesting a prolonged role of the cerebellum in brain networks over development. Given prior assertions that the centrality of hubs increase their susceptibility to insults (Crossley et al., 2014; Aerts et al., 2016; Bullmore and Sporns, 2012), and given prior reports of the importance of the cerebellum for early development, our results provide new data suggesting the cerebellum may be a particularly vulnerable brain region in early life.

4.4. Hubs as early markers of neurological and psychiatric brain disorders

Our data suggest that the identified hubs may already have a key role in facilitating efficiency in the brain network before birth. Global efficiency of the fetal network decreased more when computationally removing hub nodes than when removing random nodes. This finding is in line with Hwang et al. (2013), who reported that lesioning functional hubs significantly reduced efficiency compared with lesioning random nodes across all age groups studied (10–12, 13–17, and 18–20 years of age). Additionally, Gao et al. (2011) reported that maturation of network topology and hubs make the infant brain at age 2 years more resilient to both random errors and targeted attack than the newborn brain. Building on this latter, hubs may be particular vulnerable in the prenatal and early postnatal period and abnormal functioning of hubs during this period may serve as useful early markers of neurological and psychiatric brain disorders. There is accumulating evidence available for altered hub topology in psychiatric disorders, such as autism and schizophrenia (Crossley et al., 2014; Bassett et al., 2008; Itahashi et al., 2014; van den Heuvel et al., 2013). More research is necessary to fill the gap in our knowledge about hub development in both typically and abnormally developing fetuses.

4.5. Limitations

Several challenges are inherent in using resting-state fMRI to study the fetal brain (reviewed by van den Heuvel and Thomason, 2016). An important issue in fetal imaging that is relevant to the current study is the lack of fetal atlases. As a result, there are limits on the specificity of nomenclature used for regions isolated as hubs in the current study. Taking this limitation into account, we have restricted our report to very broad areas (left/right, medial/lateral, lobe), and report localization of hubs in fusiform facial and Wernicke's areas as putative, in the fetal brain. Another critical issue is movement of the fetus during scanning. Motion in the fetus and mother contribute to changes in image signal intensity that is difficult to separate from signals of interest (van den Heuvel and Thomason, 2016; Ferrazzi et al., 2014). To mitigate movement related errors, we have developed a 3-step method: 1) we select frames of quiescence, 2) we quantify total movement for each subject and eliminate frames or cases as needed based on strict quality and motion criteria (translational: > 1.7 mm max excursion, 0.6 mm mean; rotational: $> 2.5^{\circ}$), and 3) we eliminate any cases with frame number less than 100 frames. A limitation of this approach is that, by discarding high motion frames, we may have biased our results to reflect a particular behavioral (*i.e.*, quiet sleep) or neurological state. Another limitation that warrants mentioning is that our approach of cropping and concatenating data may have caused edge artifacts that can affect autocorrelation correction, potentially creating an artificial pattern of correlations. Finally, the relatively wide age range of our sample (~ 8 weeks) could be considered as a limitation as well. This limitation is partly tackled by normalizing and realigning all data to a 32 weeks of gestation template. Although realignment to one template

likely has a positive effect on the stability of our group level connectivity patterns, the wide age range could still be considered a limitation given the very rapid development *in utero*. Future studies should include more restricted age range or compare several age groups to test for developmental effects of hub development. A final concern is the fact that more male than female fetuses were scanned in this study. Since research has shown sex differences in brain connectivity maturation over development in childhood/adolescence (De Bellis et al., 2001; Satterthwaite et al., 2015) and even in infancy (Gao et al., 2015), this has potential to influence our results.

5. Conclusion

In sum, we report that within the connectional functional organization of the human fetal brain there are hubs, or regions that are central to the connectional architecture of neural circuitry. Putative hubs were identified in visual and motor areas, as expected, but also in association cortices. Interestingly, several of the derived connectivity hubs were localized in cerebellar regions, supporting the novel theory that hubs emerge in areas that are early to myelinate. It could be speculated that, because of their high centrally in the network, hub regions produce high levels of neural activity, which, in turn, stimulates myelin development in these regions. Furthermore, results from computationally attacking hubs compared to random nodes demonstrated that hubs are important for global efficiency of the fetal brain, emphasizing their importance in the fetal network. These results indicate that the fetal brain network may not be wired to solely support tasks that are of a perception–action nature, as previously been thought, but instead, prepare the brain for higher order cognitive functioning that develops later in life. This work raises the intriguing question as to whether the areas we have identified as hubs in the fetal brain also constitute areas of selective vulnerability, and whether functional connectivity profiles of these regions may in the future illuminate ontological bases of neurodevelopmental disorders or serve as biomarkers for later life neurodevelopmental health.

Conflict of interest

The authors report no biomedical financial interests or potential conflicts of interest.

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