International Journal of Radiology Sciences
www.radiologyjournals.com
Online ISSN: 2664-9829, Print ISSN: 2664-9810
Received: 12-10-2021, Accepted: 26-10-2021, Published: 13-11-2021
Volume 3, Issue 1, 2021, Page No. 22-29

Resting-state FMRI: canonical networks in normal children

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Abstract
Independent Component Analysis of resting-state FMRI studies allows to extract a number of neural networks form task-less studies. There is a paucity of reports on normal networks and their variants found in normal children. We describe in this study the characterization of neural networks found in a group of normal children.
Methods: 40 datasets of normal children who underwent rs-fMRI were analyzed with ICA utilizing MELODICA from FSL library. For each subject, the independent components were classified between neural networks and non-neural networks based on a heuristic approach and performed by an expert on the field. Further characterization of the neural networks was accomplished based on localization of maxima, frequency of oscillation, and profile of oscillation. Frequency of network yield across subjects was assessed along frequency of the network oscillation and main variants.
Results: 24 distinct neural networks were found. Oscillation frequency ranged from 0.0168 to 0.072 Hz. Main pattern variants consist of network-merging and iterations of the same network at different frequencies. The most frequently found networks across the subjects were the right executive network (97.5%), the precuneus (82%) and visual (77.5%). The networks less frequently found were the hippocampus (7.5%) and the amygdala (10%).

Keywords: FMRI, rs-FMRI, Resting-state, children, ICA

Introduction

Functional MRI (FMRI) can be divided in two types: task-based and task-less FMRI. Task-based FMRI requires the subject to perform a task. This task may be "active," in which the subject is required to produce an overt motor or verbal response; or "passive," in which the subject receives a sensory stimulation of any type (visual, auditory, somatosensory, proprioception, pain, etc). The task-less FMRI does not require any task. This type of FMRI is known as "Resting-state Functional Magnetic Resonance "or simply rs-FMRI. The technique is gaining momentum in clinical research and even medical practice.

The standard FMRI is based on the Blood Oxygen Level Dependent (BOLD) effect [1]. Briefly, the BOLD effect is produced when the ratio between de-oxyhemoglobin and hemoglobin are locally changed in the brain cortex specifically activated by a task. The local magnetic properties result altered by the levels of de-xyhemoglobin, a bio-molecule with magnetic properties. The level of this molecule is a function of the delivery/consumption ratio of the O2 exerted by the firing neurons. Regional magnetic changes, therefore, make possible to map the brain's response to a stimulus.

The rs-FMRI is based in the same BOLD effect but, in this case, the neural firing variations are not due to extrinsic factors (stimuli). Instead, the rs-FMRI is based on the presence of normal oscillations of brain activity, known as "spontaneous low-frequency oscillations." These oscillations are pseudo-periodic variations of the neural activity that form like tides, involving different brain areas in synchrony.

RS-FMRI sequences may be analyzed in multiples ways. The local and remote synchrony of neural spontaneous activations may be utilized to reveal connectivity of a specific chosen area or region of interest (ROI). The ROI is selected because it has been previously established as a hub of an important cognitive, behavioral or sensory/motor function circuitry. The ROI becomes a "seed" and its mean profile of oscillation is used as a regressor for the rest of the brain. Areas with similar pattern of oscillations are accepted as connected to the seed ROI. This type of analysis is termed the ROI-based functional connectivity MRI. A further sophistication in the analysis allows to depict the connectivity between hubs via graphs utilizing the Graph-Theory technique. A third approach in the rs-FMRI data analysis is to analyze all the possible correlated signals within the data by clusterization of the entire space. The procedure is termed "Independent Component Analysis (ICA)". It has the advantage of not to rely in any a-priory hypothesis. The ICA provides a number of possible signal correlations including all types of motion, noise and the spontaneous low-frequency brain oscillations.

Several different oscillations occur in the brain grouping functional regions in networks. All these oscillations have the particularity to oscillate at very low frequencies - between 0.01 and 0.08 Hz. The spontaneous low-frequency oscillations involve mostly the gray matter and have striking characteristics. They are preserved during sleep and light sedation; they have been found in small vertebrates (rats); and some networks diminish their activity when the subject is involved in cognitive-loaded tasks. These networks involve the posterior cingulate gyrus, the mesial frontal areas and lateral parietal and prefrontal areas. The system is also known with the name: the default mode. Notice that the default mode is only a part of the many brain's spontaneous oscillating networks. There are excellent detailed reviews in rs-FMRI networks if the reader wants to deepen into the topic [2, 3].

There is a pronounced scarcity of normalcy studies that may provide standardization on networks types, network number,

intra- and inter-subject variability of the ICA-resolved networks in adults. The situation is worse with respect the pediatric population.

The lack of standardization of rs-FMRI findings processed with ICA, presents a further burden. In contrast to the regular MRI or an X-ray, in which the settings of standard performance are well known and concerted by the radiology practitioners, the FMRI sequences, in general, do not have a technique to which a majority of scholars or recognized authors agree on. Less on rs-FMRI. Things as basic as the number of time points, that is, the length of the study depends highly on the preference of the clinical or research group. Many other variables may differ. However, it seems important to remark at this moment that the number of network-yield of the ICA method mostly rely on the number of time points. Too few time points and the networks may appear collapsed as the algorithm falls short of data to differentiate neighboring oscillation profiles; too many time points and normal networks involving in synchrony both hemispheres may dissociate into two or more components. These errors are called underfitting and overfitting, respectively [4]. In the preprocess of the ICA technique, the user may predefine how many components to search for. This is type of threshold that has to be selected cautiously as it may also produce the same underfitting and overfitting errors.

Another important consideration to take into account is a subdivision of the resting-state neural networks based on "stability." Neural networks are in general quite stable across subjects, sessions and even subject cognitive status. There is, however, more stability across subjects with respect control systems (salient, executive and default mode networks) than processing networks (multimodal-areas). For a review on this topic, the reader is remitted to the work of Gratton C, *et al*, (2018) [5]. In our experience, intrasubject, trans-session network stability is best seen for networks representing unimodal functional areas (primary visual, foveal visual area, primary auditory, primary somatosensory and primary motor areas). The default mode and executive (fronto-parietal) networks follow. These networks may be termed "stable networks". In addition, networks that have been previously described on normal subjects and are felt to have some commonality in group studies are called canonical. A term denoting prudence for not to brand them directly as "normal'. In contrast, non-canonical networks lack of those features, and are probably related to structural or functional abnormal conditions.

However, much of these characterizations have been established in normal adults, but there is scarcity of work to characterize the frequency and distribution of the main rs-FMRI neural networks in the children population.

The AIM of this paper is to describe the presence, yield-frequency, distribution, inter-network associations and oscillatory frequency of the rs-FMRI networks in a population of normal children, utilizing ICA.

Methods

Subjects. 40 rs-FMRI data sets were utilized from the normative data of the project "ADHD200", a repository publicly available at http://fcon_1000.projects.nitrc.org/indi/adhd200. From all the candidates available, the selection criteria include: age 18 or below; right handedness; minimum Verbal IQ of 85; minimum Full-Scale IQ of 80; and absence of neurological conditions. Each dataset was visually inspected by one of the authors, who is a senior neuroscientist with more than 20 years of experience in fMRI in children. Any case with overt head motion was discarded. The final dataset was comprised of 19 male and 21 female subjects, from 7 to 18 years of age, mean/SD: 11/2.64. The study was approved by the Western IRB.

MRI sequence: the rs-FMRI sequences were obtained utilizing an echo-planar sequence sensitive to the BOLD effect with the following parameters: 180 time points (scan time 6:00 min), TR: 2000 ms, TE: 15 ms, 1 average, flip angle: 90, standard shim mode; voxel size 3.0 x 3.0 x 4.0 mm, 33 axial interleaved slices with no gap, FOV 240 mm, slice thickness 4.0 mm.

Pre/post-processing: each subject's FMRI was visually inspected in a dynamic presentation utilizing the Mango Tool (http://ric.uthscsa.edu/mango/). Patients exhibiting overt motion were discarded. Preprocessing was performed utilizing MELODIC (Multivariate Exploratory Linear Optimized Decomposition into Independent Components) a module of the Functional MRI Brain Software Library (FSL) version 3.0, public-available at https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/MELODIC. Each study was run separately as we were more interested in variability than commonality. Therefore spatial normalization was not necessary. Each dataset was motion-corrected, spatially smoothed with a FWHM of 5 mm, and filtered with a high pass filter (all tools available on MELODIC). Automatic dimensionality estimation was chosen to threshold the number of network yield. This estimation balance under-fitting and over fitting, and usually is set at 20% of the number of time points. The maps of Independent Components are presented by MELODICA in a webpage report. Clusters are color coded and superimposed in a template from the mean of all time points of the echoplanar sequence. High to low intensities appear in a spectrum between yellow and red colors. Negative correlated signals are presented in blue color.

Network classification: In the next step the neural networks were selected and classified. The task was accomplished by the author who has an experience with rs-FMRI in children of 10+ years and more than 500 clinical and normal cases. For this purpose, the following algorithm was utilized: To discard: noise-related components; CSF pulsation components; vascular pulsation components; motion (body and eyes) component. This procedure was based on component localization of maxima, cluster morphology, frequency of oscillation and profile of oscillation (See Figure 1). From the pool of remnant components the following 3 criteria were required for a component to be classified as a Neural Network:

a. Cortical (mainly) localization
b. Shape and contours segmenting a canonical functional area
c. 3D-gaussian distribution of intensities
d. Oscillation profile (Figure 2)
e. Frequency of oscillation between 0.01 and 0.1 Hz

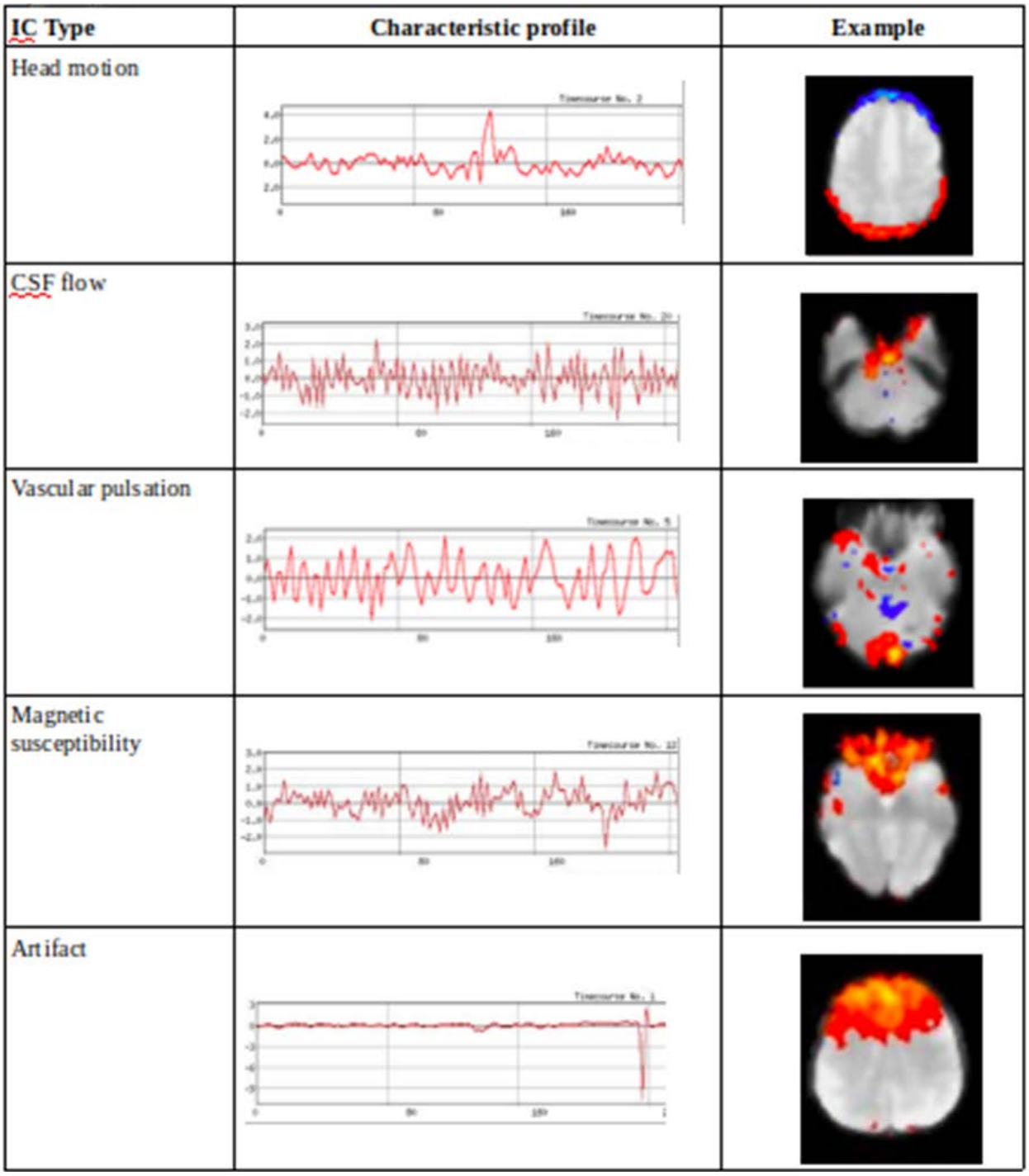


Fig 1: Non-neural network Independent Components Types**.**

Examples of oscillation profiles and typical image of 5 types of non-neural conforming components. Head motion profile is identified for its sharp low frequency-high amplitud power change. The most typical aspect is the observed double horse-shoe shaped positive/negative intensities in extra-axial antipodic localization. CSF Flow profile consist of rhytmic high frequency oscillations with the maxima of the component located extra-axially in the cysterns, ventricles and convexital spaces. Vascular pulsations have a profile of sharp waves at frequencies way above 0.1 Hz. The typical component maxima is located extra-axially at the level of great sinuses of arteries. In the example, the torcula component is presented. Magnetic susceptibility may appear in the profile as a mixture of slow and high frequencies. The localization however is more evident. In the example the component is segmenting the transition between the low intensities below the anterior fossa (paranasal sinuses and orbit) and the brain. Artifacts are easy to identify, as they do not present any pattern of oscillation. They show up as sharp high amplitude signal changes. The pattern of “activation” is unusually large and its shape not conforming with any known functional or anatomical segmentation.

3D-Gaussian distribution of intensities means that a true cluster should exhibit the highest intensities in a core and lower values in the periphery. A cluster is judged as noise by the presence of large or irregular clusters that do not match any functional or anatomical brain segmentation. Noise, in general may be due to random artifacts due to software/hardware temporal glitches, or magnetic susceptibility. The latter is identified as gross clusters

located in or around of areas of sharp transition between quite distinct intensities. They are frequently seen around the petrous bone or the anterior cranial fossa. CSF pulsation may be detected by its clear extra-axial localization. The frequency is higher than 0.1 HZ. However, this factor may vary as the CSF passages vary in size with subsequent changes in its frequency. CSF components may be seen in the ventricles, prepontine cystern, peri-chiasmatic cystern, in the sylvian fissures (when they are open) and in the hemispheric convexities. Vascular-pulsation and balistocardiogram components may be distinguished by their localization in midline (superior and inferior sagital sinus; torcula), cranial base (cavernous sinus and carotid arteries); and postero-lateral in the posterior fossa (transveral and sigmod sinus). Their frequency is usually distinctive but is not rare to find them within the spontaneous.

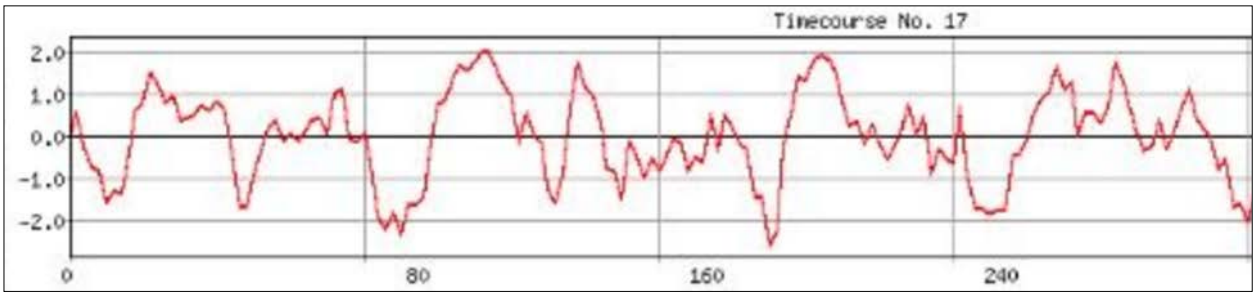


Fig 2: Typical oscillation pattern (profile) of a neural network. In the plot, the X axis represents (partially) the time (in seconds), and the Y axis represents an arbitrary signal intensity scale. Notice the double modulation of high frequency (small spikes) and the involving very low frequency characteristic of the spontaneous brain oscillations.

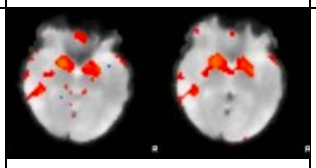
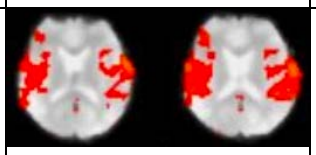
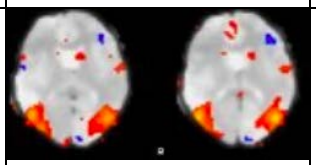
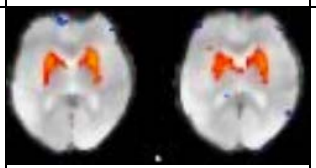
Brain oscillations range. Body (head) motion may be detected by the characteristic horseshoe-shaped artifacts it produces. Intensities are seen high in one side and low in the oposite. Eyes movement are easy to detect due to the localization of the clusters. Motion components may show frequencies within the spontaneous brain oscillations range but they lack the typical oscillation profile.

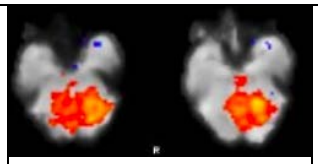
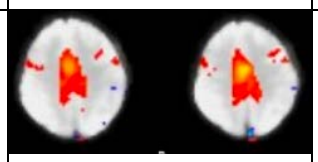
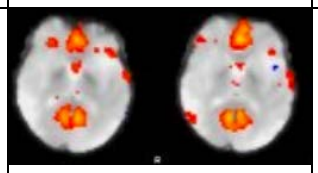
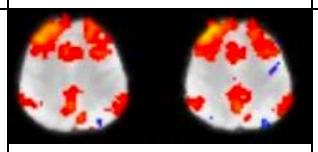
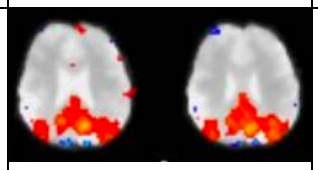
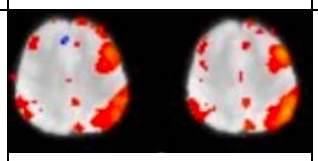
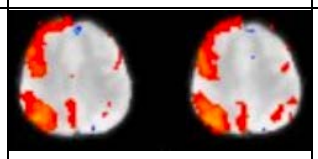
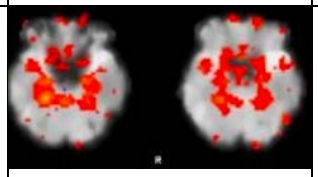
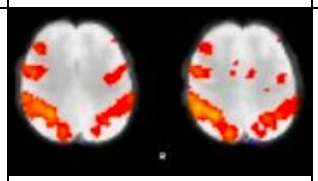
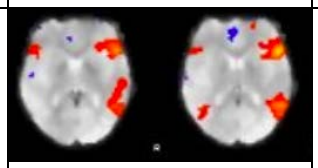
Clusters were named by its localization, shape and localization of maxima. The following categories were utilized for neural-networks classification (Table 1):

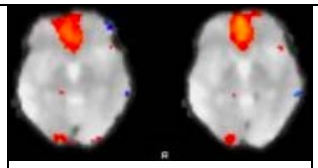
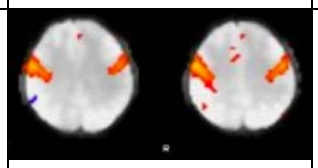
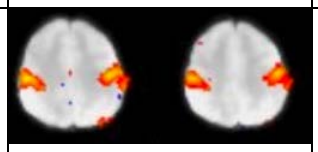
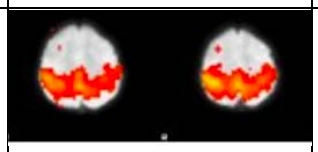
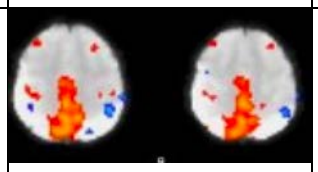
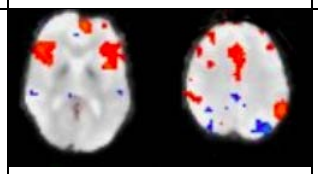
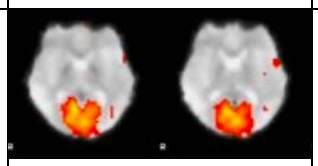
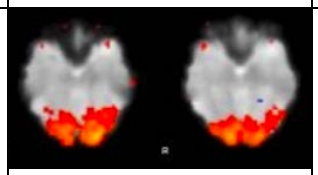
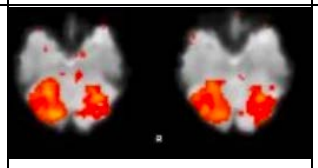
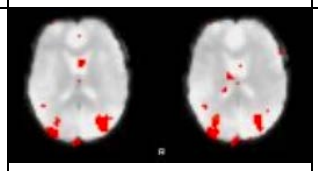
Primary area- networks (unimodal): visual, auditory, somatosensory, motor. They are bilaterally present, more or less symmetrical. Primary visual may be represented in two different components: polar visual and mesial occipital. Sensory and motor components may also be subdivided between mesial, dorsal and lateral components.

Secondary area- networks (multimodal): visual down stream, visual upstream, precuneus, inferior parietal lobule, intraparietal, prefrontal, BA40 (secondary somatosensorial area); BA39.

Table 1: ICA neural networks in a population of normal children. Conventions: Cog/cont: cognitive or control type; Sym: Symmetric in # of subjects; UL: present only in one side; FOS: Frequency of Oscillation

Network Name/Type	Example	Present in (%)	Sym (%)	FOS (Mean/SD)	Main Characteristics and Variants
Amygdala (Cog/cont)		10	75% (UL: 0%)	0.026/0.016	Usually encompassing activation of rostro-basal striatum
Auditory (Primary)		50	75% (UL: 0%)	0.0168/0.0072	May conjoin with sensory or posterior parietal networks
BA 19/39 (Secondary)		52.5	52.4 UL: 10%	0.02/0.013	May conjoin with fronto-temporal or fronto-parietal areas. Is part of the default mode.
Basal Ganglia (Cog/cont)		17.5	85.7 UL: 0%	0.022/0.01	May be seen conjoined with the amygdala

Cerebellum (cont)		15	50 UL: 0%	0.052/0.075	Notice the subcortical activation of the nuclei.
CG (middle) (Default mode)		12.5	100 UL: 0%	0.025/0.017	May appear merged with posterior or anterior default mode traits
CG (posterior) (Default mode)		57.5	95.7 UL: 0%	0.025/0.028	Connecting to areas of the ADM (as shown in the picture) or convexital subcomponents of PDM.
Default mode (anterior) (Default mode)		57.5	95.7 UL: 0	0.027/0.025	Usually conjoined with PDM clusters (as shown in the picture).
Default mode (Posterior) (Default mode)		75	76 UL: 0	0.02/0.0094	May appear partially represented with the rest of the modules in other component. May also appear conjoined with the ADM
Executive (left) (Cog/cont)		70	0	0.023/0.009	Also know as the fronto-parietal network. Clusters may peak at parietal or frontal areas.
Executive (Right) (Cog/cont)		97.5	0	0.024/0.015	The most stable and frequently present network in this sample
Hippocampus (Cog/cont)		7.5	100 UL:0	0.014/0.002	May involve the amygdala as well.
Intraparietal (Cog/cont)		70	78.6 UL: 0.5%	0.03/0.033	May appear connecting to motor and pre-motor areas (as shown in the image)
Language (Cog/cont)		57.5	0	0.021/0,01	Identification may be problematic, particularly if showing in the right hemisphere. Nodes in Wernicke's and Broca's putative areas are necessary for categorization (as shown here).

Mesial Frontal (Default mode)		55.5	91 UL: 0%	0.02/0.01	Connectivity with ACG amy be observed.
Motor (Primary)		40	100 UL: 0	0.024/0.01	The motor network usually appears in two different components: lateral (face and tongue) and high convexital for the hand.
Somatosensory (Primary)		75	86.7 UL: 0	0., 022/0.01	May appear conjoined with either motor, pre-motor networks or with auditory networks.
Paracentral (Primary)		50	100 UL: 0%	0.02/0.01	This network corresponds to a segmentation of the somatosensory network that represents the feet and the trunk.
Precuneous (Default mode)		82	87.9 UL: 0%	0.02/0.01	May be merged with other posterior neural networks (PDM, PCG).
Salience (Cog/cont)		60	79 UL: 0	0.02/0.011	The network connects the anterior insula, the frontal opercula and the ACG.
Visual Primary (Primary)		77.5	90.3 UL:0	0.0252/0.0113	May appear conjoined with visual secondary areas
Visual Polar (Primary)		42.5	100	0.02/0.01	May merge with visual down-stream network
Visual Down-Stream (Secondary)		62.5	92 UL:0	0.024 / 0.017	May marge with visual polar and up-stream
Visual Up-stream (Secondary)		37.5	86.75 UL: 0	0.024/0.088	May marge with visual polar and visual down-stream or precuneus.

***Cognitive and control networks*:** language, opercular, prefrontal, executive frontoparietal network, salience network.

***Default mode*:** anterior default mode (ADM) and Posterior default mode (PDM).

Based on the reports in adults and the experience of the author, the following priors were taken into account. All

networks but three are more or less symmetrical. The fronto-parietal executive networks (right and left) are usually singled out independently. The language network appears also asymmetrically. The default mode involves areas of the posterior and anterior cingulate gyrus (PCG, ACG), BA39 and prefrontal lateral cortices. It is a common finding to have them split into its anterior and posterior parts. The salience network involves the insula, fronto-opercular areas, prefrontal lateral and the ACG. Networks appearing less frequently (less stable) in the normal population (at least in adults) are: the mesial frontal, the frontopolar and the amygdala/hippocampal networks; several iterations of the cingulate gyrus anterior, middle and posterior (appearing separatedly). In some subjects the basal ganglia and the cerebellar nuclei show characteristics of spontaneous low-frequency oscillations.

Per each network and across the subjects we computed: (1) the percentage of frequency (within the entire population); (2) the percentage of symmetrical appearance (contrasted to unilateral appearance); (3) the range and mean of the oscillation frequency and (4) the most relevant norm-deviation in terms of conjuction with other networks or involvement of distal areas.

Results

All patient-data yielded between 32 and 44 independent components (IC). In average, the component exhibiting the maxima variability was the CSF explaining and average of 6.95% of variability. The range of neural network yield was between 11 and 26. Six networks were judged as Primary functions; 3 were Secondary functions; 9 of cognition or control and 6 of the default mode system. The oscillatory frequency mean/SD were 0.0168/0.072 Hz. Main variants consist in several patterns of network-merging (See Table 1). Usually, in cases of merging, one of the networks was of the unimodal type (e.g., somatosensory, visual, auditory). In some few cases there were iterations of the same network at different frequencies. The auditory and hippocampus networks showed the slowest frequency of oscillation (Mean 0.016 and 0.014 SD: 0.008 and 0.002, respectively); while the intraparietal network showed the highest frequency (Mean 0.03) and SD (0.033). The networks more frequently found across the subjects were the right executive network (97.5%), followed by the precuneus (82%) and visual (77.5%). The networks less frequently found were the hippocampus (7.5%) and the amygdala (10%).

Findings by Network. Table 1 summarized the main findings in all the 24 networks found in our sample.

Discussion

We have characterized the neural networks in a population of pediatric normal subjects utilizing a method of ICA on a rs-FMRI study. 24 different networks were found. The frequency of appearance and their symmetry have been assessed. We also describe the main variations on each of these networks.

To our knowledge this is the first time a description of the neural networks is accomplished in a population of pediatric normal subjects with characterization of their localization, oscillation frequency and assessment of their yield frequency (percentage of subjects exhibiting the network).

There is scant data on the standardization of neural networks in normal pediatric subjects. A PubMed search (accessed 10/14/2021) utilizing the following string (((rs-fmri [Title/Abstract]) AND (children[Title/Abstract])) NOT (patient[Title/Abstract]): yield 103 hits. However only 10 articles were based on normal subjects; the rest describe clinical cases, encompassing mostly epilepsy, ADHD, Autism, and psychiatric disorders. After reviewing the details of the 10 articles on “normal children” only 3 articles deal with normality on rs-FMRI in children. (The rest deal with different techniques including graph theory and ROI-based functional connectivity or connectivity related to specific cognitive functions, e.g., numerosity or emotion regulation). None of these 3 studies grapple with the characterization of normal networks in children: (1) Thomason M, et al [6]; studied the test-retest reliability of the rs-FMRI findings processed with the ICA in 65 children. They made comparisons across spatial, temporal and frequency domains, utilizing six cognitive and sensorimotor networks and sought for differences both within and between scan sessions. They found that “measures from resting-state data in children were consistent across multiple domains (spatial, temporal, and frequency)”. Their conclusion is that Resting-state connectivity is a reliable method for assessing large-scale brain networks in children. They do not sought for a description of the total yield of ICA or the characterization in terms of frequency of group-yield of oscillatory frequency of each component; (2) Thornburgh, Cl, et al [7] compared adult vs children’s correspondence of resting-state networks based on spatial overlap. They concluded that all the major networks described in adults and older children may be also found in young children (ages 6 and 7 years). Interestingly, this group describe a total yield of 20 networks, close to our number. However, they utilized a group-ICA method that may discard some few networks not passing the threshold for commonality; and (3) White T, et al. [8] aimed to find the time of acquisition length for the networks to come stable in a group of 84 six-to-eight year-old children,. They did not aim to standardize the rs-FMRI yield or characterize the networks in any way. They found that eight networks, including the default mode, salience, frontal, left frontoparietal, right frontoparietal, sensorimotor, auditory, and visual networks, all stabilized after ∼5½ m. Of note is that the sequence length utilized in our study was of 6 minutes, supporting the soundness of the technique.

Our study may have some shortcomings. The yield of some networks seem lower than expected. The selected data is part of a control group of the ADHD project. We have chosen carefully the subjects whose rs-FMRI settings are more similar to those we use in clinical cases. We have experience on patients with several clinical conditions including epilepsy, brain trauma, ADHD and psychosis. In our experience, the visual and PDM networks are present in more than 90% of awake cases, a significant higher yield than what we found and report here. We do not have information about the specific demands during the rs-FMR session of the cases we selected. Usually we request our patients to focus their attention in their breath. We ask them they keep their eyes open, as well. We feel these two conditions should not explain the relative low yield of the two networks in the sample.

We have intentionally chosen not to performer a group-ICA. This procedure is relevant to find commonalities but fail to show normal variants. It is well known that some rs-networks are unstable and dependable under specific conditions of the mental status of the subject. Commonality

may be of interest to contrast groups and make inferences on statistically significant deviations that make serve as biomarkers of pathology. Instead, in clinical praxis, the practitioner should be aware of all normal variations to be able to focus in those outliers prompting for explanation and that could be related to functional deviant activity of the brain. Take for example, epilepsy. It has been recently described how non-canonical networks (those non-conforming with established patterns in normal subjects) are related with epilepsy onset areas in intractable epilepsy in children [9]**.** We have also rely on an expert opinion in the selection and categorization of networks as the normal praxis of neuroradiology is usually based in such approach. Automatic pattern selection is in its infancy and difficult to implement in clinical settings.

Our finding provide an easy approach for rs-network classification from studies performed with ICA. ICA is the easiest way to perform functional connectivity as it is not hypothesis driven and is less intensive in pre and post-processing. We also provide for the first time a complete survey on neural network classification in children normative data. Non-canonical networks may be explained as brain reorganization, lack of synchrony, partial involvement of a network, or lack of activation due to pathology or functional disturbances. In patients with developmental delay, intractable epilepsy and brain lesions it is common to find hemispheric dissociation of networks (each side's component appearing appart); lack of counterpart (bilateral networks showing unilaterally); or lack of activation. Our work is a contribution to the field as no other studies have explored the normal neural networks in the way we have demonstrated in this paper.

Conclusions

We have demonstrated 24 neural canonical networks in a sample of 40 normal children who performed a rs-FMRI, utilizing ICA. These canonical networks have certain specific characteristic and may not necessarily appear in each instance of exploration. This study provides a departure point for detection of non-canonical networks that may prompt brain dysfunction or structural pathology.

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