Functional Connectivity Magnetic Resonance Imaging Reveals Cortical Functional Connectivity in the Developing Brain

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Abstract

Background and Purpose: Resting functional MRI was utilized to depict brain regions exhibiting temporal synchronization, also known as resting brain functional connectivity (rfc). This study aims to determine the temporal and spatial patterns of rfc in healthy pediatric subjects between 2wks and 2yrs old.

Methods: Rfc studies were performed on 85 children: 38 neonates (2-4 wks), 26 1yr olds and 21 2yr olds. All subjects were imaged while asleep; no sedation was employed. Six regions-of-interest (ROIs) were chosen, including the primary motor, sensory, and visual cortices in each hemisphere. Mean signal of each ROI was used to perform correlation analysis pixel-by-pixel throughout the entire brain, identifying regions with high temporal correlation.

Results: Fc is observed in all subjects in the sensorimotor and visual areas. The percent brain volume exhibiting rfc and the strength of rfc continue to increase from 2wks to 2yrs. The growth trajectories of the percent brain volume of rfc appear to differ between the sensorimotor and visual areas while the z-score is similar. The percent brain volume of rfc in the sensorimotor area is significantly larger than that in the visual area for 2wks (p=0.008) and 1yr (p=0.017) olds but not for the 2yr olds.

Conclusions: These findings suggest that rfc in the sensorimotor precedes the visual area from 2wks to 1yr but becomes comparable at 2yrs. In contrast, the comparable z-score values between the sensorimotor and visual areas for all age groups suggest a disassociation between percent brain volume and the strength of cortical rfc.

Introduction

Biswal et al ^{1,2} have observed the synchronization of low frequency blood oxygen level dependent contrast (BOLD) signal in the brain. Specifically, time series T2*-weighted images were acquired rapidly while subjects lie resting inside a MR scanner. A temporal low pass filter (cutoff frequency ~0.08Hz) was applied pixel-by-pixel to the acquired images. Subsequently, a brain region with known function was selected and signal in this region was temporally correlated with each pixel throughout the entire brain. Interestingly, brain regions with a function similar to this predefined area exhibited high temporal correlation. In addition, these temporally correlated regions resembled the brain activation maps of the same subject actually performing an activation paradigm known to activate these brain areas, finger tapping in their case, during a conventional functional MRI (fMRI) study. Biswal et al ¹ thus suggested that the observed high temporal correlation among functionally similar brain regions may represent the putative resting cortical functional connectivity and referred to this imaging approach as resting functional connectivity MRI (rfcMRI). Since then, similar findings have been replicated by many investigators in both normal subjects ³⁻⁶ and patient populations. ⁷⁻¹⁰

Unlike conventional fMRI where external sensory/cognitive paradigms are needed to specifically activate different regions of the brain, rfcMRI acquires images in the absence of cognitive demands (a resting condition) and detects brain regions, which are highly temporally correlated. Since rfcMRI does not require subjects to follow specific instructions, and subjects are at a resting condition, this approach could, potentially, be a powerful tool for delineating resting brain functional connectivity in young pediatric subjects. To this end, this study focuses on how resting functional brain connectivity may be present in very young pediatric subjects (0-2yrs). Specifically, this study aims to investigate the following two questions: 1) does resting cortical functional connectivity exist in healthy pediatric subjects between 0-2yrs old and 2) if so, how does resting cortical functional connectivity change with age?

MATERIALS AND METHODS

The children scanned were part of a larger ongoing prospective study of brain development in normal and high risk children.¹¹ For the investigation of resting functional connectivity, only the normal subjects who met the following inclusion and exclusion criteria were included for final data analysis. Inclusion criteria were birth between the gestational ages of 35 and 42 weeks, weight that was appropriate for gestational age, and the absence of major pregnancy and delivery complications as defined in the exclusion criteria. Exclusion criteria included maternal pre-eclampsia, placental abruption, neonatal hypoxia, or any neonatal illness requiring greater than 1 day NICU stay, mother with HIV, any mother actively using illegal drugs/narcotics during pregnancy, or any chromosomal or major congenital abnormality. In addition, a board-certified neuroradiologist (JKS) reviewed all images to verify that there were no apparent abnormalities in the acquired MR images.

Connectivity studies were performed on a total of 85 children: 38 neonates (2-4 wks), 26 1yr olds and 21 2yr olds. Informed consent was obtained from the parents prior to the imaging studies. All images were acquired on a Siemens 3T head-only MR scanner, and

the study was approved by the Institutional Review Board. All of the subjects were imaged while asleep; no sedation was employed.¹¹

Imaging protocols

A 3D MP-RAGE sequence was used to provide anatomical images, co-register among subjects, and define region-of-interest (ROIs) to obtain the reference signal for subsequent temporal correlation analysis. The imaging parameters were as follows: repetition time (TR) = 1820ms; echo time (TE) = 4.38 ms; inversion time = 1100ms; 144 slices; and voxel size = $1x1x1mm^3$. For the rfcMRI studies, a T2*-weighted EPI sequence was used to acquire images. The imaging parameters were as follows: TR = 2sec, TE = 32 ms; 33 slices; and voxel size = $4x4x4 mm^3$. This sequence was repeated 150 times so as to provide time series images. In 6 neonates and 5 1yr old children, a TR of 750ms instead of 2 sec was used to acquire the rfcMRI images in order to determine whether or not the choice of TR (sampling rate) would affect our ability to discern resting brain functional connectivity. With the reduction in TR, only 12 slices covering the primary motor and sensory cortices were acquired while the remaining imaging parameters were identical. This sequence was repeated 400 times so that the total acquisition time was identical to that of TR=2sec.

Image Processing

To obtain resting brain functional connectivity maps, all of the acquired images were processed with the following procedures. The first 10 sets of images were excluded from data analysis to allow magnetization to reach a steady state condition. A more detailed description of each step is provided below. 1) Time shift correction: Since images at different anatomical locations were acquired at a different time, it is imperative to correct for the time differences between different slices. This was done through a phase rotation in the frequency domain. 2) Motion correction: To minimize the potential confounds resulted from motion between scans, the Automated Image Registration (AIR)^{12,13} package with 6-parameter rigid-body transformation was used to co-register all time series images to the images of the first scan that was used for data analysis. Subsequently, signal intensity as a function of time at multiple randomly selected locations was visually inspected to further ensure that motion artifacts were minimal. Experimental data was excluded from subsequent analysis if we could not identify 90 consecutive motion free time points, and/or severe motion artifacts were identified. 3) Filtering: A spatial 3D-Gaussian ($8 \times 8 \times 8mm^3$ and $\sigma = 2mm$) filter was applied to the images in order to improve signal-to-noise ratio. Subsequently, Fourier transform was used to convert the signal-vs-time of each pixel to the frequency domain, and a low-pass filter with a cutoff frequency of $0.08H_z$ was applied. The filtered signal was then inverse Fourier transformed back to the time domain. 4) ROI selection: In order to ensure the consistency of defining ROIs for obtaining the reference function for the subsequent correlation analysis, the rfcMRI images were co-registered to the MP-RAGE images using the AIR package for each subject.^{12,13} Subsequently, a neuroradiologist manually drew three ROIs in each hemisphere for a total of 6 ROIs using two MP-RAGE images, including the right and left primary motor, sensory and visual cortices for each subject, respectively. Representative examples of the anatomical locations of these ROIs are shown in Fig. 1a and Fig. 1b for the sensorimotor and visual areas, respectively. These

manually selected regions were subsequently mapped onto the co-registered T2*weighted EPI images to obtain averaged MR signal intensity. Although both the primary motor and sensory cortices were separately identified in the MP-RAGE images, the limited spatial resolution of the T2*-weighted EPI images made it difficult to definitively analyze motor and sensory signals, separately. Therefore, signal intensities from voxels identified as the primary motor and sensory cortices were averaged to derive a single reference function to delineate the sensorimotor area. As a result, a total of four reference functions were obtained from the predefined ROIs, including the right (rsm) and left (lsm) sensorimotor and right (rv) and left (lv) visual cortices, respectively. 5) Correlation: Temporal correlation was conducted by correlating each of the four reference functions pixel-by-pixel throughout the entire brain, resulting in four separate correlation maps. 6) Correlation normalization: It has been suggested by Lowe et al 3 that the distributions of in vivo correlation coefficients may not be the same between individuals, across trials in the same subject, or even across voxels within one experiment. As a result, a direct average of the cc values to obtain group mean may introduce biases. Therefore, it is imperative to normalize the effects of the intrinsic correlations prior to conducting group analysis. To this end, correlation coefficients were converted to t-values according to $t=cc/sqrt(cc^2+v^2)$, where v is the number of the acquired time points and represents the degrees of freedom.³ The calculated t was then converted to Z-statistics according to $z=(t-\langle t \rangle)/\sigma$, where $\langle t \rangle$ and σ are the expected value and standard deviation of the Gaussian fit to the full-width at half-maximum (FWHM) of the t distribution. This step is necessary since t-values can not be used directly to obtain group mean maps. 7) Group Analysis: The MP-RAGE images from one of the subjects in each age group were randomly chosen as the template and MP-RAGE images from the remaining subjects were co-registered onto the template using the FMRIB's Linear Image Registration Tool (FLIRT). 8) Visualization: Finally, in order to better visualize regions exhibiting the putative resting brain functional connectivity, Freesufer was employed to generate the brain surface maps. To reveal resting functional connectivity of the sensorimotor area, the z-score maps obtained using the reference function obtained from the right and left sensorimotor areas were superimposed on the brain surface maps. Similar procedures were used to generate the resting functional connectivity of the visual area. Only pixels with a z-score > 1 were shown.

Data Analysis

To quantitatively compare the extent of resting brain functional connectivity as a function of age, several different parameters were calculated. First, the maximum and minimum percent signal changes in each subject were separately identified for visual and sensorimotor areas. The differences between the two values were then calculated. Second, the total brain volume exhibiting a z-score greater than 1 was divided by the total intracranial brain volume for each subject, accounting for the differences in head size across different ages. Third, the mean z-score values in brain regions exhibiting resting functional connectivity were recorded for each subject. Finally, a normalized histogram analysis was also conducted in regions exhibiting resting functional connectivity for each age group.

Statistical Analysis

For the group comparison, one-way analysis of variance (ANOVA) with Tukey's multiple comparison was employed. In contrast, a two-tailed paired t-test was employed for comparison between the two cortical areas, namely the sensorimotor and visual cortices for each age group. A p < 0.05 was considered significance.

RESULTS

Motion artifacts were observed in 18 neonates, 6 1yr olds and 8 2yr olds with TR=2 sec and 2 neonates and 2 1yr olds for TR=750ms. In addition, 2 neonates, 6 1yr and 6 2yrs old were either born prematurely (<35wks), with birth complication, with ICU stay > 24hrs, an/or offspring of parents with psychiatric disorders. All of these subjects were excluded from final data analysis. As a result, 12 neonates, 9 1yr olds and 7 2yr olds with TR=2 sec and four neonates and three 1yr olds with TR=750ms were included for final data analysis.

An example demonstrating manually selected voxels for the primary motor, sensory and visual cortices is shown in Fig. 1a and 1b using the MP-RAGE images of a 2yr old subject. Representative mean MR signals of these manually selected voxels from three separate subjects, one for each age group, are shown in Fig. 1c and 1d for sensorimotor and visual cortices, respectively. Note these signals have been processed using the steps outlined in the methods section. Evidently, the signals resemble a pattern typically observed in task-activated fMRI studies where both "on" and "off" states are observed ("on" and "off" reflect the increase and decrease of MR signal). The differences between the maximum and minimum percent signal changes for each subject in the sensorimotor and visual cortices are shown in Fig. 1e and 1f, respectively. An age dependent pattern is clearly apparent; the percent signal differences increase as age increases. Although the medians vary significantly among the three groups (p=0.02), statistical differences are only observed between the 2wk and 2yr groups for both sensorimotor and visual areas (p<0.05) after correcting for multiple comparisons.

Resting cortical functional connectivity

Resting cortical functional connectivity in the sensorimotor area for all three age groups using a TR = 2sec is shown in Fig. 2, respectively. Regions showing high temporal correlation are located in the primary sensorimotor area for all three groups. In addition, both the area and the strength (z scores) increase as a function of age. Similar findings are also observed in the visual area (Fig. 3) for the three age groups.

Quantitative comparisons

Quantitative comparisons of the normalized brain volumes of cortical connectivity (defined as the brain volume exhibiting cortical connectivity divided by the ICV) and the mean z-score values in regions exhibiting temporal correlation are shown in Fig. 4. In the sensorimotor area (Fig. 4a), both the 1yr (p<0.05) and 2yr old (p<0.001) groups exhibit significantly larger percent brain volumes than the 2wk old group while no differences are observed between 1 and 2 yr old groups. In contrast, no differences are observed between 2k and 1yr in the visual area whereas both 2wk (p<0.001) and 1yr (p<0.01) groups are significantly smaller than that of the 2 yr old group (Fig. 4b). A paired t-test between the sensorimotor and visual areas in each age group reveals that the

percent brain volume of cortical connectivity in the sensorimotor area is significantly larger than that in the visual area for both the 2wk (p=0.008) and 1 yr (p=0.017) old groups but not for the 2yr old group.

Significant differences of the z-score values among all three age groups are observed in the sensorimotor area (p<0.001 for 2wk vs 1yr and 2wk vs 2yr; p<0.01 for 1yr vs 2yr); the 2wk old group has the lowest z-score values, followed by the 1yr and 2yr old groups, suggesting that the "strength" of cortical connectivity increases with age (Fig. 4c). In contrast, while significant differences are also observed for the visual area, the 2wk old group is highly significantly lower than the 2yr group (p<0.001) while the differences between 1yr vs 2yr and 2wk vs 1 yr are smaller (p<0.05) (Fig. 4d). Paired t-test reveals no differences between the sensorimotor and visual areas for each age group, suggesting that the growth trajectory of the strength of cortical connectivity is similar between the visual and sensorimotor areas. Finally, the distributions of z-scores for all three age groups in the sensorimotor and visual areas are shown in Fig. 4e and 4f, respectively. Notice the population toward higher z-score values continue to increase from 2wk to 2yr in the sensorimotor area, while this increase is more subtle between 2wk and 1yr old groups and followed by a marked increase between 1yr and 2yr old groups in the visual area, consistent with that shown in Fig. 4c and d.

In order to determine whether or not the choice of TR affects rfcMRI, a comparison of the results obtained using TR = 2sec (left panel) and 0.75 sec (right panel) at the sensoimotor area is shown in Fig. 5. The results are comparable between the two TRs, suggesting that the effects of TR may be minimal.

DISCUSSION

While MR has been widely employed to gain insights into early brain development, most of the studies to date have focused on structural instead of functional development^{14,15}. particularly in the very young age population (i.e., <2yrs in our study). This lack of focus on functional development may not be surprising since fMRI requires subjects to follow specific instruction which is difficult to comply for young pediatric subjects. In contrast, rfcMRI acquires images while subjects are at a resting condition and/or asleep. Therefore, rfcMRI serves as an idea tool to potentially assess brain functional development in very young and healthy pediatric subjects. This study posed to address 1) whether or not resting cortical functional connectivity exists in young and healthy children at 2wks, 1yr and 2 yrs old and 2) if so, how does it depend on age? Our findings demonstrate that resting cortical functional connectivity exists as early as 2wks old in both the sensorimotor and visual cortices. This finding is similar to that reported by Fransson et al¹⁶ where infants born at a low gestational age (~ 25 wks) were imaged at a term-equivalent age. Another major finding is that the percent brain tissue exhibiting resting cortical functional connectivity as well as the z-score values are highly agedependent in both the sensorimotor and visual areas.

While these results are encouraging and demonstrating that rfcMRI may be an invaluable tool for the investigation of resting cortical functional connectivity during early brain development, definitive interpretation of these results may be hampered by the lack of

understanding of the exact underlying physiological origins contributing to the observed temporal correlation among functionally similar cortical areas. Some have questioned if rfcMRI truly reflects physiologically related information or are consequences of imaging artifacts.^{17,18} Particularly, the typically slow sampling rate makes rfcMRI vulnerable to temporal aliasing originating from cardiac and respiratory effects. Lowe et al ³ compared rfcMRI using two different TRs: 134ms vs 2sec where the shorter TR should eliminate aliasing effects attributed by both cardiac and respiratory while results obtained using TR=2 sec (similar to our study) could potentially be contaminated by aliasing artifacts. Although the specificity was lower with TR=2sec when compared with that obtained using TR=134ms, similar resting cortical functional connectivity was observed between the two TRs. Similar findings were also reported by Kiviniemi et al.¹⁹ These findings are consistent with our observation where rfcMRI results are similar between TR=2sec and TR=0.75 sec (Fig. 5). Therefore, although cardiac and respiratory components could potentially affect the performance of rfcMRI, rfcMRI most likely provides insights into resting brain functional connectivity rather than imaging artifacts.

While one can rule out imaging artifacts as one of the explanations for rfcMRI, the underlying physiological origins contributing to rfcMRI remain under extensive scrutiny. One of the most popular hypotheses is that rfcMRI is directly related to fluctuations of capillary blood flow and oxygen metabolism which indirectly link rfcMRI to neuronal activities. Biswal et al ¹ demonstrated that regions exhibiting high temporal correlation are similar to that obtained using task-activated fMRI. Cordes et al²⁰ further compared results of rfcMRI and fMRI in multiple cortical areas and observed spatial similarity between rfcMRI and fMRI, although there was not one-to-one correspondence. In a different study, Biswal et al ² employed experimental hypercapnia and reported that the magnitude of rfcMRI was reversely diminished during hypercapnia. They also pointed out that MR observed low frequency BOLD fluctuation resembled spontaneous flow fluctuation using laser-Doppler flowmetry. Peltier and Noll ²¹ reported that the normalized signal changes of rfcMRI linearly depend on TE. Wise et al ²² further demonstrated a significant correlation between low frequency fluctuation and spontaneous fluctuations of arterial pCO2 in volunteers at rest.

More recently, several lines of evidence further demonstrated the potential links between rfcMRI and neuronal activity.²³⁻²⁶ Leopold et al ²³ examined fluctuations in band-limited power of local field potential in the visual cortex of monkeys during different behavioral states. Although the band-limited power exhibited fluctuations at several different time scales, they reported a particularly large amplitude at very low frequencies, <0.1 Hz, similar to the frequency range in rfcMRI. In addition, these fluctuations exhibited a high temporal correlation among the electrode pairs, suggesting the existence of spatial coherence among these local field potential fluctuations. Simultaneous rfcMRI and EEG recording have been conducted by several investigators ²³⁻²⁶. Although there are some discrepancies regarding regions of the brain correlated with the EEG signal, all of the studies consistently demonstrated a negative correlation between BOLD signal fluctuation and the alpha power. All of the above reported results strongly support the potential links between rfcMRI and neuronal activity, underscoring the importance of rfcMRI in the study of resting brain functional connectivity.

In our study, we observe resting cortical functional connectivity in all age groups, suggesting that resting cortical functional connectivity exists as early as 2wks after birth. In addition, the resting cortical functional connectivity appears to increase with age, including an increase in the percent brain volume as well as the z-score from 2wks to 2 yrs old. Specifically, an age dependent increase in percent brain volume exhibiting high temporal correlation is observed in both sensorimotor and visual areas, although the trajectories appear to differ between the two areas. A progressive/linear increase in percent brain volume from 2wks to 2yrs old is observed in the sensorimotor area (Fig. 4a); the 2wks old group exhibits a significantly smaller volume of resting cortical connectivity than that of 1yr and 2yr old groups while no differences were observed between the 1 and 2yrs old groups, suggesting a striking increase in percent brain volume from 2wk to 1yr. In contrast, the percent brain volume in the visual area is similar between 2wk and 1yr (p>0.05) groups, followed by a marked elevation from 1yr to 2yrs, suggesting that the major increase in percent brain volume of the visual area occurs between 1yr and 2yrs old (Fig. 4b). A paired t-test comparing percent brain volume exhibiting resting connectivity between the sensorimotor and visual areas at the same age indicates a significant difference for both 2wk and 1yr old groups while no differences were observed in the 2yrs old group; the percent brain volumes in the sensorimotor area are significantly larger than that in the visual area at both the 2wk (p=0.008) and 1 yr (p=0.017) old groups. Together, these findings suggest that the development of resting functional connectivity in the sensorimotor area may pre-date that in the visual area.

One immediate follow-up question to the above findings is if the strength of resting cortical connectivity (z-score values) also follows the similar temporal pattern as that of percent brain volume. Significant differences in the z-score values are observed in both the sensorimotor and visual areas for all three groups; the strength of resting cortical connectivity continues to improve from 2wks to 2yrs in both the sensorimotor and visual areas (Fig. 4c and d). However, although the significant levels are higher in the sensorimotor than that in the visual areas, the temporal increase of z-score appears to be more linear in both sensorimotor and visual areas. As a result, the paired t-test of the z-score values at the same age exhibits no differences between the sensorimotor and visual areas. Therefore, these findings suggest that there may be a disassociation between volume and strengths of cortical connectivity where a large percent brain volume exhibiting resting cortical connectivity does not necessarily translate to a stronger connection (a higher z-score).

While the exact physiological underpinnings are poorly understood to account for the observed discrepancies in the trajectories of senorimotor and visual resting cortical connectivity from 2wks to 2yrs, Chugani et al ²⁷ reported that the most prominent area of metabolic activity in the cerebral cortex is the primary sensorimotor area in infants based on the measurements of local cerebral metabolic rates for glucose (ICMRGlc). The ICMRGlc at the sensorimotor area is about 93% and 125% of that in adults at 0-1yr and 1-2yrs, respectively. In contrast, ICMRGlc at the occipital cortex is about 76% and 113% of the adults at 0-1yr and 1-2yrs, suggesting the temporal differences in glucose utilization between the sensorimotor and visual areas. Therefore, it appears that our

results are consistent with those reported using ICMRGlc. Nevertheless, more systematic studies will be required to further elucidate the links between the alteration of glucose utilization and rfcMRI in the developing brain.

Several technical issues warrant additional discussion. First, the limited spatial resolution with an EPI sequence makes it difficult to separately analyze primary motor from sensory areas for rfcMRI. Although one could argue that the signal-to-noise ratio at 3T may allow acquiring images with a higher resolution than that used in our studies, the choice of the spatial resolution was determined based on the following two factors. In order to cover the entire brain while keeping the TR short since a long TR may increase the likelihood of being contaminated by cardiac and respiratory components, a slice thickness of 4 mm is needed. In addition, since co-registration was used to co-register images between rfcMRI and anatomical images, an isotropic resolution would provide a more accurate co-registration than non-isotropic resolution. Therefore, we have elected to employ a resolution of 4x4x4mm3 in our studies. This limitation can potentially be alleviated using parallel imaging approaches in the future.^{28,29} Second, the pre-selection of the ROIs for obtaining reference function could potentially introduce biases. Alternatively, a spatial independent component analysis (ICA) approach ^{16,30-32} can be utilized to provide brain regions exhibiting temporal correlation. Specifically, Fransson et al ¹⁶ employed the ICA approach and imaged pre-term infants at a term-equivalent age. In addition to depicting the sensorimotor and visual network, three additional networks were identified in their studies, including the superior and posterior parts of the temporal cortex and the inferior parietal cortex, bilateral superior parietal cortex, and dorsolateral section of the prefrontal cortex. While their findings are of critical importance, the lack of behavioral data makes it difficult to conclusively interpret the implication of these three networks. In addition, since the subjects in their studies were born at an extremely low gestational age, more studies will be needed with full-term normal and healthy infants and a larger sample size.

Finally, although several lines of evidence have suggested that both fMRI and rfcMRI can still be observed during a sleep condition ³³⁻³⁶, it is unclear how a varying degree of sleep states may affect rfcMRI since this was not monitored in our study. With visual evoke potential (VEP) in full-term neonates, Apkarian et al ³⁷ reported that behavioral states (quiet sleep, active sleep, quiet wakefulness and active wakefulness) would affect the VEP. Nevertheless, the major differences were between awake and sleeping states. Therefore, although a varying degree of sleep states among subjects may affect our results, it should not alter the overall findings of our studies.

Conclusions

RfcMRI was employed to delineate the development of resting brain functional connectivity in healthy pediatric subjects from 2wk to 2yrs old. Our results demonstrate that rfcMRI is capable of consistently revealing resting brain functional connectivity in both sensorimotor and visual cortices across all subjects. In addition, the percent brain volume exhibiting resting functional connectivity increases as function of age although the trajectories differ between sensorimotor and visual areas. These results highlight the

temporal and spatial dynamic of the development of resting brain functional connectivity from 2wk to 2yrs old.

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Figures



Fig. 1: The anatomical locations for defining the

sensorimotor and visual areas are shown in **a** and **b**, respectively. The crosses, filled circles, squares, asterisks, left triangles and right triangles represent the right motor, right sensory, left motor, left sensory, left visual and right visual cortices, respectively. Representative processed MR signals at the sensorimotor and visual areas using the approaches outlined in the Method section for a neonate (upper row), 1yr (middle row) and 2yrs (bottom row) old children are shown in **c** and **d**, respectively. The percent signal difference between the maximum and minimum signals for the sensorimotor and visual areas are shown in **e** and **f** for each age group. The error bars represent the standard deviations.



Fig. 2: The averaged group results demonstrate the brain regions exhibiting resting functional connectivity when the right and left sensorimotor ROIs were employed to obtain the reference functions for correlation analysis and superimposed on the brain surface for neonates, 1yr and 2yrs old groups. The color bar represents the z-score values and the "L" and "R" represent the left and right hemispheres, respectively.



Fig. 3: The averaged group results demonstrate the brain regions exhibiting resting functional connectivity when at the right and left visual ROIs were employed to obtain the reference functions for correlation analysis and superimposed on the brain surface for neonates, 1yr and 2yrs old groups. The color bar represents the z-score values and the "L" and "R" represent the left and right hemispheres, respectively.



Fig. 4: Quantitative comparisons of the percent brain volume exhibiting resting functional connectivity (**a** and **b**), the z-score values (**c** and **d**) and the histograms (**e** and **f**) for the three age groups at both the sensorimotor and visual areas are shown. The error bars in **a**-**d** represent the standard deviations. The y-axis in **e** and **f** represents the normalized populations.



Fig. 5: A comparison of two different TRs for obtaining resting cortical functional connectivity at the sensorimotor area is shown. The left and right sensorimotor ROIs were used to obtain the reference functions for correlation analysis for both TRs. The "L" and "R" represent the left and right hemispheres, respectively and the color bar represents the z-score values.