Summary

To support the findings reported in the manuscript - Growing together and growing apart: Regional and sex differences in the lifespan developmental trajectories of functional homotopy, we conducted several supplementary analyses. These include (1) regression model selection using Akaike's Information Criterion (AIC); (2) examination of VMHC test-retest reliability; (3) comparability of findings across samples and (4) examination of the impact of potential confounding factors (e.g., brain morphometry and registration) on developmental changes in VMHC. We also provide additional discussion of our findings of regional variation in development trajectories.

Supplementary Validation

1. AIC-based regression model selection

In the current study, we identified linear, quadratic and cubic age-related changes in VMHC (i.e., life-span trajectories). Accordingly, we performed three separate multiple regression analyses including age, age^2 and age^3 as regressors (see equation (1) in main text). AIC was then used to select the best-fit regression model (Akaike, 1974). AIC reflects a trade-off between the likelihood and complexity (i.e., number of parameters) of a model.
We adopted a finite sample corrected AIC, namely $AIC_c$ (Hurvich and Tsai, 1989),

$$AIC_c = n \ln(2\pi) + n \ln(\hat{\sigma}^2) + n + \frac{2n(k + 1)}{n - k - 2}, \quad (1)$$

where $\hat{\sigma}^2$ is the variance of the residuals, $k$ is the number of predictors in the regression model, and $n$ is the number of samples. The regression model with the lowest $AIC_c$ value was chosen as the best model to fit the data.

2. Test-Retest Reliability

We used a publicly available (http://www.nitrc.org/projects/nyu_trt) test-retest (TRT) resting-state fMRI (R-fMRI) dataset collected from 25 healthy adults to evaluate the TRT reliability of VMHC. Each R-fMRI scan comprised 197 contiguous EPI functional volumes (TR = 2000 ms; TE = 25 ms; flip angle = 90, 39 slices, matrix = 64x64; FOV = 192 mm; acquisition voxel size = 3x3x3 mm$^3$). Scans 2 and 3 were conducted in a single scan session, ~45 minutes apart, which took place 5-16 months (mean 11 ± 4) after Scan 1. All individuals were asked to relax and remain still with their eyes open during the scans.

R-fMRI data preprocessing was the same as described in the main text. Individual VMHC maps were calculated for all 75 scans (i.e., 3 scans per participant x 25 participants). In line with our previous studies (Shehzad et al., 2009; Zuo et al., 2010a; Zuo et al., 2010b), we computed intra-class correlation coefficients (ICC) to assess TRT reliability. Multiple variants of ICC exist, each with different advantages and limitations. The specific form used here is:
$$\text{ICC}_c = \frac{MS_p - MS_e}{MS_p + (d-1)MS_e}. \quad (2)$$

In this equation, $d$ is the number of scans, $MS_p$ is the between-participants mean square and $MS_e$ is the error mean square.

At each voxel, intra-session (i.e., short-term) TRT reliability was calculated as the ICC$_c$ between Scan 2 and Scan 3. Inter-session (i.e., long-term) TRT reliability was computed as the ICC$_c$ between Scan 1 and the mean of Scans 2 and 3. Given the close temporal proximity of Scans 2 and 3 relative to Scan 1, we averaged Scans 2 and 3 together for our estimation of the long-term TRT reliability, rather than using a single scan from the second session (i.e., either Scan 2 or Scan 3). This decision was made to obtain the best possible estimate for the second session data, and thus long-term TRT reliability.

3. Effects of Brain Registration

The co-registration of functional and structural images and brain normalization by means of registration to a standard template are processing steps that inherently introduce some degree of error and noise to the data. To account for the quality of registration between functional space and the structural template (MNI 152 space), we calculated the spatial correlation between the base functional image (the 8$^{th}$ image of the functional volume) and the structural template, for each participant. Because registration of midline regions is typically poorest, we also limited the calculation of the spatial correlation to a 3-D box that extended 10 mm from the midline into the left and right hemispheres. We then included each of these two measures of spatial correlation as a covariate in our
regression models to investigate the impact of the quality of registration on our findings. Figure S5 shows that registration errors had little effect on our main findings.

4. Comparability of Findings for NYU and ICBM Samples

An obvious concern that arises is the possibility that the combination of datasets across two sites may introduce artifactual age–related effect, especially given the different age distributions of the NYU and ICBM samples. Two factors mitigate this concern. First, site was modeled as a covariate in all analyses, thereby statistically removing any associated variance. In addition, the age-ranges of the two samples overlap substantially (NYU: ages 7-49; ICBM: ages 19-85; overlapping ages: 19-49; percentage of subjects within overlapping range: 52%), further reducing concerns about combining the two datasets. Nonetheless, it is possible for overall young-old group differences to confound linear models, causing an artifactual linear relationship with age. The fact that lifespan linear trends were not detected when the focus was restricted to participants aged 7-21 years old from the NYU site (Figure S6C) suggests that a young vs. old group difference may exist in the data. However, when we examined the ICBM data (ages 18-85) separately from the NYU data (7-49), we did observe overlap between the regions exhibiting linear relationships in the combined ICBM/NYU sample and in the ICBM sample alone (Figure S6A-B). As for why the same regions did not demonstrate linear effects in the analysis of participants aged below 21 years old (a subset of the NYU sample), it is most likely that the age-range (7-21 years) was too limited to reveal the linear developmental effects.
Noting that the ICBM dataset spanned a broader range of ages surrounding the inflection points (i.e., peak-ages) identified in our quadratic analyses, we repeated our analyses using the ICBM dataset only. For both linear and quadratic regressions, the statistical estimates for the ICBM dataset were strongly related to those obtained for the NYU/ICBM combined dataset (Linear: Pearson r = 0.7; Quadratic: Pearson r = 0.5). This is especially notable given that the ICBM dataset is less than 25% of the size of the combined dataset. Additionally, as demonstrated in Figure S7A, a high degree of concordance, as measured by eta$^2$ (Cohen et al., 2008), was observed between the quadratic curves fitted for the NYU/ICBM dataset and the ICBM dataset separately. To examine the similarities in curve fits across the three datasets (i.e., NYU, ICBM, NYU + ICBM), we performed the linear and quadratic curve-fitting procedures on the three datasets separately, and plotted the curves for two typical clusters showing significant linear or quadratic age-related changes in homotopic RSFC (Figure S6B; Figure S7B).

Finally, to demonstrate the importance of including a late lifespan sample on the detection of quadratic trajectories, we performed both linear and quadratic curve fittings using only the 79 participants aged 7 to 21 years (a subset of the NYU sample). No significant quadratic effect of age on homotopic RSFC was detectable (Figure S6C). Although linear effects were observed in some regions, as discussed above, it is likely that the age-range was too limited to appreciate the full spectrum of linear developmental effects observable in the combined NYU/ICBM dataset.

5. Brain Morphometry Homotopy
To investigate the effect of structural homotopy on functional homotopy, we used FSL's voxel-based morphometry (VBM)-style analysis pipeline (http://www.fmrib.ox.ac.uk/fsl/fslvbm/index.html) to compute gray matter volume measures for each participant (Good et al., 2001a; Good et al., 2001b). Each participant’s high-resolution anatomical image was skull-stripped to remove non-brain material, and segmented into gray matter (GM), white matter and cerebrospinal fluid. The resultant GM images were registered to the ICBM152 GM template (included with FSL) with 2×2×2 mm³ resolution, using a nonlinear registration (FNIRT). These images were then averaged together with their mirror images, in order to create a symmetric study-specific GM template. Each individual’s native GM image was then registered to this study-specific GM template using FNIRT nonlinear registration, and each voxel was subsequently divided by the Jacobian of the nonlinear warp field in order to compensate (“modulate”) for contraction or enlargement caused by the non-linear transformation. The modulated normalized GM images were then smoothed with an isotropic Gaussian kernel with a sigma of 3mm (analogous to a 7 mm FWHM).

In order to measure structural homotopy, we introduced a structural variant of VMHC, namely voxel-mirrored homotopic morphometry (VMHM). VMHM is defined as

\[
\text{VMHM} = 1 - \frac{|L_{GM} - R_{GM}|}{L_{GM} + R_{GM}},
\]

where \(L_{GM}\) and \(R_{GM}\) are the gray matter morphometry measures for each voxel within the left and right hemispheres, respectively. Practically, VMHM is calculated by subtracting each participant’s modulated normalized GM image from its L-R flipped mirror image.
We examined the relationship between and global VMHM by averaging VMHM values across all brain voxels within a unilateral hemispheric gray matter mask (as there is only one VMHM value for each pair of homotopic voxels). The mask was created as the conjunction between a unilateral mask created using the MNI152 gray matter tissue prior included with FSL, thresholded at 40% tissue-type probability and the mean (across all participants) GM image. Linear, quadratic and cubic curve fittings were applied to these data (Figure S2).

We controlled for the effects of VMHM on our models of VMHC by including the 3-D VMHM volumes for each participant as a voxel-dependent covariate in the group analyses of VMHC. As for all the main analyses, voxel-wise group analyses were performed using the FSL program flameo, and corrected for multiple comparisons at cluster-level (p<0.05) using easythresh (voxel-wise minimum Z > 2.3) within one hemisphere only.

**Supplementary Discussion**

*Regional Variation in VMHC*

On the lateral surface, we observed the strongest homotopic connectivity within motor, premotor, and visual cortex (see surface view in Figure 1A). Within these zones, ventral somatosensory and motor cortex (i.e., secondary areas) and the premotor cortex exhibited homotopic resting-state functional connectivity (RSFC) similar in strength or even stronger than that of primary areas. Within parietal areas, several interesting dissociations were observed. For instance, while medial areas (precuneus), anterior supramarginal and angular gyri exhibited strong homotopic RSFC, posterior parietal
cortex and the intraparietal sulcus exhibited weaker homotopic RSFC. Strong homotopic RSFC was also observed between insular cortex, and virtually all medial temporal and subcortical structures, including the striatum, amygdala and thalamus (see slice view in Figure 1A).

Homotopic RSFC was generally higher on the midline than on the lateral surface, particularly between homotopic regions of anterior and posterior cingulate cortex, medial visual, parietal and motor cortex. Despite the overall high level of homotopic RSFC, an anterior-to-posterior gradient of increasing RSFC is observable, as well as a gradient of decreasing homotopic RSFC with increasing distance from the midline, such that areas within the paracingulate sulcus showed weaker homotopic RSFC than areas on the cingulate gyrus. The general pattern of greater homotopic connectivity medially than laterally is consistent with Stark et al. (2008), i.e., it was observable independent of the relationship between hierarchical subdivisions and interhemispheric correlation. The medial/lateral difference in the strength of homotopic RSFC is not likely to be an artifact of spatial smoothing, as data that had not been spatially smoothed showed the same spatial distribution, although the overall strength of connectivity was reduced relative to smoothed data (Figure 1B). Finally, lateral temporal and lateral and orbital frontal cortex exhibited the weakest homotopic RSFC. The consistency between the present findings and those of Stark et al. (2008) is not simply due to commonalities among the resting state datasets included in the two studies. Although the data used for Stark et al. (2008)’s analyses were included here, they constitute less that 30% of the total dataset, as they were supplemented by a second, equally large dataset collected at NYU, and by a third dataset (ICBM).
As discussed by Stark et al. (2008), regional variations in homotopic connectivity strength are broadly consistent with the brain’s functional hierarchy (Mesulam, 1998). That is, while areas involved in lower-order sensory and motor processing exhibited the strongest homotopic RSFC, areas known to exhibit lateralization of function, such as lateral temporal and lateral and orbital frontal cortex exhibited the weakest homotopic RSFC. While this hierarchical organization held true for the lateral surface, areas on the medial surface exhibited a generally high degree of homotopic RSFC, with RSFC further increasing along an anterior-to-posterior gradient. The deviation of midline areas subserving higher order cognitive functions (e.g., cingulate and precuneus) from the hierarchical pattern was previously noted in Stark et al. (2008). In that paper, an independent relationship between distance and correlation strength was observed, although that relationship could not statistically explain the homotopic correlation strength of these regions. We speculate that one of the brain’s organizational principles is that processes that demand greater degrees of interhemispheric interaction are located near the midline, thus minimizing transmission distances. Furthermore, many of the midline areas exhibiting strong homotopic VMHC (e.g., precuneus, medial prefrontal cortex, cuneus) are highly connected “hubs” within the brain’s overall intrinsic functional architecture (Fransson and Marrelec, 2008; Ghosh et al., 2008; Hagmann et al., 2008; He et al., 2009; Honey et al., 2009). Their extensive connectivity with the rest of the brain may explain their high degree of homotopic connectivity, as RSFC can be driven by both interregional interactions, as well as by coordinated interactions with tertiary areas (Fingelkurts and Kahkonen, 2005; Horwitz, 2003).
Table S1. Lifespan trajectories of voxel-wise homotopic RSFC

<table>
<thead>
<tr>
<th>Region/Type of Trajectory</th>
<th>Cluster Size (mm$^3$)</th>
<th>Peak Coordinates (X, Y, Z) in MNI152 Space</th>
<th>Peak Z-stat</th>
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<tr>
<td><strong>Linear: positive</strong></td>
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<td>Caudate and Thalamus</td>
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<td>Parieto-Occipital Fissure</td>
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<tr>
<td>Postcentral Gyrus</td>
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<td>38 -22 50</td>
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<tr>
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<tr>
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<tr>
<td><strong>Quadratic: negative</strong></td>
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<td>Cerebellum (Crus I)</td>
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<tr>
<td><strong>Cubic: positive</strong></td>
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<tr>
<td>Superior Frontal Gyrus</td>
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<td>24 30 56</td>
<td>4.4</td>
</tr>
<tr>
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<td>3.5</td>
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<tr>
<td>Medial Frontal Cortex and Anterior Cingulate Cortex</td>
<td>349</td>
<td>12 -12 60</td>
<td>-5.4</td>
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Supplementary Figures

Figure S1. Whole brain homotopic resting-state functional connectivity (RSFC) pattern. A multiple linear regression examined the relationship with the whole-brain mean voxel-mirrored homotopic connectivity (VMHC) at each voxel with different levels of smoothing applied during preprocessing. The maps show the results of one-sample t-tests (against 0). Multiple comparisons correction was carried out within one hemisphere (as there is only one correlation for each pair of homotopic voxels) based on Gaussian random field theory (min Z > 2.3, cluster-level p < 0.05, corrected). The final statistical maps are visualized as symmetric axial slices for both 6mm FWHM (A) and 0mm FWHM (B) spatial smoothing preprocessing strategies.
Figure S2. Scatter plots of global homotopic gray matter volume similarity. The global homotopic gray matter volume similarity was obtained for each subject by averaging voxel-mirrored homotopic morphometry (VMHM) values across all gray matter voxels within a predefined gray matter mask (40% threshold) in MNI152 standard space. Linear, quadratic and cubic curve fittings were applied to these data. Three fit curves are plotted. The quadratic fitting was chosen as the best-fit model using AIC-based model selection; its peak age is plotted.
Developmental trajectories of voxel-wise homotopic resting-state functional connectivity (RSFC). Multiple linear regressions modeled the linear (A), quadratic (B) and cubic (C) age effects on voxel-mirrored homotopic connectivity (VMHC) at each voxel. AIC was used to select the best model among these three. One-sample tests on the regression coefficients of age, age² and age³ variables were performed for both positive and negative contrasts. Multiple comparisons correction was carried out within one hemisphere (only one correlation for each pair of homotopic voxels) based on Gaussian random field theory (min Z > 2.3, cluster-level p < 0.05, corrected). All six statistical Z-maps (3 models x 2 contrasts) were visualized as six hemispheric surfaces (cortical regions) and six hemispheric axial slices (subcortical regions).
Figure S4. **Peak age of quadratic age-related changes in homotopic resting-state functional connectivity (RSFC).** Warm colors indicate peak ages for positive quadratic trajectories (i.e., U-shaped) and cool colors indicate peak ages for negative quadratic trajectories (i.e., inverted U-shaped).
Figure S5. Effects of brain registration on development of homotopic resting-state functional connectivity (RSFC). The maps show the results of analyses including full brain registration error (A) or midline registration error (B) as a covariate. The figure demonstrates that registration errors had little effect on our main findings (compare with Figure 3).
Figure S6. **Effects of combining samples on linear development trajectory of homotopic resting-state functional connectivity (RSFC).** The voxels showing significant linear age-related changes in voxel-mirrored homotopic connectivity (VMHC) are depicted for the ICBM (green) and NYU/ICBM combined (blue) samples (A), and for the 7-21 year-old subset of the NYU sample (C). In (B), the individual data and resultant curves were plotted for two sample clusters as indicated by the orange arrows: NYU (black), ICBM (green) and NYU/ICBM combined samples (blue).
Figure S7. **Effects of combining samples on the quadratic development trajectory of homotopic resting-state functional connectivity (RSFC).** The quadratic curve fits were performed for both ICBM and NYU/ICBM combined samples (A). $\eta^2$ was used to measure the degree of concordance between the two quadratic curves fitted for the NYU/ICBM combined dataset and the ICBM dataset. In (B), the quadratic curve-fitting procedures were performed on the three datasets (i.e., NYU, ICBM, NYU + ICBM) separately. The data and resultant curves were plotted for two clusters as indicated by the orange arrows: NYU (green), ICBM (blue) and NYU/ICBM combined samples (red).
Figure S8. **Effects of brain homotopic morphometry on development of homotopic resting-state functional connectivity (RSFC).** The analyses of VMHC developmental trajectories (A) and their sex-related effects (B) were repeated to include voxel-mirrored homotopic morphometry (VMHM) as a voxel-dependent covariate. Comparisons with Figures 3 and 6 show that developmental and sex-related effects on morphometric interhemispheric asymmetry had little effect on our main findings (compare with Figure 3 and Figure 5).
References


