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Original Article

ORIGINAL ARTICLE

DNA Methylation and Resting Brain Function Mediate the Association between Childhood Urbanicity and Better Speed of Processing

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Abstract

Urbanicity has been suggested to affect cognition, but the underlying mechanism remains unknown. We examined whether epigenetic modification (DNA methylation, DNAm), and brain white matter fiber integrity (fractional anisotropy, FA) or local spontaneous brain function activity (regional homogeneity, ReHo) play roles in the association between childhood urbanicity and cognition based on 497 healthy Chinese adults. We found significant correlation between childhood urbanicity and better cognitive performance. Multiset canonical correlation analysis (mCCA) identified an intercorrelated DNAm-FA-ReHo triplet, which showed significant pairwise correlations (DNAm-FA: Bonferroni-adjusted *P*, $P_{bon} = 4.99E{-}03$, rho = 0.216; DNAm-ReHo: $P_{bon} = 4.08E{-}03$, rho = 0.239; ReHo-FA: $P_{bon} = 1.68E{-}06$, rho = 0.328). Causal mediation analysis revealed that 1) ReHo mediated 10.86% childhood urbanicity effects on the speed of processing and 2) childhood urbanicity alters ReHo through DNA methylation in the cadherin and Wnt signaling pathways (mediated effect: 48.55%). The mediation effect of increased ReHo in the superior temporal gyrus underlying urbanicity impact on a better speed of processing was further validated in an independent cohort. Our work suggests a mediation role for ReHo, particularly increased brain activity in the superior temporal gyrus, in the urbanicity-associated speed of processing.

Key words: cognition, DNA methylation, spontaneous brain activity, superior temporal gyrus, urbanicity

Introduction

Cognition affects many important life outcomes (Peter 2010), including both physical and mental health, longevity, marital success and educational and occupational attainment. The missing heritability of general cognitive function in twin studies has led to interest in environmental influence (Haworth et al. 2010; Plomin and Deary 2015). Urbanicity, the impact of living in urban areas at a given time (Vlahov and Galea 2002), shows connections to better performance in working memory (Linnell et al. 2013; Tine 2014), speed of processing, and verbal learning (Gupta et al. 2011). However, little is known about the underlying neurobiological mechanism.

DNA methylation (DNAm) has been postulated to play a role in translating environmental impact into phenotypic traits, supported by the paradigm of environmentally induced DNAm alterations (Turecki and Meaney 2016; Hannon et al. 2018) and the involvement of DNAm in human disorders and behaviors, including cognitive processes (Halder et al. 2016). A recent study has demonstrated that early childhood malnutrition, the rate of which differs between urban and rural areas, leads to long-lasting DNAm signatures associated with attention (Peter et al. 2016).

Another plausible mediation candidate is brain structure or function. Accumulating evidence suggests the tangible environmental influence in the brain (Tost et al. 2015), for example, the urban living and upbringing affect brain structure (Haddad et al. 2015) and function related to social stress processing (Lederbogen et al. 2011). Moreover, the connection between brain development with cognitive development and performance has been well established (Luna et al. 2001). Fractional anisotropy (FA), the most commonly investigated measure of white matter microstructure based on diffusion tensor imaging (DTI) (Basser and Pierpaoli 1996), has been revealed to be closely associated with cognition development during childhood (Haas et al. 2014; Moran et al. 2015; Bathelt et al. 2018; Thijssen et al. 2020). Regional homogeneity (ReHo) measures local functional homogeneity on a voxel-wise basis and reflects coordination in regional neural activity (Zang et al. 2004). Varieties of studies has successfully applied ReHo to investigate the association between brain activity and cognitive performance (Gau and Shang 2018; Harrison et al. 2019; Liu et al. 2019; Ma et al. 2019; Respino et al. 2020).

The cognitive development during childhood is sensitive to environmental exposures (Gluckman et al. 2007), such as urbanicity. Certain exposure may exert an influence on the rapid and dynamic brain development during the childhood period (Dubois et al. 2014; Gilmore et al. 2018) and lead to long-lasting effect on behaviors in adulthood (Knudsen 2004). Therefore, we proposed a hypothesis that the impact of childhood urbanicity on cognition is partially mediated by DNAm, and brain structure or function.

To test this hypothesis, we recruited 497 healthy Chinese adults with different childhood urbanicity and performed the following analyses: 1) investigating the association between childhood urbanicity and cognitive performance; 2) measuring the urbanicity-related joint pattern among DNAm, brain structure (FA), and brain function (ReHo) and its association with cognition; 3) investigating possible mediation role of this joint pattern in the association between childhood urbanicity and cognitive performance; and 4) validating the primary mediation findings in an independent sample. As urban populations are growing rapidly worldwide (Dye 2008), exploring how urbanicity affects cognition may provide insights into the healthy living and society.

Materials and Methods

Participants

We included 497 healthy participants (discovery study: $N = 280$; validation study: $N = 217$, Table 1) in the present study from the local community with the following recruitment conditions: 1) Chinese of Han ancestry; 2) age ranging from 18 to 45; 3) no current or history of neurological or psychiatric disease met the criteria of Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision (DSM-IV-TR), assessed by psychiatrists using the Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Non-patient Edition (SCID-I/NP); 4) no history of loss of consciousness for more than 5 min; 6) no abnormalities on subsequent magnetic resonance images, confirmed by a radiologist; and 7) currently living in Beijing for at least 1 year. This study was approved by the institutional ethics review board of Peking University Sixth Hospital. Written informed consent was obtained from all participants.

Data Collection and Preprocessing

Assessment of Urbanicity

The present study assessed childhood urbanicity based on urban birth and upbringing to 18 years old. Urban areas included cities with populations greater than 100 000, and rural areas refer to agricultural regions with populations less than 10 000. Samples were divided into four urbanicity groups: born and raised in rural areas from birth to 18 years old (Group I); born in rural areas and migrated to urban areas between 12 and 18 years old (Group II); born in rural areas and migrated to urban areas before 12 years old (Group III); and born and raised in cities (Group IV). We also applied the urbanicity score (URS) to assess urbanicity by considering the population size (Mortensen et al. 1999; Lederbogen et al. 2011). Areas with different population sizes were scored as follows: a super city with more than 5 000 000 residences (4/year), a large city with 1 000 000–5 000 000 residences (3/year), a small or medium city with 100 000–1 000 000 residences (2/year), and an administrative town or village with fewer than 10 000 residences (1/year). Then, we calculated the URS using the URS per year multiplied by the years spent in each area, from birth to 18 years old. The Kruskal–Wallis test revealed that the URSs were significantly different among the four groups (discovery study: $P = 3.87E{-}38$; validation study: $P = 1.77E{-}30$).

Cognitive Assessment

Cognitive function was assessed using the Chinese version (Shi et al. 2015) of the MATRICS Consensus Cognitive Battery (MCCB) (Nuechterlein et al. 2008). Raw MCCB scores were standardized to T scores (mean = 50, SD = 10). To control confounding effect, generalized linear regression calculated a residual value for each MCCB score with the effect of age, sex, and educational attainment regressed out. The between-group differences in cognitive performance were evaluated based on residual MCCB scores using the Kruskal–Wallis test, and the correlation between URS and residual MCCB scores was also examined using Spearman's rank correlation analysis.

Table 1 Demographic data

Sample	N	Sex (male:female)	Age (year)	Educational attainment (year)	MATRICS Consensus Cognitive Battery, T score	Urbanicity score
Discovery study						
Group I	70	35:35	25.53 (2.05)	17.61 (2.10)	50.7 (4.38)	23.35 (16.95)
Group II	70	35:35	24.89 (2.58)	17.51 (2.44)	51.82 (4.65)	25.67 (13.21)
Group III	70	35:35	24.71 (2.32)	17.26 (2.08)	53.64 (3.57)	37.99 (15.59)
Group IV	70	35:35	23.4 (4.79)	15.99 (2.54)	54.93 (4.51)	67.74 (12.03)
Validation study						
Group I	63	32:31	26.06 (3.69)	17.10 (2.74)	47.93 (11.80)	18.07 (5.40)
Group II	49	26:23	23.59 (5.13)	15.20 (2.52)	50.42 (9.19)	21.67 (4.30)
Group III	55	20:35	23.29 (5.24)	15.8 (2.21)	49.76 (12.90)	29.95 (8.86)
Group IV	50	23:27	24.42 (2.73)	17.58 (2.01)	50.96 (15.80)	64.86 (19.84)

Note: MCCB, MATRICS Consensus Cognitive Battery.

DNA Methylation

The genome-wide methylation profile in peripheral blood was measured by an Illumina Infinium Human Methylation EPIC Beadchip covering over 850 000 CpG sites genome-wide. After quality control, normalization, and correction for batch and cell type, 275 samples with 800 587 DNAm probes remained. A threshold of false discovery rate-corrected P (P_{FDR}) < 0.05 was applied to obtain URS-associated DNAm probes, leaving 30 538 probes that were enriched in promoter regulatory regions.

Brain Imaging

Neuroimaging data of resting-state functional MRI (rs-fMRI) and DTI for all participants were acquired by a 3.0T GE Discovery MR750 scanner in the Center for MRI Research, Peking University. After preprocessing, 274 samples with brain maps of ReHo for rs-fMRI and FA for DTI were obtained. The three-dimensional brain image of each subject was reshaped into a one-dimensional vector and stacked, forming a subject by voxel matrix (FA: $274 \times 59\,155$; ReHo: $274 \times 77\,039$).

Details for data collection and preprocessing are provided in Supplementary Methods.

Data Analysis in Discovery Study

Multiset Canonical Correlation Analysis

We adopted mCCA, to assess the linked alternations among DNAm, ReHo, and FA. The mCCA is developed as an extension of CCA to find linear transforms that simplify the correlation structure among a group of random vectors, which takes multiple stages, where in each stage, a linear combination is found for each random vector such that correlation among the group of resulting variates, that is, the canonical variates, is maximized (Kettenring 1971; Li et al. 2009). It has been successfully used to identify joint patterns in psychiatric disorders (Sui et al. 2013; Sui et al. 2015; Modabbernia et al. 2021). Three modalities, including the URS-associated DNAm probes and preprocessed brain maps of FA and ReHo, of four urbanicity groups were applied in mCCA, with sex, age, educational attainment, height, and weight regressed out. The mCCA jointly decomposed each feature into loading parameter (A_k, $k = 1, 2, 3$) and spatial map (S_k, $k = 1, 2, 3$, Supplementary Fig. S1). Each pairwise modality correlation between A_i and A_j is maximized and sorted from high to low. Eight components for each modality were estimated based on the minimum description length (Li et al. 2007). We evaluated pairwise correlations for all component pairs from mCCA. After Bonferroni correction, the significantly correlated ReHo-FA-DNAm link with the highest correlation was included for subsequent analysis. We further evaluated the intercorrelations of the ReHo-FA-DNAm link with urbanicity grouping controlled to ensure that the correlation was not driven by grouping.

A Z-score was calculated for each brain region and each DNAm probe to evaluate its contribution to the corresponding component. We adjusted the rural upbringings > the urban upbringings for all modalities on the mean of loading parameters. Thus, a positive Z-value (red regions in the spatial map) indicated a higher contribution in the rural upbringings than the urban upbringings, and vice versa. The top contributing brain regions and DNAm loci were defined using a threshold of |Z-score| > 2. The code for mCCA is available through the Fusion ICA Toolbox (http://mialab.mrn.org/software/fit).

Functional Enrichment

To investigate biological implication in the DNAm component, we applied the top contributing DNAm probes (802 probes, 645 genes) in a functional enrichment analysis of pathways and Gene Ontology (GO) using the WEB-based Gene SeT AnaLysis Toolkit (Web Gestalt v.2017) (Wang et al. 2017). Tissue enrichment analysis was conducted in the FUMA Web platform (https://fuma.ctglab.nl/) using 53 tissue types in the Genotype-Tissue Expression (GTEx) consortium (v7) (Watanabe et al. 2017). A significant threshold was set as $P_{FDR} < 0.05$.

Statistical Analysis

The Kruskal–Wallis test and Wilcoxon signed-rank test were used to investigate possible difference among urbanicity groups. The Spearman's rank correlation analysis was used to evaluate the correlation between measures with non-Gaussian distribution. The possible confounding effect of individual factors was regressed out with the generalized linear modeling before correlations analysis with Spearman's rank correlation analysis.

Causal Mediation Analysis

The causal mediation effect, defined as X leading to Y through M, was calculated by two statistical models with 5000 simulations in bootstraps. The first linear regression model defined M as the outcome, and X and covariates as the treatment. The second linear regression model used Y as the outcome, and M, X, and

covariates as the treatment. Causal mediation analysis was performed for the following hypothesis: 1) the ReHo-FA-DNAm link (M: each component) mediates the urbanicity impact (X: URS) on cognition (Y: MCCB scores); 2) the DNAm component (M) mediates the urbanicity effect (X: URS) on the brain (Y: ReHo and FA components); and 3) the brain (M: ReHo and FA components) mediates the DNAm component (X) modulating cognitive performance (Y: MCCB scores). Causal mediation analysis was performed using the "mediation" (Tingley et al. 2014) package in R, with age, sex, and educational attainment as covariates.

Blood–Brain Correlations of DNAm Probes

As DNAm in the present study was measured with DNA obtained from peripheral blood mononuclear cells, we assessed the likely correlation between blood and brain methylation in three databases: the Blood Brain DNA Methylation Comparison Tool (Hannon et al. 2015), the Blood–Brain Epigenetic Concordance (BECon) (Edgar et al. 2017), and the Iowa Methylation Array Graphing for Experimental Comparison of Peripheral tissue & Gray matter (IMAGE-CpG) (Braun et al. 2019).

Independent Validation Study

In an independent cohort, the identified mediation effect in the discovery study was validated, that is, ReHo mediates the urbanicity impact on speed of processing. First, we identified brain regions with URS-associated ReHo in independent samples by applying whole-brain regression analysis of the URS in SPM12 using individual ReHo images, with age, sex, and educational attainment as covariates. Next, we evaluated the association between URS-associated ReHo (mean Z-score of each significant region) and cognition (residual MCCB scores) using Spearman's rank correlation analysis. Then, we applied causal mediation analysis to examine the mediation effect of ReHo (M: ReHo) in urbanicity (X: URS) influence in the correlated cognitive performance (Y: MCCB scores).

We further evaluated the consistency between discovery findings and validation findings. For this purpose, we first identified regions of interest (ROIs) according to the findings of whole-brain regression analysis of the URS in the validation study and separately calculated mean Z-score of each ROI in the ReHo component individually in the discovery cohort. Then, correlation analysis with MCCB scores was also performed.

Results

Urbanicity Is Correlated with Better Cognitive Performance

Groups with different childhood urbanicity were well matched in sex, but not in age and education attainment in both discovery and validation samples (Table 1 and Supplementary Table S1). The residual MCCB composite score was significantly different among urbanicity groups in the discovery cohort (P_{FDR} = 1.46E−05, Epsilon squared = 0.108, 95% confidence interval [95% CI]: [0.056, 0.201]) and in the validation cohort (P_{FDR} = 1.32E−04, Epsilon squared = 0.111, 95% CI: [0.052, 0.211]), with the highest urbanicity group (Group IV) showing the highest MCCB composite score and vice versa (Table 1 and Supplementary Table S2). Consistently, URS was significantly positively correlated with residual MCCB composite score (discovery study: P_{FDR} = 3.66E−06, rho = 0.299, 95% CI: [0.188, 0.410]; validation study: P_{FDR} = 2.09E−04, rho = 0.276, 95% CI: [0.153, 0.406]). Participant with a higher URS tend to perform better in speed of processing, attention/vigilance, working memory, verbal learning, reasoning, and problem solving in both the discovery and validation cohorts (Table 2). Additionally, given the modest sample size, a post hoc power analysis was performed based on the mean correlation coefficients between URS and MCCB scores in the discovery (rho = 0.171) and validation (rho = 0.179) cohort, identifying the statistical power as 0.821 for discovery study and 0.757 for validation study, which approximate to the recommended 0.80 level (Cohen 2013).

A Joint DNAm-FA-ReHo Pattern

The mCCA identified one significant intercorrelated DNAm-FA-ReHo triplet with significant pairwise correlations among the URS-associated DNAm probes (30 538 probes), and preprocessed FA and ReHo maps. The three components of the intercorrelated DNAm-FA-ReHo triplet showed significant pairwise correlations (DNAm-FA: Bonferroni-adjusted P, P_{bon} = 2.00E−02, rho = 0.216, 95% CI: [0.093, 0.331]; DNAm-ReHo: P_{bon} = 4.08E−03, rho = 0.239, 95% CI: [0.123, 0.353]; ReHo-FA: P_{bon} = 1.69E−06, rho = 0.328, 95% CI: [0.212, 0.425]). After controlling urbanicity grouping, intercorrelations of this triplet remained significant (DNAm-FA: P = 6.25E−03, rho = 0.165, 95% CI: [0.045, 0.287]; DNAm-ReHo: P = 9.60E−04, rho = 0.198, 95% CI: [0.093, 0.308]; ReHo-FA: P = 6.42E−07, rho = 0.295, 95% CI: [0.178, 0.412]), indicating similar association patterns among the triple components in all groups.

The spatial maps of the FA and ReHo components with a threshold at $|Z\text{-score}| > 2$ are shown in Figure 1*a*,*b*. Compared with rural upbringings, urban upbringings have lower ReHo mainly in the frontal gyrus and higher ReHo in the middle temporal gyrus, thalamus, lingual gyrus, and cerebellum. Only lower FA was observed in the urban upbringings, mainly in the bilateral anterior corona radiata (ACR), the corticospinal tract, and the bilateral posterior thalamic radiation (PTR). Anatomical information of the identified ReHo component is shown in Supplementary Table S3. The corresponding genes for the DNAm component were significantly enriched in the cadherin and Wnt signaling pathways and 38 GO terms (Supplementary Table S4). Tissue enrichment analysis revealed that those genes were upregulated in several brain regions involved in the ReHo component (Supplementary Table S5, Supplementary Fig. S2). As a portion of DNAm probes is tissue-specific, we interpreted the blood–brain correlation of five DNAm probes with the highest Z-score. Four probes had significant blood–brain DNAm correlations (Supplementary Figs S3 and S4 and Supplementary Table S6), especially cg15864601 (r = 0.48, P = 0.03) and cg05353869 (r = 0.86, $P < 2E{-}16$). More details are demonstrated in Supplementary Results.

The Joint DNAm-FA-ReHo Pattern Was Associated with Urbanicity and Cognition

Each identified component in the joint DNAm-FA-ReHo pattern showed a significant correlation with URS (Fig. 1*c*, FA-URS: P = 4.10E−05, rho = −0.245, 95% CI: [−0.351, −0.134]; ReHo-URS: P = 2.46E−04, rho = −0.220, 95% CI: [−0.332, −0.107]; DNAm-URS: P = 4.54E−20, rho = −0.516, 95% CI: [−0.603, −0.421]) and a significant difference among urbanicity groups (Fig. 1*d*, FA: P = 8.94E−04, Epsilon squared = 0.060, 95% CI: [0.021, 0.138]; ReHo: P = 1.66E−02, Epsilon squared = 0.038, 95% CI: [0.011, 0.098]; DNAm: P = 6.61E−16, Epsilon squared = 0.270, 95% CI: [0.190, 0.374]). The strongest evidence of pairwise differences was

Table 2 The association between urbanity and cognitive performance

MCCB		Discovery study ($N = 280$)				Validation study ($N = 217$)			
		Urbanicity group		Urbanicity score		Urbanicity group		Urbanicity score	
		P_{FDR}	Effect size, 95% CI	P_{FDR}	rho, 95% CI	P_{FDR}	Effect size, 95% CI	P_{FDR}	rho, 95% CI
Speed of Processing	Brief Assessment of Cognition in Schizophrenia: Symbol Coding (BACS-SC)	0.629	0.006, [0.002, 0.046]	0.514	0.039, [−0.085, 0.153]	0.099	0.031, [0.007, 0.103]	0.026	0.159, [0.020, 0.285]
	Trail Making Test, Part A (TMT-A)	0.039	0.034, [0.008, 0.094]	0.005	0.177, [0.057, 0.297]	0.091	0.036, [0.009, 0.110]	0.025	0.170, [0.039, 0.293]
	CF-AN	0.003	0.059, [0.019, 0.139]	0.002	0.204, [0.083, 0.322]	0.002	0.078, [0.028, 0.172]	0.002	0.229, [0.091, 0.364]
	Speed of Processing Total Score (SoP)	0.004	0.056, [0.021, 0.130]	0.001	0.210, [0.096, 0.325]	6.23E−04	0.093, [0.040, 0.192]	2.09E−04	0.279, [0.156, 0.400]
Attention/Vigilance	Continuous Performance Test-Identical Pairs (CPT-IP)	0.014	0.043, [0.012, 0.107]	0.009	0.166, [0.046, 0.290]	0.102	0.030, [0.008, 0.099]	0.026	0.166, [0.046, 0.296]
Working memory	Wechsler Memory Scale-3rd Ed.: Spatial Span (WMS-SS)	0.058	0.029, [0.008, 0.093]	0.024	0.142, [0.020, 0.261]	0.099	0.032, [0.006, 0.100]	0.043	0.143, [0.009, 0.282]
Verbal learning	Hopkins Verbal Learning Test-Revised (HVLT-R)	3.27E−04	0.080, [0.033, 0.162]	2.81E−04	0.240, [0.117, 0.357]	0.050	0.044, [0.012, 0.122]	0.025	0.168, [0.045, 0.285]
Visual learning	Brief Visuospatial Memory Test-Revised (BVMT-R)	0.625	0.007, [0.001, 0.045]	0.105	0.100, [−0.015, 0.221]	0.099	0.033, [0.007, 0.108]	0.083	0.121, [−0.005, 0.261]
Reasoning and problem solving	Neuropsychological Assessment Battery: Mazes (NAB-Mazes)	0.007	0.050, [0.018, 0.124]	0.003	0.192, [0.086, 0.302]	9.44E−05	0.121, [0.064, 0.216]	0.002	0.236, [0.102, 0.364]
Social cognition	Mayer–Salovey–Caruso Emotional Intelligence Test: Managing Emotions (MSCEIT-ME)	0.417	0.012, [0.002, 0.061]	0.076	0.112, [−0.009, 0.230]	0.848	0.004, [0.001, 0.051]	0.712	0.025, [−0.116, 0.155]

Notes: Each MCCB score was the residual MCCB score with the effect of age, sex, and educational attainment regressed out. The between-group differences in MCCB scores were evaluated using the Kruskal–Wallis test, with the effect size measured by Epsilon-squared. The associations between urbanity score and MCCB scores were calculated using Spearman's rank correlation. We calculated a 95% CI for each statistical analysis with 1000 replications.

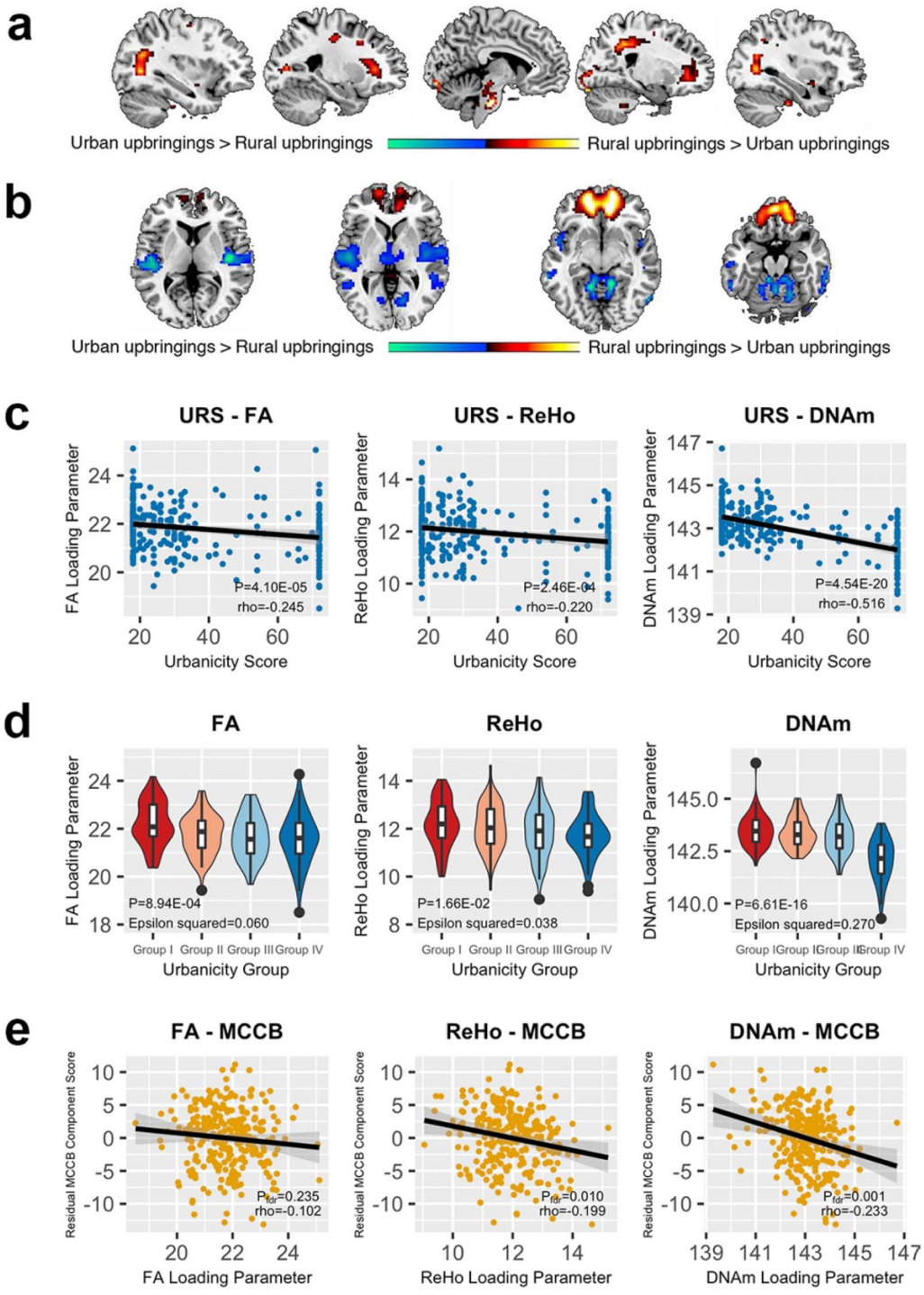

Figure 1. The joint pattern among DNAm, FA, and ReHo. The mCCA identified a joint DNAm-FA-ReHo pattern. (*a*) Spatial maps of the FA component in the joint pattern. Red indicates urban upbringings < rural upbringings, while blue regions are urban upbringing > rural upbringings. (*b*) The spatial maps of the ReHo component in the joint pattern. (*c*) Spearman correlation analysis showed significant correlations between each modality and URS, controlling for age, sex, and educational attainment. (*d*) Each modality was significantly different among the four groups. (*e*) Significant correlations of each modality with MCCB composite score using Spearman correlation analysis, with age, sex, and educational attainment controlled.

found between Group I (rural birth and upbringing) and Group IV (urban birth and upbringing), with significant differences in all three modalities (Supplementary Table S7).

ReHo and DNAm components showed significant correlation with residual MCCB composite scores and several specific tests (Fig. 1*e* and Supplementary Tables S8 and S9). No significant correlation between FA component and cognitive test was observed (Fig. 1*e* and Supplementary Table S10). After controlling urbanicity grouping in each component, a group of correlation remained significant, including the correlation of ReHo with

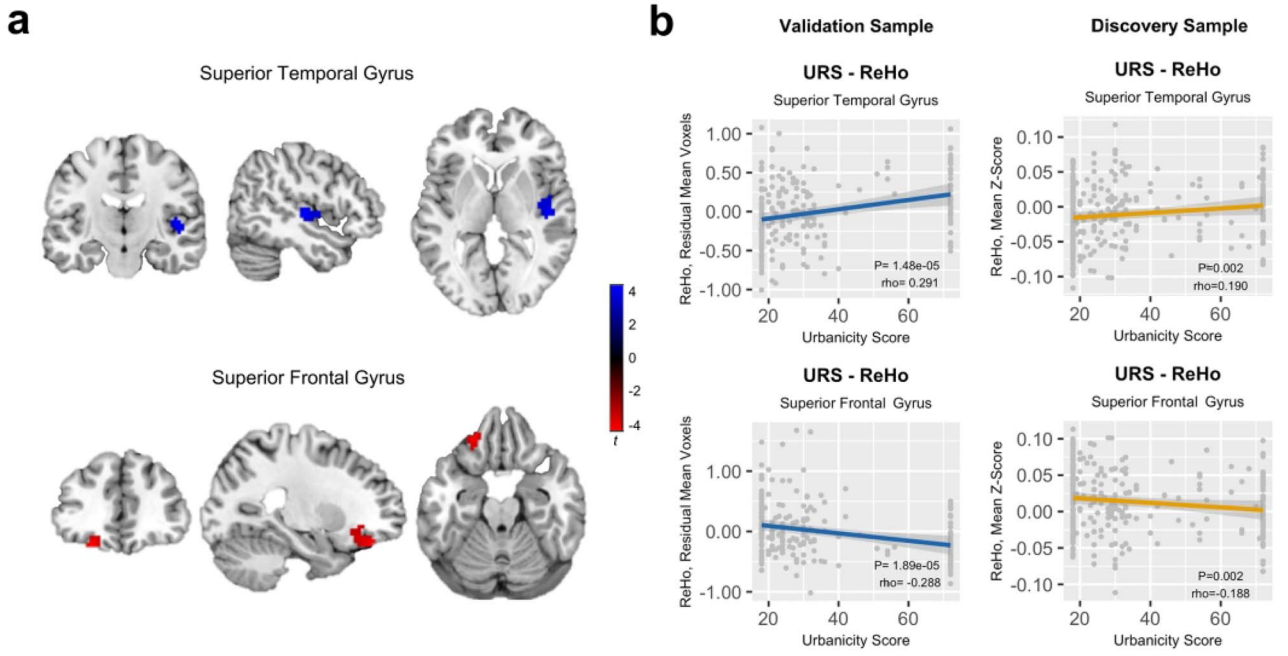

Figure 2. Urbanicity-associated ReHo in an independent validation study. (*a*) In the validation study, ReHo in the STG was positively associated with URS, and ReHo in the SFG was negatively correlated with URS, after regressing confounding effect of age, sex, and educational attainment ($P < 0.05$, cluster-level FWE corrected within the whole brain). In order to be consistent with the presentation pattern of ReHo component in the discovery study, the region with positively correlation was in blue, and the region with negatively correlation was in red. (*b*) In the discovery sample, the correlation analysis of the mean Z-value of ReHo component with URS based on the ROIs in the validation study showed significantly positively correlation in the STG and negatively correlation in the SFG.

MCCB composite score ($P = 0.023$, rho = −0.137, 95% CI: [−0.250, −0.012]) and with Category Fluency: Animal Naming (CF-AN, $P = 0.041$, rho = −0.124, 95% CI: [−0.241, −0.008]), and the correlation of DNAm with CF-AN ($P = 0.043$, rho = −0.122, 95% CI: [−0.240, 0.006]) and with Brief Visuospatial Memory Test (BVMT, $P = 0.015$, rho = −0.147, 95% CI: [−0.261, −0.029]). The CF-AN score revealed cognitive performance in speed of processing and the BVMT referred to visual learning.

A Casual Mediation Model Suggested an Urbanicity Effect on Cognition

Then, we performed causal mediation analysis to verify a possible mediation role of DNAm and ReHo underlying the association between urbanicity and cognitive performance. Since the visual learning test, BVMT, was not significantly associated with any measure of urbanicity, we only included CF-AN and MCCB composite score. As shown in Supplementary Table S11, we found that 1) ReHo mediated urbanicity effects on the speed of processing (CF-AN: $P = 4.72E{-}02$, mediation proportion = 10.86%); 2) DNAm mediated the urbanicity impact on ReHo ($P = 1.88E{-}02$, mediation proportion = 48.55%); and 3) ReHo showed significant mediation effect in the association between DNAm and the MCCB Composite score ($P = 4.00E{-}03$, mediation proportion = 15.25%) and marginally mediation effect in the association between DNAm and the speed of processing. In summary, the above results suggested a mediation model: urban upbringing → DNAm → ReHo → speed of processing.

Independent Validation of the Mediation Role of ReHo

The whole-brain analysis in an independent cohort ($N = 217$) revealed that URS is significantly positively correlated with ReHo in the superior temporal gyrus (STG) and is negatively correlated with ReHo in the superior frontal gyrus (SFG) ($P < 0.05$, cluster-level FWE corrected within the whole brain, Fig. 2*a* and Supplementary Table S12). The connection between URS and ReHo was consistent with findings in the discovery study that urban upbringings showed a higher ReHo component in the STG and a lower ReHo component in the SFG compared with rural upbringings (Fig. 1*b* and Supplementary Table S3). Additional analysis based on those two ROIs revealed that the URS was significantly positively correlated with the ReHo in the STG and negatively correlated with that in the SFG in both the discovery and validation samples (Fig. 2*b*), suggesting urbanicity influences the activity of the STG and SFG in opposite directions.

ReHo in the STG and the SFG was correlated with residual speed of processing total score in opposite direction (STG: $P_{FDR} = 0.002$, rho = 0.246; SFG: $P_{FDR} = 0.036$, rho = −0.159; Supplementary Table S13). ReHo in the STG also showed significantly positive correlations with other tests of processing speed, including symbol coding, Trail Making Test, and Category Fluency (BACS-SC, $P_{FDR} = 0.010$, rho = 0.207; CF-AN, $P_{FDR} = 0.036$, rho = 0.155; TMT, $P_{FDR} = 0.036$, rho = 0.151). ReHo in the SFG was also negatively correlated with BACS-SC ($P_{FDR} = 0.018$, rho = −0.184).

Furthermore, causal mediation analysis validated that ReHo in the STG mediated the urbanicity effect on BACS-SC ($P = 0.04$, mediation proportion = 43.98%, Supplementary Table S14) and Speed of Processing Total Score ($P = 2.00E{-}03$, mediation proportion = 22.84%).

Discussion

The present study provided initial evidence for the mediate role of DNAm modification and resting-state brain activity in

childhood urbanicity influence on speed of processing. In particular, the mediation effect of spontaneous brain function in STG underlying the correlation between a higher childhood urbanicity and a faster speed of processing was validated in an independent sample.

The mediation analysis suggested that childhood urbanicity influenced DNAm levels and local spontaneous brain function activity and further affected cognition. These findings are consistent with previous reports. Several studies have shown that urban upbringing and living correlated with better performance in cognitive tests (Gupta et al. 2011; Chen 2012; Tine 2014). The early-life environment and experience alter brain structure and function, thus affecting behaviors (Roth and David Sweatt 2011). The epigenetic regulation of genes alters adult neurons and contributes to the influence of the environment and experience on brain function and behavior (Casey et al. 2009). A growing number of studies have investigated associations between DNAm and neuroimaging markers of human behavior and cognition in order to better understand the role of DNAm in brain health and disease (Wheater et al. 2020). However, the link between the DNAm and MRI features has not been well established. The findings in the discovery samples suggested that the effect of childhood urbanicity on DNAm was related to the brain function measured with ReHo, in line with the crucial role of DNAm in regulating neurogenesis and brain plasticity (Fan et al. 2001, 2005). Our findings also emphasized the Wnt signaling and cadherin pathways in this process. The Wnt signaling and cadherin pathways play a role in neuronal plasticity, brain development, learning, and memory (Maguschak and Ressler 2011; Hirano and Takeichi 2012). Therefore, the current findings provided an insight for neurobiological mechanisms underlying the environmental effects on human behaviors.

More importantly, our study highlighted the spontaneous brain function of STG in the childhood urbanicity influence on the performance of processing information in adulthood. STG has been suggested as a key structure in extracting information from speech input, which is an important part of processing information (Bigler et al. 2007; Luo et al. 2018). This was supported by the greater left temporal activation during category fluency (Mummery et al. 1996; Gourovitch et al. 2000) and a category fluency deficit in patients with temporal lobe lesions (Janowsky et al. 1989). Thus, the current findings add to and extend a mediation role of spontaneous brain function in STG in urbanicity impact on cognitive functions. In addition, our discovery study reported that higher ReHo values in the thalamus, lingual gyrus, and cerebellar played a part in urbanicity-associated better speed of processing. These results were in accordance with reported findings that the lingual gyrus was involved in the recognition of words and semantic processing (Mechelli et al. 2000; Heath et al. 2012), greater cerebellar activation correlated with faster processing speed (Genova et al. 2009), and a verbal fluency task reliably activated the cerebellum (Hubrich-Ungureanu et al. 2002).

Although we failed to find any mediation effect of FA, the FA component showed that urban upbringing was related to a lower FA in the bilateral ACR, the corticospinal tract, and the bilateral PTR. The ACR is a part of the limbic–thalamo–cortical circuitry, connecting the thalamus, mPFC, and STG, and the PTR refers to fiber pathways linking the thalamus and lingual gyrus. As the thalamus, mPFC, STG, and lingual gyrus contributed to the ReHo component, this may explain the correlation in the joint DNAm-FA-ReHo pattern.

The findings of this study should be tempered by the following limitations. First, the sample size was relatively small, requiring further validation of our finding in a larger cohort. Second, age and educational attainment were not well matched between groups. Of note, all the participants were in their middle twenties with the group with the lowest educational attainment (Group IV) showed the best cognitive performance, and we controlled for potential confounding effect during the whole study, suggesting that age and educational attainment could not essentially drive our findings. Third, DNAm was only examined in the peripheral blood without any gene expression data. Although public databases revealed high blood–brain correlations for the key DNAm probes, further validation using DNAm and gene expression in brain tissues is still needed.

In conclusion, our research suggests that urban birth and upbringing are linked to a faster processing speed, mediated by DNAm and ReHo, and highlights the role of STG activity in this process. This current study integrated multi-omics data and provided new insight into the biological and neurological mechanism underlying environmental influence on human behaviors. As various environmental factors differ between urban and rural upbringing, the contributing environmental determinants and their effects remain to be further explored in future work.

Supplementary Material

Supplementary material can be found at *Cerebral Cortex* online.

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