Structural and functional influences of urban and rural childhoods on the medial prefrontal cortex

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Abstract

Urbanization has been implicated as having an important influence on brain development and risk for psychiatric disorders but the mechanisms in brain and the role of genetic background have been unresolved. Here we explored major urbanization changes in recent history in China, and its correlates with brain structure and function in a genetically controlled sample with similar current education and socioeconomic status. Principal component analyses showed no global genomic differences across urban and rural groups. Subjects with rural childhoods had relatively increased medial prefrontal cortex (mPFC) gray matter volumes. The engagement of medial prefrontal cortex during working memory was relatively reduced under increased interpersonal stress. Further, increased trait anxiety and depression was associated with reduced stress-related mPFC engagement but only in subjects with urban childhoods. This is the first study to examine the impact of childhood urbanicity on brain development and function controlling for genetic and socioeconomic variation. We find that urbanicity during childhood is associated with changes in mPFC structure and function that are consistent with conceptualizations of how urbanicity might increase the influence by which interpersonal stressors are neurally processed.

Introduction

Urbanicity, the experience of the urban environment, has been an emerging characteristic of world societies for centuries. But this change from rural agrarian communities has accelerated in recent history. From less than 20% of the world's population in the 1970s, the urban population has rapidly increased several-fold such that by 2007, the majority of the world's population is living in urban areas for the first time in history. The urban population is expected to further rise to 66 percent in 2050, driven by urbanization in developing countries in Asia and Africa[1]. Modern urban cities are characterized by dense population and housing, and an array of industries and services, offering improved educational, health and income opportunities[2]. Although urban environments can facilitate a higher standard of living on average, it also is accompanied by increasing risk for non-communicable diseases like cancer, obesity, diabetes, chronic respiratory diseases and psychiatric disorders[3] – the latter an indirect focus of this report. Many studies have been performed to examine the relationship between urbanicity and mental health, mostly in developed countries. Indeed, population-based studies have suggested a relationship between childhoods in urban cities and an increased risk for schizophrenia[4-9], autism spectrum disorders[10], alcohol and drug dependence[11], as well as mood and anxiety disorders[12-14].

In pursuit of a link between the urban environment and risk for mental disorders at the level of brain, recent work has suggested that the prefrontal cortex is a particular target of urban experience[15-17]. The prefrontal cortex (PFC) continues to develop and integrate experiences through childhood and early adulthood[18,19], with the dorsolateral PFC (dlPFC) guiding thoughts, attention and actions[20], and the orbital (oPFC) and ventromedial PFC (vmPFC) regulating emotion[21]. Gray matter changes as measured on MRI in ventral and medial PFC regions are evident in mood disorders and in post-traumatic-stress disorder[22-26]. The perigenual

anterior cingulate cortex and nearby mPFC has been particularly implicated in childhood urbanicity[15]. This work suggests that relatively dysfunctional medial prefrontal engagement under interpersonal stress occurred in relation to exposures to childhood urbanicity, and that urban childhoods might sensitize medial prefrontal dysfunction to the more prevalent interpersonal stressors there. Nevertheless, the biologic mechanisms underlying this observation are unknown and prior studies have not been replicated in a different ethnic population, or excluded genetic background in the context of the differing environmental experiences.

Studies of mPFC function suggest that this region is critical for weighing the value of conflicting information, and in affective regulation and social cognition[27]. In a recent experiment on social hierarchy, the mPFC selectively mediated the updating of knowledge about one's own social position, as opposed to that of another individual[28]. The vmPFC also appears to track self-estimates of performance[29]. There are findings showing that gray matter volume measured on MRI in the vmPFC and oPFC relate to interpersonal relationships, social network size and potentially, resilience to stress[30,31]. Reduced medial prefrontal regional gray matter volumes and altered activation have also been implicated in patients with depression and in the expression of anxious and depressive traits[23-26]. In comparison to healthy controls, patients with depression show altered mPFC activation during increased negative self-referential processing, another function thought to be subserved by mPFC[32].

Indeed, if childhood in urban environments does influence medial prefrontal dysfunction in response to interpersonal stressors[15,17] independent of genetic ancestry, we might expect that these environmental effects could result in brain structural and functional changes associated with states and traits related to interpersonal stress at these medial prefrontal brain regions. These effects should also occur in non-European populations, and the environmental factor would not be dependent on genetic background. To test these hypotheses, we

examined a relatively genetically homogeneous sample of healthy adult individuals living in Beijing who experienced differing levels of rural or urban childhood environments during China's recent two decades of rapid and widespread urbanization[33]. We further designed an event-related working memory (WM) task with and without interpersonal stress based on previous work[34,35]. Specifically, we predicted that individuals from urban childhoods, and particularly those individuals with higher trait anxiety-depression, might have a more aberrant physiological response to interpersonal stress in medial prefrontal cortex. The converse might occur for individuals with more rural childhoods where they might have a relatively less dysfunctional medial prefrontal stress response.

Results

Demographics

We studied 490 healthy adult subjects with structural MRI at 3 Telsa (see Methods). This resulted in a sample of individuals with differing childhoods in urban (N=249, living in urban settings since before age 12) and rural (N=241, living in rural settings from birth to beyond age 12) environments. Subjects with urban childhoods were slightly younger and taller, and had higher parental education and parental social-economic status, and older fathers (Table 1). Both groups, however, had similar gender distribution, were currently living in Beijing, and had similar current educational and occupational levels. They were genetically homogeneous with no significant differences across the first 20 principal components from whole genome genotyping (Supplementary Figure S1). Note, we also have similar demographic, and subsequent MRI structural and functional findings (below) if we increased the resolution by which childhood urban-rural exposures are defined (see Methods, Supplementary Figures S2, S3, S5).

Behavioral results:

We adapted an event-related "number working memory task" based on previous work[34,35], but with induced interpersonal stress states occurring in half the trials in an event-related block design (Figure 1, see Methods). Following quality-control exclusions for the functional MRI paradigm (see Methods), an overlapping sample of 394 healthy subjects was studied (Table 2). During working memory (WM) maintenance, trials with interpersonal stress were associated with increased accuracy ($p < 0.001$) and faster reaction time ($P < 0.001$). This effect was not seen, however, during WM manipulation, but there was a trend of faster RT under stress ($p = 0.07$). When comparing urban vs rural childhoods, there were no differences in accuracy, but relatively faster reaction time in the urban group in WM manipulation, and WM maintenance, during the stressed, as

well as less-stressed conditions ($p < 0.01$). There, however, were no urbanicity-by-stress interactions on accuracy, or reaction time performance in WM manipulation, or in WM maintenance.

We found that trait anxiety-depression as measured on the Eysenck Personality Questionnaire Neuroticism subscore was significantly higher in the urban group (Table 2). Note though, that none of the subjects were diagnosed with a current or past mood or anxiety disorder.

Effects of urban vs rural upbringing on gray matter volume

Total gray matter volume (GMV) was relatively higher in individuals in the more urban compared with rural childhoods ($p=0.02$), as well as in males ($p<0.001$). Despite this global effect of urban childhoods, controlled for age, the second-degree polynomial expansion of age[36], gender, education and total gray matter volume, we found that individuals with rural childhoods had relatively increased regional gray matter volume in the mPFC in Brodmann Area (BA) 11 ($x=-6$, $y=59$, $z=-20$; $T=5.37$; Cluster Size $=332$; $p<0.05$, family-wise error (FWE) corrected) and BA8 ($x=6$, $y=35$, $z=40$; $T=4.95$; Cluster Size $= 40$; $p<0.05$, FWE-corrected) (Figure 2). No other brain regions differed between childhoods in urban and rural environments at these thresholds. We have similar results if we increased the resolution of this grouping, stratifying the groups to individuals who were born in and continue to live in cities, vs those who have lived in cities since before age 12, vs those who lived in rural areas between birth and age 18, vs those lived in rural areas for >18 years since birth. We also have similar results using a childhood urbanicity score[6,15] (See Supplementary Figures S2 and S3).

Functional MRI Results

During WM maintenance and manipulation, regions in the prefrontal cortex, parietal lobe, temporal lobe, striatum, were robustly engaged, while there was a decrease in engagement of the mPFC (Figure 3,

Supplementary Table 1). Relative to WM maintenance, WM manipulation engaged greater activity at the prefrontal cortex, parietal lobe, temporal lobe, and striatum, as well as more reduced engagement of the mPFC; these occurred in stressed, as well as less stressed conditions (Supplementary Table 2). The interpersonal stress vs less stressed condition was associated with reduction in the engagement of prefrontal cortex, including the mPFC during WM manipulation, as well as WM maintenance (Figure 3, Supplementary Table 3). In WM maintenance as well as WM manipulation tasks, whether under stress or less stress, faster reaction time was associated with relatively more engagement of mPFC, posterior cingulate cortex, bilateral insula and somatosensory association cortex (Supplementary Figure S4); accuracy was not significantly correlated with brain activation, consistent with the event-related design considering only correctly performed trials. All the above statistical thresholds were $p<0.05$ whole brain family-wise error (FWE) corrected.

We then examined the hypothesized childhood urbanicity effects on states and traits related to stress. Given the observed differences in trait anxiety-depression across urbanicity, differences in mPFC gray matter volumes, and that mPFC had more reduced engagement under stress, consistent with the suppression needed during WM tasks of multifaceted self-referential processes at mPFC, including interpersonal stress[23,37,38], we then examined how urbanicity and trait anxiety-depression might modulate WM function under stress at the mPFC functional region of interest (ROI, see Methods). Main effects of urbanicity and trait anxiety-depression did not significantly influence mPFC function. These negative findings in mPFC, however, appeared to arise because of an interaction of urbanicity and trait anxiety-depression during WM manipulation ($x=8$, $y=54$, $z=-4$, $T=3.37$; $p<0.05$ FWE corrected within the functional ROI; Figure 4). In the group with more urban childhoods, increased trait anxiety-depression appeared to result in a larger reduction in mPFC engagement during stress. On the other hand, this effect was not apparent in the rural group, where trait anxiety-depression instead appeared to be associated with relatively less reduction in mPFC engagement

during stress. We did not observe significant effects at the thresholds indicated during WM maintenance across these same contrasts. There was no significant relationship between trait anxiety-depression and accuracy or reaction time during WM maintenance or manipulation; there was also no urbanicity group by accuracy or reaction time interactions for WM maintenance or WM manipulation. Similar results were obtained if we increased the resolution of the childhood rural-urban exposure groups (Supplementary Figure S5).

Discussion

In this study, we examined the effects of urban and rural childhoods on brain structure and function in a large and genetically homogeneous sample of healthy Han Chinese who have had similar current educational and occupational status, but experienced different extents of urban and rural childhoods during China's rapid urbanization. Prior work has highlighted the role of mPFC in mediating the experience of social stress, but no previous study has controlled for genetic ancestry and for current environmental circumstances, which are critical in interpreting otherwise circumstantial associations. Our findings thus extend that by Lederbogen et al[15] on how childhood urbanicity may affect medial prefrontal cortex function through mechanisms related to increased sensitivity to interpersonal stress. While we also find that childhood urbanicity may affect the functional states of medial prefrontal cortex under stress, we suggest that this occurs in part through potentially more enduring effects of trait anxiety-depression, and may also affect brain structure, at least as measured on MRI. In our sample, individuals with relatively more urban childhood experience had reduced measures of medial prefrontal gray matter volumes. We found that in the accurate performance of specifically WM manipulation under interpersonal stress, suppression of presumably stress-related self-referential processes at medial prefrontal cortex[23,37,38] was greater in individuals with urban childhoods in relation to

increasing trait anxiety-depression. On the other hand, in individuals with more rural childhood experience, trait anxiety-depression did not result in reduced stressed medial prefrontal function but appeared to relate in the opposite direction, suggesting a more physiologically resilient and protective state.

Our findings of relatively reduced medial prefrontal gray matter volumes in relation to urban childhoods appear consistent with work implicating this effect with increased adverse stress exposures. Indeed, larger effects of gray matter volume reductions were observed in cumulative adversity[39], chronic pain[40], depression[25] and post-traumatic stress disorder[41]. Reduced medial prefrontal gray matter was also found in childhood maltreatment[42,43], and in relation to increased trait anxiety in adulthood, and sensitivity to life stress[26]. We note, however, that none of our subjects suffered from a psychiatric illness or have evidence of childhood maltreatment on the clinical interviews. In comparison with our results at mPFC, urban childhoods in Mannheim, Germany, were associated with relatively reduced lateral prefrontal gray matter volumes[16]. It may be argued that lateral and medial prefrontal changes are part of a similar network mediating adverse experiences in urban childhoods, given that medial and lateral PFC are linked in regulating emotional experience[44,45], and in the adaptive cognitive appraisal of emotional experience[46]. It could also be that differences between our results indicate specific differences in the urbanicity experience between countries, or that our findings are due to our substantially larger sample size.

Nevertheless, further evidence suggests that urbanicity may be characterized by a more stressful social environment and greater social disparities[47], manifestations of which include more fragmentation of social and family ties[48,49]. Indeed, a recent study in China also revealed higher levels of negative life stress in urban relative to rural youth[50]. Moreover, there is evidence that enduring personality traits, particularly trait anxiety-depression, may be increased by childhood adversity, including in urban environments[51-53]. At the

extremes, childhood abuse and genetic risk predisposes to depressive illness[54-57]. Trait anxiety-depression also modulates how one cognitively processes interpersonal stress, and higher trait anxiety correlates with lower levels of problem-focused coping and poorer clinical outcomes[58]. Specifically, it has been suggested that trait anxiety-depression contributes to states of stress through tendencies to mis-appraise events as highly threatening and coping resources as low[59-62]. These cognitive re-appraisal processes strongly engage executive function and lateral and medial prefrontal cortex[44]. There is also converging evidence that prefrontal executive cognition is particularly sensitive to stress, and this is likely to at least in part involve dopaminergic processes[22]. Indeed, maladaptive self-referential ruminations associated with trait anxiety-depression[63] is related to mPFC function, which under cognitive task demands need to be suppressed[23,37,38,64,65]. These prior studies suggest that if indeed urbanicity affects cognitive function under stress, and relates to medial prefrontal structure and function, it would impact an interaction between enduring traits related to the processing of stress, and executive function such as WM manipulation under stress. These are consistent with our findings, where urban childhoods potentiate the effect of even normal variation in trait anxiety-depression to affect a greater reduction in medial PFC function under stress during, specifically, more complex WM manipulation, rather than WM maintenance.

On the other hand, we suggest the rural childhood environment may moderate the effects of interpersonal stress on executive function and trait anxiety-depression through the medial PFC. Consistent with this, a recent study suggested that experience of nature relative to urban traffic moderated negative ruminations and medial PFC function in healthy young adults[66]. If indeed part of the rural vs urban upbringing engages some of these processes, we might predict that anxiety or stress-related behavioral patterns and personality traits would be somewhat less in individuals with rural upbringing, as we have found. It is also conceivable that rural environments influence stress-related medial PFC function as being less reactive to anxiety-related traits[67,68].

Indeed, we found that individuals with more rural childhoods who scored higher on trait anxiety appeared to engage the medial PFC in a pattern of response opposite to those with more urban childhoods. It remains to be understood if this could suggest that the individuals with more rural childhoods would require higher levels of trait anxiety or stress before medial PFC function falls, or that they are thus are more tolerant of stress before brain function decreases. Nonetheless, all this may be likely given the inverted-U relationship that scales across many levels of stress and behavioral mechanisms, from prefrontal systems level physiology[69,70] to dopaminergic sensitivity in prefrontal neurons[22,71]. It might therefore be conceivable that rural upbringing biases the stress-response curve, at least in relation to medial PFC function to the right of individuals with more urban childhoods. This would imply they require a greater amount of stress or trait anxiety to tip them over. This could be consistent with rural upbringing having some degree of physiological resilience at least at the level of medial PFC function during executive function such as WM manipulation under stress. While compelling, these conjectures would need to be more directly tested in a larger range of stress and anxiety traits than in our study.

There are also potential advantages of urban childhoods that we have yet to fully develop here, such as the higher total gray matter volumes, parental education, and faster reaction time on cognitive tasks. Though in our data they appear unrelated to trait anxiety or medial PFC function, they may relate to other early childhood socioeconomic and educational differences[72,73] for future study. Nevertheless, it appears that there is at least one factor that has allowed our sample of individuals with rural childhoods to do comparably well living in Beijing as adults as the more urban group. This appears to relate to the advantageous structural and functional effects at the medial PFC that we describe as potentially associated with some degree of resilience to stress and trait anxiety. We also show that these effects are unlikely to be due to genetic differences per se across urban-rural exposures. Future work would be needed to identify more specific mechanisms related to these

findings at an individual level, in particular potential genetic variation predisposing risk or resilience in an interaction with childhood environmental factors.

Methods

Participants

This study was approved by the Institutional Review Boards of the Peking University Institute of Mental Health and the Johns Hopkins University School of Medicine. Five hundred and twenty-two healthy subjects were recruited from the local community in Beijing, and written informed consent was obtained from each subject. We recruited subjects by advertising the study using social media and posters in the community. All subjects had to be currently living in Beijing for at least one year, and had to have at least finished the national nine-year education program, suggesting their IQ is likely within the normal range. All participants were assessed by psychiatrists using the Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Non-patient Edition (SCID-I/NP) to exclude presence of a psychiatric disorder. Further, subjects met the following inclusion criteria: 18 to 45 years old; right handed; Chinese of Han ancestry; no history of psychiatric or neurological diseases and substance abuse or dependence; no history of more than 5 minutes' loss of consciousness; and no abnormalities on subsequent MR images, confirmed by a radiologist.

To determine urbanicity, all subjects provided residence details from birth to study enrollment. We defined rural areas to refer to agricultural parts of the country with a local population typically <10,000. Urban areas had to have been cities with populations typically more than 100,000 to well over several million. In this report, we divided subjects into 2 main groups: the urban group were adult subjects who have mostly lived in cities since before age 12, while the rural group were those who only moved to cities beyond age 12. We have also analyzed our data, with similar structural and functional results, by stratifying them into 4 groups: individuals who were born in and continue to live in cities, vs those who have lived in cities since before age 12, vs those who lived in rural areas between birth and age 18, vs those lived in rural areas for >18 years since

birth. Similar results were also obtained using an urbanicity score based on previous studies[6,15]. (Supplementary Figures S2, S3, S5).

We collected MRI, genetics and questionnaire data from all subjects. Of note in this report on interpersonal stress and trait anxiety-depression on brain, we used a validated Chinese translation of the Eysenck Personality Questionnaire (EPQ) to study trait neuroticism[74,75].

MRI Data Acquisition:

All subjects were scanned on a 3.0 T GE Discovery MR750 scanner in the Center for MRI Research, Peking University. T1-weighted high resolution structural image was acquired in a sagittal orientation using an axial 3D fast, spoiled gradient recalled (FSPGR) sequence with the following parameters: time repetition(TR) = 6.66 ms, time echo (TE) = 2.93 ms, field of view (FOV) = 256 × 256 mm^2, slice thickness/gap = 1.0/0 mm, acquisition voxel size=1 × 1 × 1mm^3, flip angle = 12°, 192 contiguous sagittal slices.

As for functional MRI, each echo-plannar image consisted of 33 (4.2 mm thick, 1 mm gap) axial slices covering the entire cerebrum and cerebellum (TR/TE = 2000/30 ms, flip angle = 90°, field of view = 24 cm, 64 x 64 matrix). Scanning parameters were selected to optimize the stability and quality of the BOLD signal with the exclusion of the first 4 images as dummy scans.

Structural MRI Data Analysis

Structural images were processed using SPM8 (http://www.fil.ion.ucl.ac.uk/spm), DPABI[76], and DARTEL[77]

to perform VBM analysis. DARTEL is believed to have improved segmentation results[78]. Image processing included: (1) Transforming structural images into NIFTI format. (2) Reorienting structural images iteratively so that the millimeter coordinates of the anterior commissure (AC) matched the origin. (3) T1-weighted MR images were segmented into grey matter, white matter, cerebrospinal fluid using "New Segment" in SPM8. (4) DARTEL was used to compute transformations from individual native space to Montreal Neurological Institute (MNI) space for registration, normalization, and modulation. (5) The segmented, normalized and modulated GM images were then smoothed with an 8-mm full width at half maximum isotropic Gaussian kernel.

To study the effect of relative urban-rural childhoods on brain structure, an absolute threshold of 0.2 was used to remove voxels of low intensity from the analysis and to prevent possible edge effects[79]. In the rural vs urban contrasts, we controlled for age, second polynomial of age[36], gender, education years and total gray matter volume. Significant effects were those that survived a $p<0.05$ whole brain family-wise error (FWE) correction. BrainNet Viewer[80] was used to visualize our findings. While we cannot exclude the potential effects of head motion on measures of brain structure[81], we have attempted to address this by confirming that there were no significant group differences in the six head motion dimensions from the functional MRI acquisition performed in the same MRI session.

Functional MRI Task and Analysis

We adapted an event-related “number working memory task” based on previous work[34,35], but with induced interpersonal stress states occurring in half the trials in an event-related block design (Figure 1). Subjects were trained outside the scanner for about 10 minutes. For working memory (WM), subjects encoded 2 integer numbers presented over 1s and retained in WM across a jittered interval of 3-5 seconds; in maintenance trials,

subjects then responded to which of the two numbers was "larger" or "smaller" within 2s; in the manipulation trials, subjects had to perform a mental subtraction on one of the two numbers before the "larger" or "smaller" evaluation within 2s. There were 28 trials of WM manipulation and 28 trials of WM maintenance, half of which included competition and relatively more stress. Trials were embedded within equal numbers of competition or no-competition blocks, each comprising one WM maintenance and one WM manipulation trial, counterbalanced within 2 MRI runs, each about 10 minutes. Each block of competitive or non-competitive events was preceded by an initial instructional cue, whereby for competition, the participants were led to believe they were playing against a "competitor" of the same gender and of similar age, and after each WM trial, were given win or loss feedback. Here, subjects were given negative (loss) feedback approximately 70% of the time.

In the functional MRI data analysis, we further excluded subjects with accuracy rate not higher than 50 percent on WM maintenance or manipulation (n = 37); those with head motion greater than 2 mm translation or 2 degrees rotation (n = 43); and those with image artifacts or did not complete the task (n = 16). Functional imaging analysis was performed using SPM12 (http://www.fil.ion.ucl.ac.uk/spm) and Matlab 2016b. Functional images for each subject was slice timing corrected, realigned to the first volume in the time series, and corrected for head motion. Images were then spatially normalized into standard stereotaxic space (Montreal Neurological Institute template) using a fourth degree B-spline interpolation. Spatial smoothing was applied with a Gaussian filter set at 8mm full-width at half-maximum. Each task-evoked stimulus was modeled as a separate delta function and convolved with a canonical hemodynamic response function, ratio normalized to the whole-brain global mean to control for systematic differences in global activity, and temporally filtered using a high-pass filter of 128s. Each task-evoked stimulus events were modeled for correctly performed trials. Incorrect responses and residual movement parameters were also modeled as

regressors of no interest. In our study, planned contrasts of interest were brain activity at the maintenance or manipulation task phases under less stress, stress and less stress vs stress. These contrasts were subsequently taken to a second-level urban vs rural group analysis in which inter-subject variability was treated as a random effect, with control for age and reaction time (RT) as regressors because these variables differed across group.

In order to explore the stress-related function of medial prefrontal cortex[82,83] implicated in the structural analyses (see Results), we created functional region of interests (ROI) around two 30mm diameter peaks within the left and right medial prefrontal cortex differentially implicated in the less interpersonal stress vs interpersonal stress contrast during the WM manipulation or maintenance task in the entire sample at $p<0.05$ whole brain FWE correction. Given that the trait anxiety and depression is implicated in the expression of states of interpersonal stress at medial prefrontal cortex[26,84] and that this may be modulated by childhood urbanicity[15], we then specifically tested for the interaction effects of trait anxiety-depression and urbanicity within this orthogonal mPFC functional ROI. Within this functional ROI (1264 voxels), we considered significant effects as surviving $p<0.001$ uncorrected and $p<0.05$ FWE corrected within the smaller functional ROI search volume. We also performed identical analyses for the WM maintenance task.

DNA Collection and Genotyping

Genomic DNA samples were extracted from peripheral blood using the QIAamp DNA Mini Kit (QIAGEN). Genotyping of samples was conducted using Illumina Human OmniZhongHua BeadChips, designed for the Chinese population. Normalized bead intensity data obtained for each sample were loaded into Illumina BeadStudio software, which converted fluorescence intensities into SNP genotypes. Samples were excluded according to the following criteria: (1) genotype call rate of <95%, (2) gender discordance, (3) first- or second-degree relatedness, or (4) the genetic outliers. SNPs were excluded using the following criteria: (1)

minor allele frequency (MAF) <0.01, (2) genotype call rate of <95%, (3) P values for Hardy-Weinberg equilibrium < 1e-5. Principal Component Analysis (PCA) was performed to identify genetic outliers and determine whether population stratification existed between our urban and rural samples, using EIGENSTRAT (http://genetics.med.harvard.edu/reich/Reich_Lab/Software.html). We compared the first 20 PCAs among the two groups using a two-sample t-test with statistical significance set at $p<0.05$ corrected for the number of independent components tested.

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Author Contributions

HYT, WY, H Yan, DRW and DZ conceptualized and designed the study; X Zhang, X Zhao, ZD, Xiaoxi Zhang, JL, SJ, JL, YZ, WY, H Yan, HYT and DZ performed or supervised the data acquisition; X Zhang, H Yu, SS, GY, QC, TM, H Yan, WY, DRW, DZ and HYT analyzed the data; X Zhang, H Yan, WY, DZ, DRW and HYT wrote and edited the manuscript.

Competing Financial Interests Statement

All authors declare that they have no conflicts of interest.

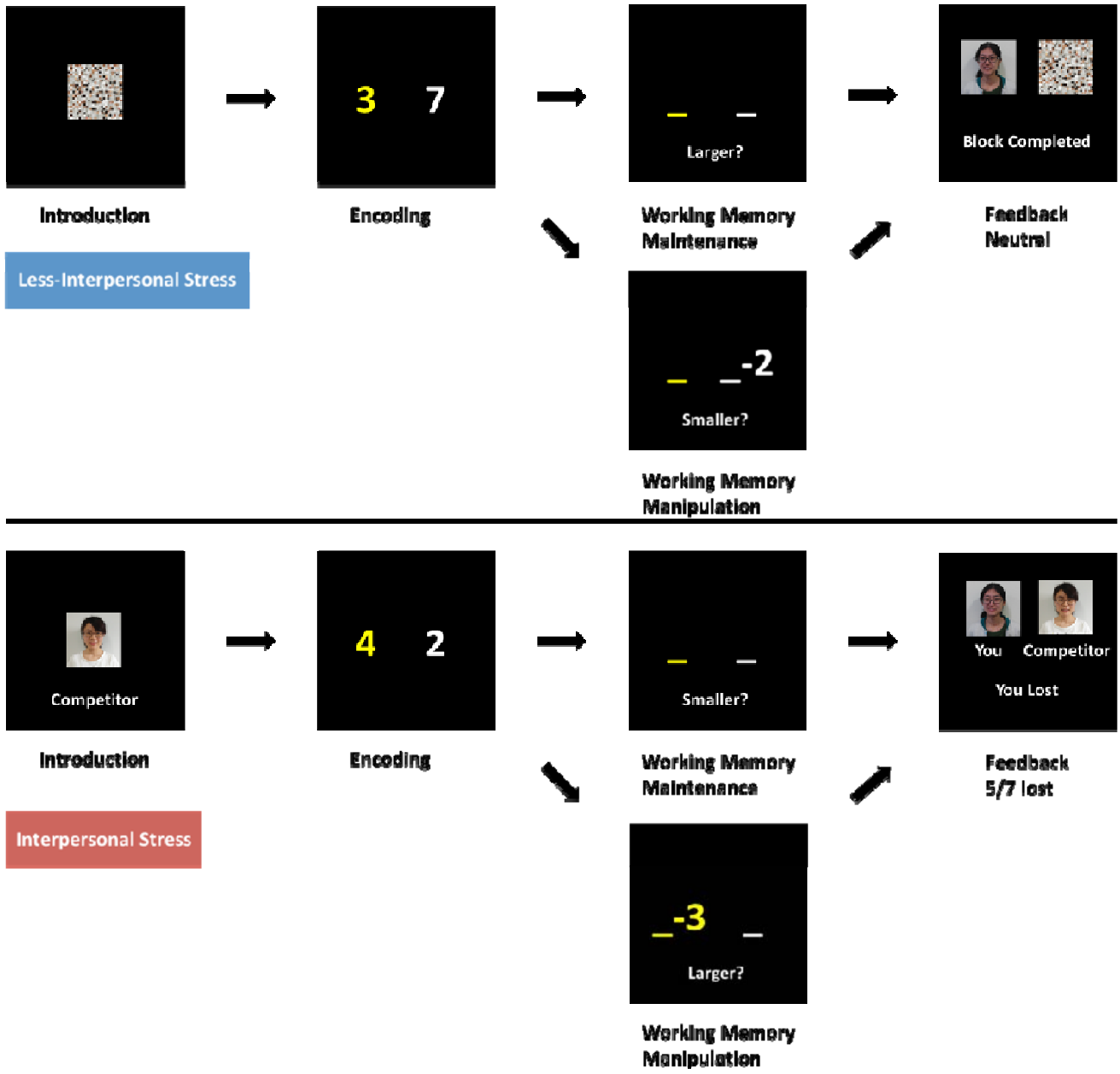

Figure 1 Working memory paradigm. Schematic of the working memory paradigm incorporating interpersonal stress vs less stress. In the stress component, subjects were led to believe they were playing against a "competitor" of the similar age and gender, and were judged win or lose based on their speed and accuracy, which subsequently resulted in ~70% loss feedback. In the less stressed blocks, there was no competitor and subjects would receive a neutral feedback. In the working memory task per se, an array of two number digits was encoded and held in working memory over 3-4s. In working memory manipulation, subjects were to perform subtraction arithmetic on one of the numbers held in working memory, followed by

answering which was larger or smaller as instructed. In working memory maintenance, they answered which of the two maintained in working memory was larger or smaller as instructed. We performed 2 runs counterbalanced for trial and stimuli presentation order over ~20 mins. All instructions were showed in Chinese.

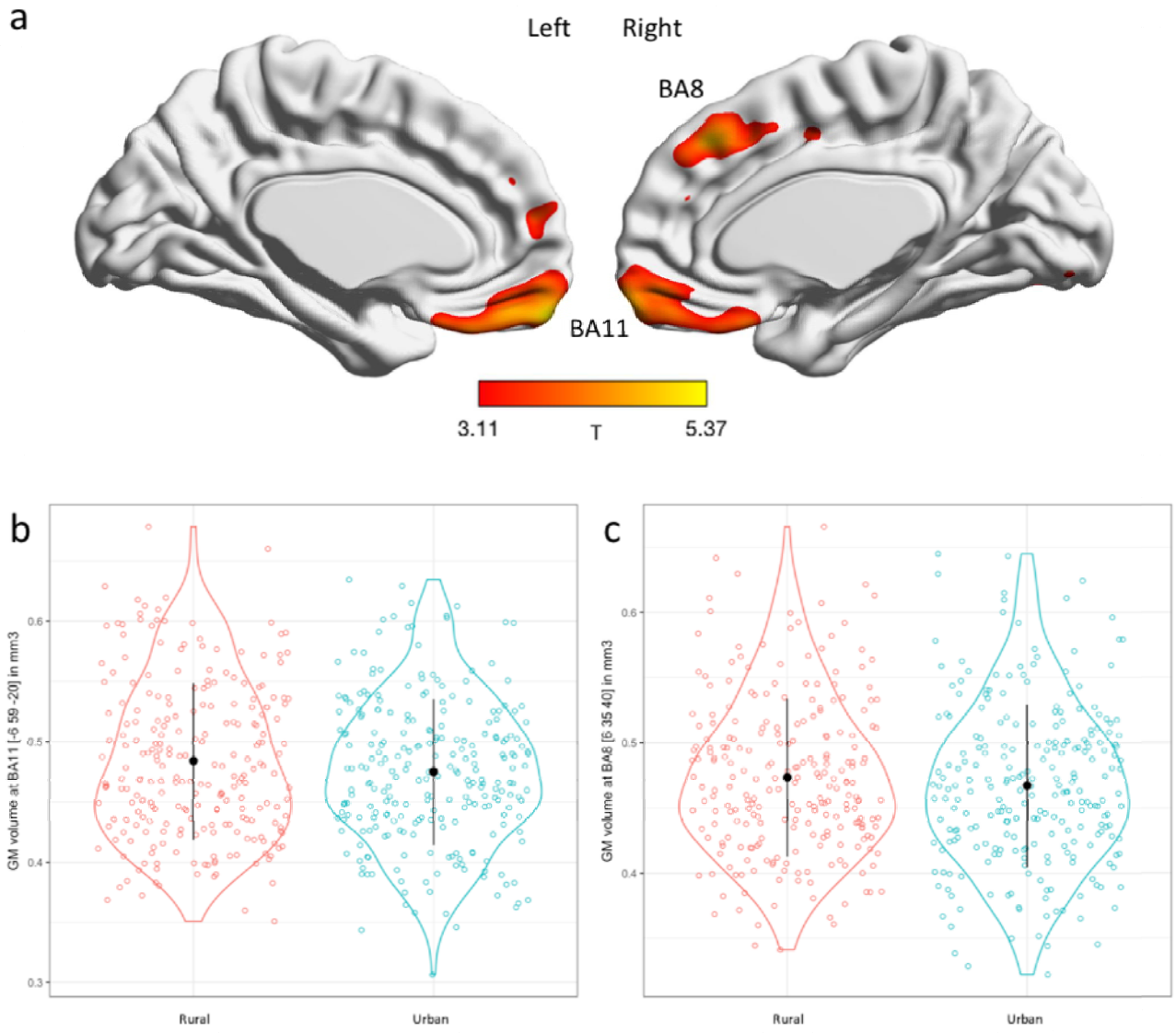

Figure 2 Early-life urbanicity effect on grey matter volume (a) T-map of the rural > urban effects controlled for age, second polynomial of age, gender, education, total grey matter volume as covariate (shown at p<0.001 uncorrected but peaks survive p<0.05 whole brain FWE corrected). (b) Scatterplot of rural > urban findings at Brodmann area 11 in medial prefrontal cortex (peak atlas = [-6 59 -20], t = 5.37, cluster size = 332, p<0.05, FWE corrected). (c) Scatterplot of rural > urban findings at Brodmann area 8 in mPFC (peak atlas = [6 35 40], t = 4.95, cluster size = 40, p<0.05, FWE corrected).

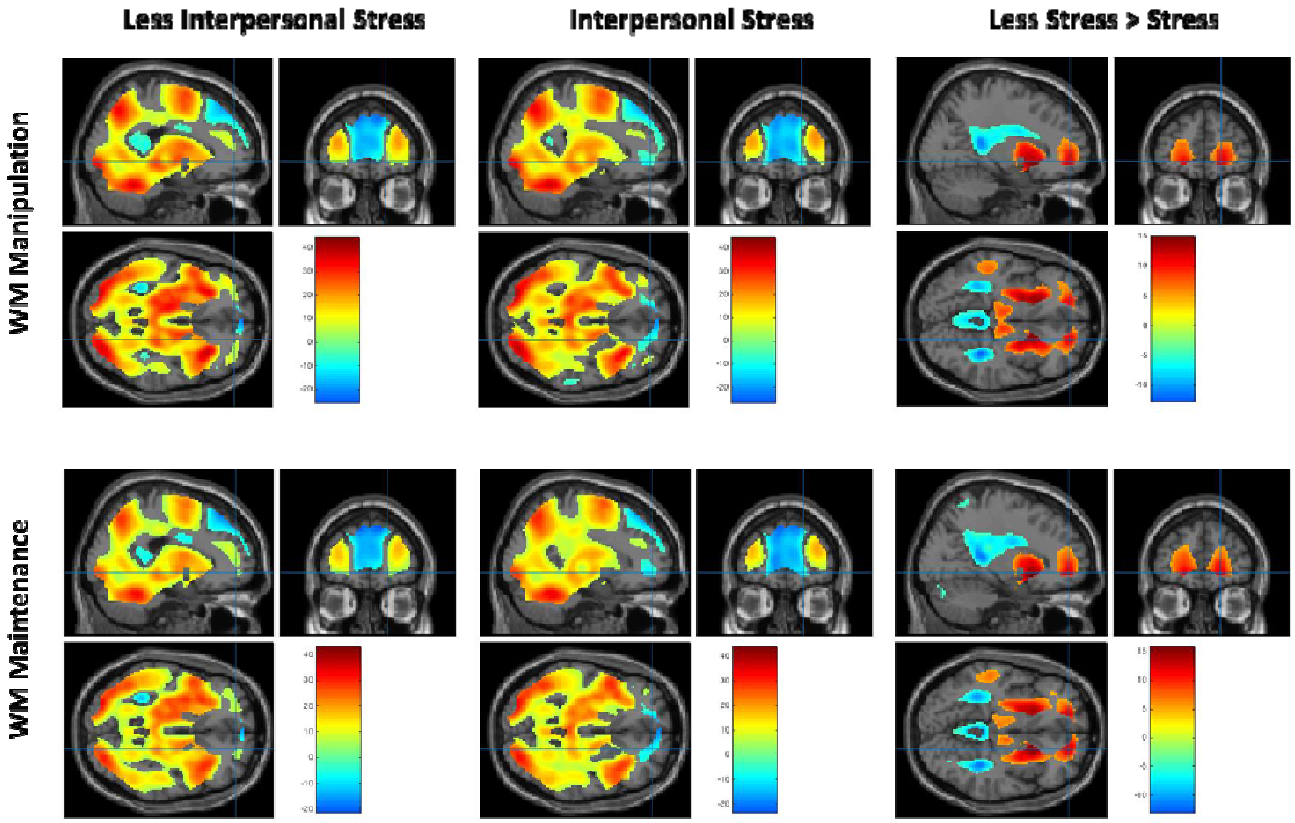

Figure 3 Brain activation under different working memory and interpersonal stress conditions. One-sample t-tests of working memory (WM) task across the entire sample (N=394, $p < 0.05$, whole brain FWE corrected, cluster size > 100) under differing stress and working memory manipulation and maintenance conditions. Medial prefrontal cortex, amongst other brain regions, was less engaged under stress.

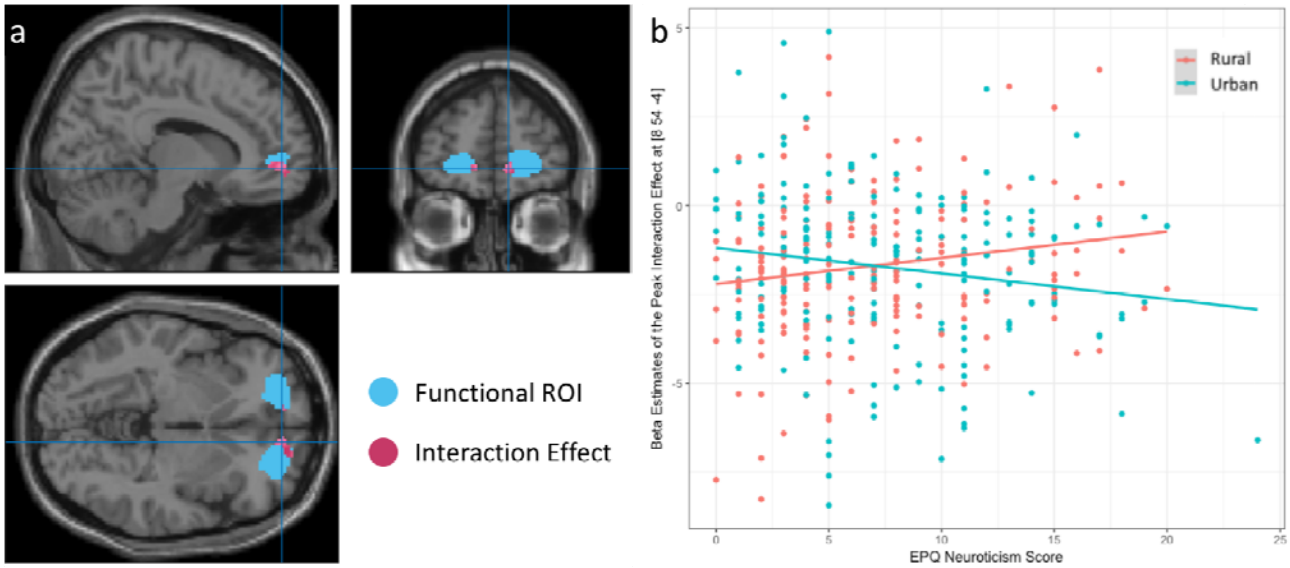

Figure 4 Interaction effects of trait anxiety-depression and urbanicity on medial prefrontal cortex engagement under interpersonal stress during working memory manipulation. **(a)** Interaction effects of trait anxiety-depression and urbanicity on medial prefrontal cortex engagement under interpersonal stress working memory manipulation task (x=8, y=54, z=-4, T=3.37; $p<0.05$ FWE corrected within the functional ROI, the interaction effect was shown with uncorrected $p < 0.005$ for visualization). **(b)** Scatter plot showing the interaction between rural or urban upbringings and trait anxiety-depression score. In the group with more urban childhoods, increased trait anxiety-depression appeared to result in a larger reduction in medial prefrontal cortex engagement during stress ($p = 0.025$; $r = -0.159$). On the other hand, subjects with more rural childhoods had relatively less reduction with increased trait anxiety-depression ($p = 0.019$; $r = 0.168$).

Tables

Table 1: Demographic Characteristics of Subjects in the Structural MRI Study (N=490)

	Urban (sd)	Rural (sd)	F/χ^2	p
Gender (no. female/male)	131/118	119/122	0.512	0.474
Age (yrs)	23.89 (3.98)	25.03 (3.45)	3.389	0.001
Education (yrs)	16.63 (2.32)	16.98 (2.62)	1.579	0.115
Height (cm)	169.27 (8.13)	167.65 (7.61)	2.265	0.024
Weight (kg)	62.42 (13.04)	61.35 (11.49)	0.961	0.337
Father's Childbearing Age (yrs)	28.45 (4.74)	27.02 (6.00)	2.909	0.004
Father's Education (yrs)	12.94 (3.57)	9.59 (3.29)	10.805	<0.001
Mother's Childbearing Age	26.63 (3.83)	25.92 (5.08)	1.746	0.081
Mother's Education (yrs)	12.42 (3.55)	7.88 (3.88)	13.515	<0.001
Current Occupation (no.)			3.724	0.590
Managers in public or private companies: Urban (5), Rural (5);				
Professionals and Technicians: Urban (46), Rural (58);				
Clerks: Urban (12), Rural (10);				
Service Personnel: Urban (13), Rural (10);				
Students: Urban (164), Rural (145);				
Others: Urban (9), Rural (13);				
Total GMV (in cm^3)	752.13 (61.89)	739.12 (61.58)	2.334	0.020

Abbreviations: GMV, Grey Matter Volume.

Table 2: Demographic Characteristics of Subjects in the Functional MRI Study (N=394)

	Urban (sd)	Rural (sd)	F/χ^2	p
Gender (female/male no.)	109/90	99/96	0.634	0.426
Age (yrs)	23.69 (3.65)	24.96 (3.13)	3.707	<0.00
Education (yrs)	16.63 (2.32)	17.20 (2.53)	2.337	0.020
EPQ Neuroticism	7.60 (4.88)	6.65 (4.66)	1.968	0.050
Stress				
Accuracy in WM manipulation	0.86 (0.10)	0.86 (0.10)	0.471	0.638
RT in WM manipulation (s)	1.43 (0.34)	1.54 (0.36)	2.901	0.004
Accuracy in WM maintenance	0.92 (0.08)	0.92 (0.10)	0.903	0.367
RT in WM maintenance (s)	1.04 (0.27)	1.11 (0.27)	2.660	0.008
Less Stress				
Accuracy in WM manipulation	0.85 (0.10)	0.86 (0.10)	0.650	0.516
RT in WM manipulation (s)	1.48 (0.34)	1.58 (0.36)	2.789	0.006
Accuracy in WM maintenance	0.86 (0.08)	0.86 (0.09)	0.442	0.659
RT in WM maintenance (s)	1.10 (0.31)	1.19 (0.31)	3.010	0.003

Abbreviations: EPQ, Eysenck Personality Questionnaire; RT, Reaction Time; WM, Working Memory.