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The Association between Lifelong Greenspace Exposure and 3-Dimensional Brain Magnetic Resonance Imaging in Barcelona Schoolchildren

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BACKGROUND: Proponents of the biophilia hypothesis believe that contact with nature, including green spaces, has a crucial role in brain development in children. Currently, however, we are not aware of evidence linking such exposure with potential effects on brain structure.

OBJECTIVE: We determined whether lifelong exposure to residential surrounding greenness is associated with regional differences in brain volume based on 3-dimensional magnetic resonance imaging (3D MRI) among children attending primary school.

METHODS: We performed a series of analyses using data from a subcohort of 253 Barcelona schoolchildren from the Brain Development and Air Pollution Ultrafine Particles in School Children (BREATHE) project. We averaged satellite-based normalized difference vegetation index (NDVI) across 100-m buffers around all residential addresses since birth to estimate each participant's lifelong exposure to residential surrounding greenness, and we used high-resolution 3D MRIs of brain anatomy to identify regional differences in voxel-wise brain volume associated with greenness exposure. In addition, we performed a supporting substudy to identify regional differences in brain volume associated with measures of working memory (d' from computerized n-back tests) and inattentiveness (hit reaction time standard error from the Attentional Network Task instrument) that were repeated four times over one year. We also performed a second supporting substudy to determine whether peak voxel tissue volumes in brain regions associated with residential greenness predicted cognitive function test scores.

RESULTS: Lifelong exposure to greenness was positively associated with gray matter volume in the left and right prefrontal cortex and in the left premotor cortex and with white matter volume in the right prefrontal region, in the left premotor region, and in both cerebellar hemispheres. Some of these regions partly overlapped with regions associated with cognitive test scores (prefrontal cortex and cerebellar and premotor white matter), and peak volumes in these regions predicted better working memory and reduced inattentiveness.

CONCLUSION: Our findings from a study population of urban schoolchildren in Barcelona require confirmation, but they suggest that being raised in greener neighborhoods may have beneficial effects on brain development and cognitive function. https://doi.org/10.1289/EHP1876

Introduction

Currently, approximately half of the world's population lives in cities, and it is predicted that by 2050, nearly 66% of people will live in urban areas worldwide (UN Department of Economic and Social Affairs 2015). Urban areas are characterized by a network of nonnatural built-up infrastructures where residents often have limited access to natural environments (Escobedo et al. 2011). Proponents of the biophilia hypothesis believe that contact with nature may have a defining role in human brain development (Kahn and Kellert 2002; Kellert 2005).

In a recent longitudinal study of >2,200 Barcelona schoolchildren (7–9 y old) (Dadvand et al. 2015a), we found that over a 12-month period, children who attended schools with higher outdoor greenness had a greater increase in working memory and a greater reduction in inattentiveness than children who attended schools with less surrounding greenness. The brain develops steadily during prenatal and early postnatal periods, which are considered the most vulnerable windows for effects of environmental exposures (Grandjean and Landrigan 2014). In this context, exposure to greenness early in life could be associated with beneficial structural changes in the developing brain. Accordingly, the overarching aim of this study was to determine whether lifelong exposure to residential surrounding greenness is associated with regional differences in brain volume based on 3-dimensional magnetic resonance imaging (3D MRI) among children attending primary school (the principal substudy). Toward this aim, we sought to confirm the beneficial nature of these differences by investigating the overlap between them and brain regions associated with cognitive function (supporting substudy I) and by studying the association between peak tissue volumes in these regions and objective measures of cognitive function (supporting substudy II).

Materials and Methods

We estimated lifelong exposure to residential surrounding greenness using satellite-based normalized difference vegetation index (NDVI) for all residential addresses of each participant since birth. High-resolution 3D MRIs of brain anatomy used to measure voxel-wise brain volume in the present study population were first obtained for studies of air pollution exposures and neurodevelopmental outcomes (Pujol et al. 2016a, 2016b). Measures of working memory and inattentiveness from a previous study of the entire Brain Development and Air Pollution Ultrafine Particles in School Children (BREATHE) cohort were based on computerized n-back and attentional network task (ANT) tests, respectively,

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that were repeated four times over a 12-month period (Dadvand et al. 2015a).

Study Participants

This study was developed in the context of the BREATHE project (Dadvand et al. 2015a). The general project design has been fully described elsewhere (Dadvand et al. 2015a). Briefly, all schoolchildren ($n = 5{,}019$) without special needs in the second to fourth grades (7–10 y old) of 39 representative schools were invited to participate by letters or presentations in schools for parents, of whom 2,897 (59%) agreed to take part in BREATHE. All children had been in the school for >6 months (and 98% >1 y) before the beginning of the study. Participating schools were similar to the remaining schools in Barcelona in terms of the neighborhood socioeconomic vulnerability index (0.46 versus 0.50, Student's t-test $p = 0.57$).

Of 2,897 original BREATHE families, 1,564 (54%) agreed to receive information about the MRI study via post, email, or telephone, and 263 completed the imaging protocol in 2013. Ten children were excluded on the basis of image quality criteria (Pujol et al. 2016b); thus, 253 participants were included in the analyses. All parents or legal guardians signed the informed consent form approved by the Research Ethical Committee of the Hospital del Mar Medical Research Institute (IMIM)-Parc de Salut Mar, Barcelona, and the FP7-ERC-2010-AdG Ethics Review Committee.

Exposure to Greenness

To assess outdoor surrounding greenness, we applied NDVI derived from RapidEye data at 5 m × 5 m resolution. NDVI is an indicator of greenness and is calculated as follows:

$$NDVI = \frac{(NIR - R)}{(NIR + R)},$$

where NIR and R are the land surface reflectance of the near-infrared and red (visible) parts of the spectrum, respectively. NDVI ranges between -1 and 1 with low values (e.g., ≤ 0.1) indicating water bodies, snow, and barren areas of rock and sand; and higher values indicating photosynthetically active vegetation (USGS 2015). RapidEye images were acquired from a constellation of five satellites 630 km above ground in sun-synchronous orbits. We generated our NDVI map using an image obtained on 23 July 2012. We used this map to estimate greenness surrounding all residential addresses for each participant from birth until the time of MRI evaluation, reported by parents at baseline enrollment into BREATHE. Given the highly developed built environment in Barcelona and the relatively short period of follow-up, the greenness in most areas would have been unlikely to change substantially during the study period. Lifelong residential surrounding greenness was abstracted as the average of NDVI in a buffer of 100 m (Dadvand et al. 2012, 2015a) around all the home addresses of each study participant since birth, weighted by the time (years) the participant stayed at each address.

Neuroimaging

A 1.5-Tesla Signa Excite system (General Electric) equipped with an eight-channel phased-array head coil was used for neuroimaging. High-resolution 3D anatomical images were obtained using an axial T1-weighted 3D fast spoiled gradient inversion recovery-prepared sequence (Pujol et al. 2016a, 2016b). A total of 134 contiguous slices were acquired with an inversion time of 400 ms, repetition time of 11.9 ms, echo time of 4.2 ms, flip angle of 15°, field of view of 30 cm, 256×256 pixel matrix, and slice thickness of 1.2 mm.

All of the anatomical images were visually inspected before analysis by a trained operator to detect any motion effect. Gray and white matter tissue volume [i.e., the volume proportion of gray matter to white matter and cerebrospinal fluid (CSF) and the volume proportion of white matter to gray matter and CSF] at a voxel level was measured using statistical parametric mapping (SPM8; FIL Methods Group 2013). SPM voxel-based morphometry (VBM) algorithms with DARTEL registration (FIL Methods Group 2013) were used to carry out the following processing steps (Pujol et al. 2016b): segmentation of anatomical images into gray and white matter tissue probability maps in their native space; normalization of images (warping) to a common group template and, later, from the common template to Montreal Neurological Institute (MNI) space by iteratively registering the individual segmented images with their average; scaling the MNI-normalized tissue probability maps by the Jacobian determinants estimated during the normalization procedure; and finally, reslicing images to 1.5 mm resolution and smoothing the resulting images with a $10 \times 10 \times 10$ full width at half maximum (FWHM) Gaussian filter before entering into the study analyses. The scaling of tissue probability maps by Jacobian determinants, a procedure known as modulation, ensures that the total volume of the tissue is preserved even though the normalization to a common template implies the stretching and shrinking of structures. Modulated VBM can be interpreted as gray/white matter local volume, affected by differences in both tissue concentration and volume of local structures. Analyses for both gray and white matter were conducted within the same standard MNI space whole-brain mask.

Cognitive Function

Details on the BREATHE cognitive assessment methodology have been published elsewhere (Dadvand et al. 2015a). Briefly, cognitive function was assessed through repeated evaluations of working memory and attention. We selected these functions because they grow steadily during preadolescence (Rueda et al. 2005; Jaeggi et al. 2010). The n-back test and Attentional Network Task (ANT) have been frequently used to assess working memory (Jaeggi et al. 2010; Shelton et al. 2010) and inattentiveness (Rueda et al. 2004), respectively. The 2-back predicts general mental abilities (hereafter referred to as working memory), and the 3-back predicts superior functions such as fluid intelligence (hereafter referred to as superior working memory) (Forns et al. 2014). We have previously shown that for the BREATHE participants, the n-back and ANT indicators have statistical relationships with age, school performance, attention deficit hyperactivity disorder (ADHD) clinical criteria, behavioral problems, and maternal education (Forns et al. 2014).

From January 2012 to March 2013, children were evaluated every three months, using computerized n-back tests (with number stimuli) and ANTs, over four repeated visits in sessions lasting approximately 40 min. Having repeated measures is expected to improve the precision of the characterization of cognitive function. Groups of 10–20 children wearing ear protectors were assessed together and supervised by one trained examiner per 3–4 children (Dadvand et al. 2015a). The n-back parameter analyzed was d prime (d'), a measure of detection that subtracts the normalized false alarm rate from the hit rate ($Z\,\text{hit rate} - Z\,\text{false alarm rate}) \times 100$). A higher d' value indicates more accurate test performance. In line with previous BREATHE studies [e.g., (Dadvand et al. 2015a)], from among the ANT measures, we chose hit reaction time standard error (HRT-SE), a measure of response speed consistency throughout the test (Conners and Multi-Health Systems Staff 2000; Dadvand et al. 2015a). A higher HRT-SE value indicates highly variable reactions related to inattentiveness.

Statistical Analysis

Principal substudy. After individual preprocessing of each 3D anatomical image (Pujol et al. 2016a, 2016b), analyses were carried out to map, voxel-wise, the association between brain tissue measures and greenness exposure by conducting a separate regression analysis for each voxel in the brain using statistical parametric maps (SPM8; FIL Methods Group 2013). The statistical map, in effect, shows clusters as accumulations of individual voxels that were statistically significantly associated with the predictor of interest. Within SPM8, voxel-wise correlation analyses were performed with the lifelong greenness variable as the predictor without adjustment for any covariates. The resulting statistical maps require a correction for multiple comparisons because the statistical test for every voxel is strongly dependent on the tests of the neighboring voxels. We addressed this issue by performing 2,000 Monte Carlo simulations using AlphaSim as implemented in the SPM REST toolbox (Song et al. 2011). Input parameters to AlphaSim included an individual voxel threshold probability of 0.01, a cluster connection radius of 5 mm, 10 mm FWHM smoothness, and a comprehensive gray matter mask volume of 301,780 voxels (1.02 L). Results were considered significant with clusters of 2.2 mL (650 voxels) at a height threshold of $p < 0.01$, which satisfied the family wise error (FWE) rate correction of $p_{\text{FWE}} < 0.05$.

Supporting substudy I. For the first supporting substudy, we reanalyzed the MRI data using working memory (2-back d'), superior working memory (3-back d'), and inattentiveness (ANT HRT-SE) as predictors to identify brain regions with volumes that were significantly correlated with each predictor.

Supporting substudy II. For the second supporting substudy, we performed a series of analyses with the three cognitive measures (2-back d', 3-back d', and HRT-SE) modeled as the outcome (dependent) variables and each brain region (cluster) that was significantly correlated with greenness as the predictor (one cognitive measure and one brain region per model). We used linear mixed effects models with child and school as random effects to account for the four repeated outcome measures within each child and the nonindependence of children within each school (Dadvand et al. 2015a). Each brain region (significant cluster) was modeled using the measured tissue volume of the voxel with the strongest association with greenness exposure (peak value) within the cluster as the independent variable.

Sensitivity Analyses

We repeated the principal analysis to identify brain regions associated with lifelong residential greenness using a 500-m versus a 100-m buffer to derive the exposure variable. We also evaluated, separately, the influences of maternal education [an indicator of individual socioeconomic status (SES)] and Urban Vulnerability Index (an indicator of neighborhood SES at the census-tract level [median area of 0.08 km^2 for the study area)] on the findings of our principal substudy. Urban Vulnerability Index is based on 21 indicators of urban vulnerability grouped into four themes: sociodemographic vulnerability (five indicators), socioeconomic vulnerability (six indicators), housing vulnerability (five indicators), and subjective perception of vulnerability (five indicators) (Spanish Ministry of Public Works 2012). For this study, we used the Urban Vulnerability index based on the 2001 Spanish Census (Spanish Ministry of Public Works 2012). Moreover, we explored the impacts of sex, age, and maternal education on our findings for supporting substudy II.

Results

Descriptive sociodemographic characteristics for the participants of the original BREATHE cohort and for those included in the present study are presented in Table 1. Participants included in the present study were similar to the original BREATHE cohort in terms of age and sex; however, they tended to have mothers with higher educational attainments, higher scores for working memory (2-back d') and superior working memory (3-back d'), and a lower score for HRT-SE (consistent with less inattentiveness). In the present study, participants' age, sex, and maternal education were not associated with their lifelong greenness exposure (see Table S1).

Principal Substudy

The principal analysis identified clusters in several brain regions with volumes that were significantly associated with lifelong exposure to greenness, including clusters mapped to gray matter in the right and left prefrontal cortex and in the left premotor cortex (Table 2 and Figure 1A) and to white matter in the right prefrontal region, in the left premotor region, and in both cerebellar hemispheres (Table 2 and Figure 1B). Adjusting for maternal education or for neighborhood SES (in separate models) reduced the size of all of the clusters, with the largest reduction after adjustment for maternal education (see Table S2). Cluster sizes for clusters in the left prefrontal cortex and for the superior white matter cluster in the right prefrontal region were no longer significant after adjustment for either variable, and the size of the left premotor region

Table 1. Description of characteristics of the study participants in the current MRI study and the original BREATHE cohort.

Characteristic	MRI study ($n = 253$)	BREATHE cohort ($n = 2{,}897$)	p-Value[a]
Age (y)	8.4 (1.3)	8.5 (1.5)	0.13
Sex (female)	49.4%	49.5%	0.97
Maternal educational attainment			0.05
None or primary school	9.6%	12.7%	
Secondary school	23.9%	28.7%	
University	66.5%	58.6%	
Residential surrounding greenness[b]	0.10 (0.06)	0.09 (0.07)	0.25
2-back (d')[c]	2.5 (1.3)	2.4 (1.3)	0.06
3-back (d')[c]	1.3 (1.0)	1.2 (0.9)	0.03
HRT-SE (ms)[c]	241.0 (112.1)	249.0 (110.9)	0.02

Note: For continuous variables, the median [interquartile range (IQR)] has been reported, and for categorical variables, the percentage of each category has been reported. BREATHE, Brain Development and Air Pollution Ultrafine Particles in School Children project; HRT-SE, hit reaction time standard error; MRI, magnetic resonance imaging.

[a] p-Value of chi-squared test for categorical variables and Mann–Whitney U test for continuous variables.

[b] Average of normalized difference vegetation index (NDVI) across a buffer of 100 m around the residential address(es) since birth, weighted by the time the participant spent in each address.

[c] Average of four repeated test values. 2-back d' and 3-back d' are indicators of working memory and superior working memory, respectively, and HRT-SE is an indicator of inattentiveness.

Table 2. Regional clusters associated with lifelong exposure to greenness.

Location	Cluster size,[a] voxels (mL)	p-Value[b]	$x\ y\ z$ Coordinates[c]	t[d]	p-Value[e]
Gray matter					
Left premotor cortex	1338 (4.5)	<0.0005	−36 2 66	3.29	0.0006
Left prefrontal cortex	1980 (6.7)	<0.0005	−38 33 27	3.03	0.001
Right prefrontal cortex					
Superior prefrontal	3233 (10.9)[f]	<0.0005	32 45 21	3.09	0.001
Inferior prefrontal (operculum)	3233 (10.9)[f]	<0.0005	47 38 0	3.46	<0.0005
White matter					
Cerebellum					
Left hemisphere	15938 (53.8)[f]	<0.0005	−29 −60 −47	3.46	<0.0005
Right hemisphere	15938 (53.8)[f]	<0.0005	30 −74 −39	3.14	0.0009
Left premotor region	981 (3.3)	0.006	−32 2 61.5	3.22	0.0007
Right prefrontal region					
Superior cluster	840 (2.8)	0.017	44 41 12	3.29	0.0006
Inferior cluster (operculum)	1373 (4.6)	<0.0005	57 27 −3	4.26	<0.0005

Note: All clusters reported correspond to cluster size p-values <0.05. Lifelong exposure to greenness was abstracted as the average of normalized difference vegetation index (NDVI) across a buffer of 100 m around the residential address(es) since birth, weighted by the time the participant spent at each address.

[a] The number of voxels each showing statistically significant association with lifelong residential surrounding greenness.

[b] Cluster size p-value that establishes the probability of the occurrence of a cluster of the specified voxel size or larger under the null hypothesis of a brain made of voxels with only spatially autocorrelated noise.

[c] $x\ y\ z$ coordinates of the voxel with maximum (peak) t-value inside the corresponding cluster provided in Montreal Neurological Institute (MNI) space.

[d] Maximum (peak) t-value within the cluster. The t-values are generated from the voxel-wise regression model.

[e] Peak p-value that establishes the probability of occurrence of the specified t-value or greater generated by the voxel-wise regression model. The p-value of each row corresponds to the maximum (peak) t-value of each cluster.

[f] Two parts of a single cluster.

white matter cluster was no longer significant after adjustment for maternal education (see Table S2). However, for all clusters and both models, associations with the peak voxel volume remained significant. When we defined residential surrounding greenness as the NDVI average in a 500-m buffer around residential addresses of the study participants (instead of the 100-m buffer used in the aforementioned analyses), the voxel-wise Pearson's correlation coefficient of the t-values of the statistical maps associated with these two sets of exposures was 0.90 for the white matter and 0.86 for the gray matter. The overlap (intersection) of clusters associated with greenness exposure across 100-m and 500-m buffers relative to the extent of the clusters associated with the 100-m buffer was 72.8% for white matter and 53.8% for gray matter.

Supporting Substudy I

With regard to gray matter, smaller ANT HRT-SE (i.e., less inattentiveness) values were associated with higher gray matter volume across a broad area of the prefrontal lobes and inferior parietal lobules bilaterally, in addition to areas in the opercula and the inferior temporo-occipital cortex (Figure 2A, red color; see also Table S3). Similarly, higher d' values in 2-back and 3-back tests (i.e., better working memory and better superior working memory, respectively) were associated with higher gray matter volumes in prefrontal, inferior parietal, and lateral temporal areas, in addition to the dorsal premotor cortex (Figure 2C and 2E, red color; see also Table S2). The gray matter clusters associated with greenness and those associated with cognitive outcomes partially overlapped at the dorsal prefrontal cortex (Figure 2A, 2C, and 2E, yellow color). This overlap was 37.4%, 22.2% and 32.2% for ANT HRT-SE, 2-back d', and 3-back d', respectively, relative to the area of the clusters associated with greenness exposure, or 6.6%, 6.3%, and 5.6%, respectively, relative to the area of the clusters associated with the corresponding cognitive test.

As shown in Figure 2, better performance on the ANT task and the n-back tests was mainly associated with greater white matter volumes in the cerebellum, the brainstem, the thalamus, part of the parietal lobe, the hippocampus, and the sensorimotor cortex extending to the premotor cortex (Figure 2B, 2D, and 2F, red color; see also Table S3). The gray matter clusters associated with greenness and those associated with cognitive outcomes partially overlapped at the cerebellar white matter and in a small portion of the premotor white matter (Figure 2B, 2D, and 2F, yellow color). This overlap was 51.2%, 17.0% and 64.2% for ANT HRT-SE, 2-back d' and 3-back d', respectively, relative to the area of the clusters associated with greenness exposure, or 1.3%, 1.2%, and 2.6%, respectively, relative to the area of the clusters associated with the corresponding cognitive test.

Supporting Substudy II

Of the gray matter clusters associated with greenness, peak volume measured in the left premotor cortex was positively associated with scores for working memory and superior working memory (2-back and 3-back d', respectively) and inversely associated with inattentiveness (HRT-SE). In addition, peak volume in the superior cluster of the right prefrontal cortex was associated with working memory; peak volume in the inferior cluster of the right prefrontal cortex was associated with superior working memory and was inversely associated with inattentiveness; and peak volume in the left prefrontal cortex was inversely associated with inattentiveness (Table 3). Of the white matter clusters associated with greenness, peak volume in both the right and left cerebellar hemispheres was inversely associated with inattentiveness; peak volume in the right cerebellar hemisphere was positively associated with working memory; and peak volume in the left cerebellar hemisphere was positively associated with superior working memory (Table 3). In addition, peak volume in the left premotor region was positively associated with working memory and superior working memory ($p = 0.06$ for the latter), and peak volume in the superior cluster of the right prefrontal region was inversely associated with inattentiveness (Table 3).

After adjustment of the analyses of supporting substudy II for age, sex, and maternal education, the associations for working memory and superior working memory remained generally consistent in terms of direction and statistical significance; however, the associations between white matter at the right cerebellar hemisphere and working memory and left cerebellar hemisphere and superior working memory lost their statistical significance (Table 4). Our observed associations for inattentiveness remained similar after this adjustment in terms of their direction; however, these associations became weaker and lost their statistical significance with the exception of the association

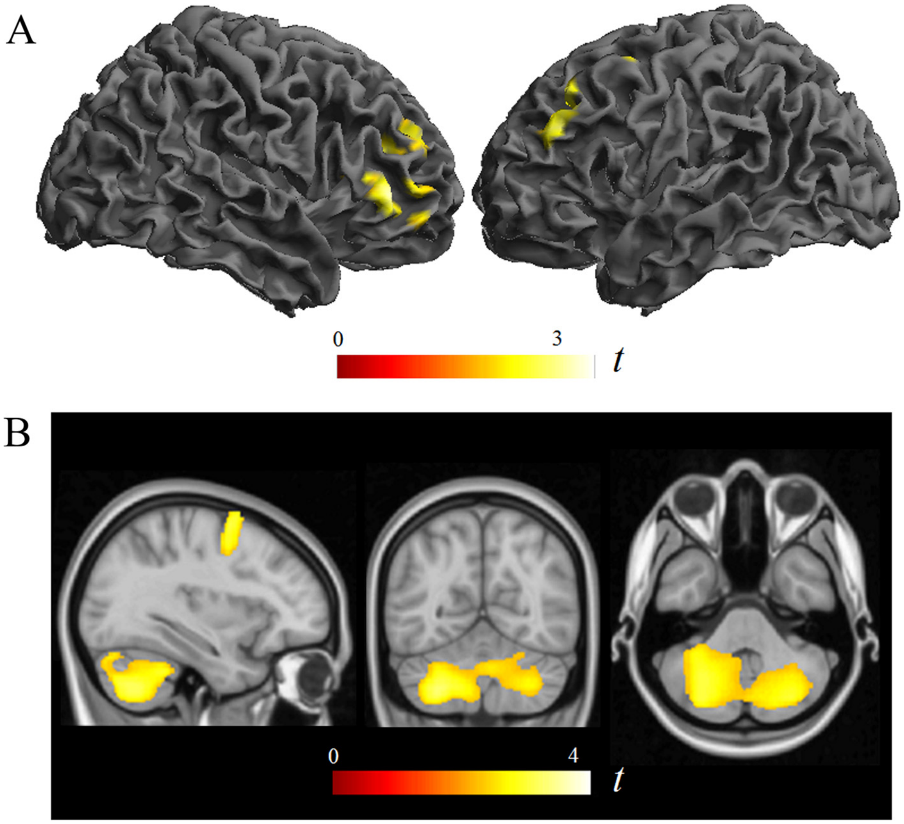

Figure 1. Regional gray and white matter volumes associated with lifelong residential surrounding greenness. Results are displayed using conventional canonical templates [Cortex_20484 surface mesh in (*A*) and MNI152_T1 template in (*B*)] in Montreal Neurological Institute (MNI) space with statistical parametric mapping (SPM8; FIL Methods Group 2013) software. Yellow and white areas indicate regional clusters with volumes positively associated with greenness (larger *t*-statistics). (*A*) Three-dimensional (3D) renderings of cortical gray matter (Cortex_20484 render) showing significant clusters in the right and left prefrontal cortex (left and right panels, respectively) and in the left premotor cortex (right panel). Results were considered significant with clusters of 2.2 mL (650 voxels) at a height threshold of $p < 0.01$, which satisfied the family-wise error (FWE) rate correction of $p_{\mathrm{FWE}} < 0.05$. (*B*) Orthogonal displays (sagittal, coronal, and axial views in the left, middle, and right panels, respectively, MNI152_T1 template) showing significant white matter clusters in the cerebellar hemispheres (all panels) and in the left premotor region (sagittal view). The right hemisphere appears on the right side of the axial and coronal views. Clusters with inverse associations between volumes and greenness would appear in cold colors (none identified.) See Table 2 for numeric data for each significant region. Residential greenness exposure was quantified based on the average Normalized Difference Vegetation Index (NDVI) within a 100-m buffer around all residences since birth, weighted by the time the participant spent at each address.

for the left prefrontal area, which remained nearly statistically significant ($p = 0.06$).

Discussion

We evaluated whether an estimate of lifelong residential surrounding greenness was associated with differences in MRI-based measures of regional brain volumes in primary schoolchildren. Greenness exposure was positively associated with gray matter volume in clusters located in the left and right prefrontal cortices and in the left premotor cortex and with white matter volume in clusters located in the right prefrontal region, in the left premotor region, and in both cerebellar hemispheres. Clusters associated with the residential greenness exposure partly overlapped with more numerous and spatially extensive clusters that were positively associated with measures of working memory and inversely associated with a measure of inattentiveness.

Interpretation of Results

As the first investigators to evaluate such an association, we did not have an *a priori* hypothesis about specific brain regions that might be affected by exposure to residential greenness. However, considerable consistency existed between the regions in white and gray matter that were identified to be associated with greenness exposure in our principal substudy. For all cortical regions found to be associated with greenness exposure (with the exception of the left prefrontal cortex), we also observed changes in their adjacent white matter region. Furthermore, clusters associated with greenness overlapped by larger clusters associated with measures of working memory and inattentiveness, and the peak volumes measured in some of the clusters associated with greenness were positively associated with working memory and inversely associated with inattentiveness, particularly before adjustment for confounding by age, sex, and maternal education. These findings are in line with the available body of evidence showing that both premotor and prefrontal areas are key elements of the dorsal attentional network and are consistently activated during working memory tasks, specifically during the *n*-back test used in our study (Owen et al. 2005). In a functional MRI (fMRI) study of a sample of nine adult males, the *n*-back test was reported to activate the cerebellum (Stoodley et al. 2012), where we found an increase in white matter volume associated with greenness exposure as well as increases in 2-back and 3-back d'. A recent review noted that evidence from MRI studies suggests that children and adults with ADHD have lower prefrontal and premotor cortex and cerebellum volumes than those without ADHD

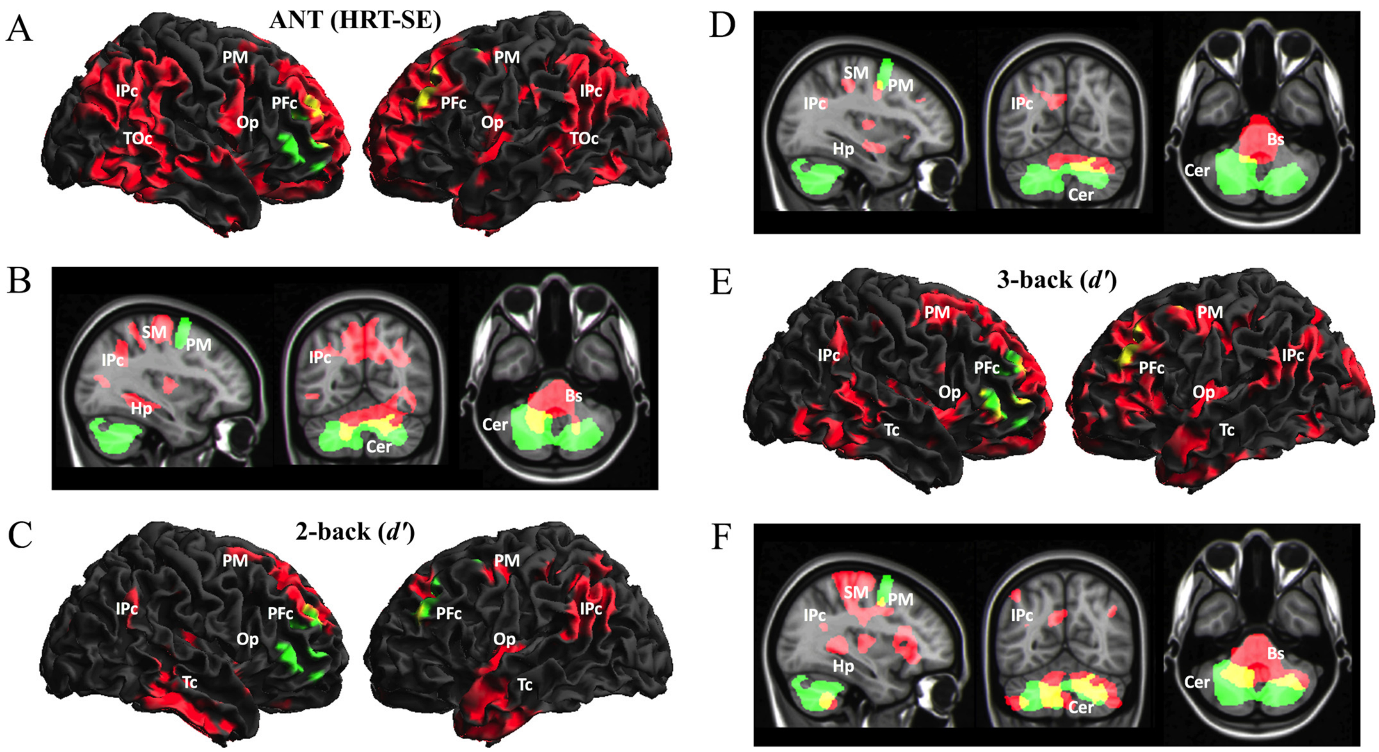

Figure 2. Regional gray and white matter volumes associated with lifelong residential surrounding greenness and cognitive performance. Results are displayed using conventional canonical templates [Cortex_20484 surface mesh in (*A*), (*C*), and (*E*) and MNI152_T1 template in (*B*), (*D*), and (*F*)] in Montreal Neurological Institute (MNI) space with statistical parametric mapping (SPM8; FIL Methods Group 2013) software. Green areas indicate regional volumes significantly associated with greenness (see Figure 1). Results were considered significant with clusters of 2.2 mL (650 voxels) at a height threshold of $p < 0.01$, which satisfied the family-wise error (FWE) rate correction of $p_{\mathrm{FWE}} < 0.05$. Red areas indicate regional clusters with volumes significantly associated with cognitive functions: hit reaction time standard error (HRT-SE; an indicator of inattentiveness) in (*A*) and (*B*); 2-back d' (an indicator of working memory) in (*C*) and (*D*), and 3-back d' (an indicator of superior working memory) in (*E*) and (*F*). The overlaps between regions associated with greenness and those associated with cognitive functions are shown in yellow. Voxels with significant results were binarized to the corresponding single color. (*A*) Three-dimensional (3D) renderings of cortical gray matter showing clusters negatively associated with HRT-SE in the right and left cortex (left and right panels, respectively). (*B*) Orthogonal displays (sagittal, coronal, and axial views in the left, middle, and right panels, respectively) showing white matter clusters negatively associated with HRT-SE. (*C*) 3D renderings of cortical gray matter showing clusters positively associated with 2-back. (*D*) Orthogonal displays showing white matter clusters positively associated with 2-back. (*E*) 3D renderings of cortical gray matter showing clusters positively associated with 3-back. (*F*) Orthogonal displays showing white matter clusters positively associated with 3-back. The right hemisphere appears on the right side of the axial and coronal views. See Table S3 for numeric data for each significant region. Residential greenness exposure was quantified based the average Normalized Difference Vegetation Index (NDVI) within a 100-m buffer around all residences since birth, weighted by the time the participant spent at each address. Bs, brainstem; Cer, cerebellum; Hp, hipocampus; IPc, inferior parietal cortex; Op, operculum; PFc, prefrontal cortex; PM, premotor cortex; SM, sensorimotor cortex; Tc, temporal cortex; TOc, temporo-occipital cortex.

(Friedman and Rapoport 2015). Consistently, we observed negative associations of inattentiveness with volumes of cerebellar vermis and hemispheres (supporting substudy I) and with peak values of greenness exposure–related clusters in cerebellar hemispheres and prefrontal cortex (supporting substudy II). However, the latter associations lost their statistical significance after controlling for age, sex, and maternal education.

Underlying Mechanisms

The biophilia hypothesis suggests that humans have important evolutionary bonds to nature (Wilson 1984; Kellert and Wilson 1993). Accordingly, contact with nature has been postulated to be essential for brain development in children (Kahn 1997; Kahn and Kellert 2002). Proponents of the biophilia hypothesis postulate that green spaces provide children with opportunities such as prompting engagement, discovery, creativity, risk taking, mastery, and control; bolstering sense of self; inspiring basic emotional states; and enhancing psychological restoration, which in turn are suggested to positively influence different aspects of brain development (Kahn and Kellert 2002; Kellert 2005; Bowler et al. 2010). In addition to exerting direct influence on brain development, green spaces might have indirect impacts mediated by other factors. For example, greener areas often have lower levels of traffic-related air pollution (Dadvand et al. 2015b) and noise (Gidlöf-Gunnarsson and Öhrström 2007). Moreover, people living in proximity to green spaces or in greener areas have been reported, albeit inconsistently, to be more physically active (James et al. 2015). Furthermore, green spaces are postulated to enrich microbial input from the environment (Rook 2013). Reduced exposure to air pollution and noise, increased physical activity, and enriched microbial input could lead to a beneficial impact of green spaces on brain development (Fedewa and Ahn 2011; Klatte et al. 2013; Rook 2013; Sunyer et al. 2015).

Limitations of the Study

Participants in the present study tended to have a better SES and to perform better on cognitive tests than the original BREATHE cohort, which might have resulted in selection bias. Although there are no known adverse effects of MRI exposure, the present study was conducted using a 1.5-Tesla magnet, which results in

Table 3. Crude associations between gray/white matter volume in the peak voxel of clusters significantly associated with lifelong residential greenness (independent variable) and cognitive test scores measured on four occasions over 12 months (dependent variables) derived using separate linear mixed effect models with random effects for child and school.

	Working memory^a		Superior working memory^b		Inattentiveness^c	
Location	Regression coefficient (95% CI)	*p*-Value	Regression coefficient (95% CI)	*p*-Value	Regression coefficient (95% CI)	*p*-Value
Gray matter						
Left premotor cortex	4.1 (1.2, 7.0)	0.01	4.1 (1.7, 6.6)	<0.01	−367 (−639, −95)	0.01
Left prefrontal cortex	1.0 (−0.4, 2.4)	0.18	1.0 (−0.3, 2.1)	0.12	−161 (−292, −31)	0.02
Right prefrontal cortex						
Superior cluster	3.1 (0.6, 5.6)	0.02	1.3 (−0.9, 3.4)	0.25	−198 (−434, 38)	0.10
Inferior cluster	0.8 (−0.7, 2.3)	0.30	1.7 (0.4, 3.0)	0.01	−150 (−287, −13)	0.03
White matter						
Cerebellum						
Left hemisphere	0.4 (−0.7, 1.4)	0.50	0.9 (0.0, 1.8)	0.05	−97 (−190, −4)	0.04
Right hemisphere	1.9 (0.1, 3.7)	0.04	1.3 (−0.2, 2.9)	0.09	−226 (−395, −58)	0.01
Left premotor region	2.8 (0.0, 5.7)	0.05	2.3 (−0.1, 4.8)	0.06	−176 (−442, 89)	0.19
Right prefrontal region						
Superior cluster	1.4 (−1.2, 4.1)	0.28	1.3 (−0.9, 3.6)	0.24	−244 (−486, −2)	0.05
Inferior cluster	−1.9 (−12.5, 8.7)	0.73	6.7 (−2.3, 15.7)	0.15	180 (−798, 1158)	0.72

Note: Adjustment was conducted for age, sex, and maternal education. Volume refers to the volume proportion of gray matter to white matter and cerebrospinal fluid (CSF) and the volume proportion of white matter to gray matter and CSF in each voxel. Voxel-wise volumes are expressed so that the total amount of tissue volume in the different brain structures is preserved during the process of normalization, which involves local stretching and shrinking of the brain structures. This is accomplished by the modulation of the segmented tissue probability maps using the Jacobian determinants derived from the spatial normalization step. CI, confidence interval.

[a] Characterized using 2-back d'. A higher d' indicates more accurate test performance.

[b] Characterized using 3-back d'. A higher d' indicates more accurate test performance.

[c] Characterized using Attentional Network Task (ANT) Hit Reaction Time Standard Error (HRT-SE). A higher HRT-SE indicates more inattentiveness.

lower exposure but also generates lower-resolution images than would be obtained using a 3-Tesla magnet. We estimated greenness exposure at all residential addresses since birth, but we did not account for greenness in the vicinity of schools, friends' homes, or other locations. Therefore, our residential exposure metric did not capture all possible exposure to greenness. Using high-resolution satellite data on greenness enabled us to account for small-area green spaces (e.g., home gardens, street trees, and green verges) in a standardized way. However, NDVI does not distinguish the types of vegetation or provide information on the quality of green spaces or on access to these spaces, which might have had implications in our study. By using an NDVI map obtained at a single point in time (2012), we effectively assumed that the spatial distribution of NDVI across our study region remained constant over the study period. The findings of our previous studies support the stability of the NDVI spatial contrast over years (Dadvand et al. 2012, 2014). We did not have data on parental cognitive status or on geographical factors such as walkability, which might have resulted in residual confounding in our results.

Conclusions

We identified several brain regions that had larger volumes in urban children with higher lifelong exposure to residential surrounding greenness. Brain regions whose volumes were increased in association with better cognitive test scores partly overlapped with some of the regions associated with greenness. In addition,

Table 4. Adjusted associations between gray/white matter volume in the peak voxel of clusters significantly associated with lifelong residential greenness (independent variable) and cognitive test scores measured on four occasions over 12 months (dependent variables) derived using separate linear mixed effect models with random effects for child and school.

	Working memory^a		Superior working memory^b		Inattentiveness^c	
Location	Regression coefficient (95% CI)	*p*-Value	Regression coefficient (95% CI)	*p*-Value	Regression coefficient (95% CI)	*p*-Value
Gray matter						
Left premotor cortex	4.3 (1.2, 7.4)	0.01	3.8 (1.2, 6.5)	<0.01	−226 (−502, 49)	0.11
Left prefrontal cortex	1.0 (−0.5, 2.5)	0.17	0.8 (−0.5, 2.1)	0.23	−127 (−2260, 7)	0.06
Right prefrontal cortex						
Superior cluster	3.0 (0.4, 5.6)	0.02	0.9 (−1.4, 3.1)	0.44	−108 (−338, 123)	0.36
Inferior cluster	0.8 (−0.8, 2.5)	0.32	1.6 (0.2, 3.1)	0.02	−78 (−222, 67)	0.29
White matter						
Cerebellum						
Left hemisphere	0.3 (−0.8, 1.3)	0.62	0.7 (−0.2, 1.6)	0.12	−53 (−146, 39)	0.26
Right hemisphere	1.8 (−0.2, 3.7)	0.08	0.9 (−0.8, 2.6)	0.31	−124 (−296, 49)	0.16
Left premotor region	3.1 (0.2, 6.0)	0.04	2.1 (−0.4, 4.6)	0.10	−112 (−371, 147)	0.40
Right prefrontal region						
Superior cluster	0.9 (−1.8, 3.7)	0.52	0.5 (−1.9, 2.9)	0.68	−39 (−284, 206)	0.76
Inferior cluster	−1.8 (−12.6, 8.9)	0.74	5.8 (−3.4, 15.0)	0.21	461 (−481, 1403)	0.34

Note: Adjustment was conducted for age, sex, and maternal education. Volume refers to the volume proportion of gray matter to white matter and cerebrospinal fluid (CSF) and the volume proportion of white matter to gray matter and CSF in each voxel. Voxel-wise volumes are expressed so that the total amount of tissue volume in the different brain structures is preserved during the process of normalization, which involves local stretching and shrinking of the brain structures. This is accomplished by the modulation of the segmented tissue probability maps using the Jacobian determinants derived from the spatial normalization step. CI, confidence interval.

[a] Characterized using 2-back d'. A higher d' indicates more accurate test performance.

[b] Characterized using 3-back d'. A higher d' indicates more accurate test performance.

[c] Characterized using Attentional Network Task (ANT) Hit Reaction Time Standard Error (HRT-SE). A higher HRT-SE indicates more inattentiveness.

peak volumes in some of the clusters associated with greenness also predicted better scores for some cognitive tests. Our findings provide new perspectives on how connections with the natural environment could potentially contribute to brain development. Further studies are needed to confirm our findings in other populations, settings, and climates; to evaluate other cognitive and neurological outcomes; to examine differences according to the nature and quality of green spaces (including specific types of vegetation) and children's access to and use of them. Moreover, whether developmental effects on the structure of the brain contribute to associations between greenness exposure and cognitive development remains an open question to be evaluated by future studies.

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