Urban upbringing has consistently been associated with schizophrenia, but which specific environmental exposures are reflected by this epidemiological observation and how they impact the developing brain to increase risk is largely unknown. On the basis of prior observations of abnormal functional brain processing of social stress in urban-born humans and preclinical evidence for enduring structural brain effects of early social stress, we investigated a possible morphological correlate of urban upbringing in human brain. In a sample of 110 healthy subjects studied with voxel-based morphometry, we detected a strong inverse correlation between early-life urbanicity and gray matter (GM) volume in the right dorsolateral prefrontal cortex (DLPFC, Brodmann area 9). Furthermore, we detected a negative correlation of early-life urbanicity and GM volumes in the perigenual anterior cingulate cortex (pACC) in men only. Previous work has linked volume reductions in the DLPFC to the exposure to psychosocial stress, including stressful experiences in early life. Besides, anatomical and functional alterations of this region have been identified in schizophrenic patients and high-risk populations. Previous data linking functional hyperactivation of pACC during social stress to urban upbringing suggest that the present interaction effect in brain structure might contribute to an increased risk for schizophrenia in males brought up in cities. Taken together, our results suggest a neural mechanism by which early-life urbanicity could impact brain architecture to increase the risk for schizophrenia.

Key words: environmental risk/urbanicity/schizophrenia/social stress hypothesis/cortical volume/voxel-based morphometry
shared risk-associated behaviors associated with urban life. Notably, to date, there is little empirical support for a role of differential biological exposures such as toxic pollution or infectious agents.9 Instead, and in line with findings of a crucial role of social stress in schizophrenia risk,10 the majority of authors favor the hypothesis that differences in social conditions along urban-rural gradients might account for the effects of urban upbringing.11 Although these results should currently be interpreted with caution given the complexity of the examined variables, there is thus convincing evidence in favor of the social stress hypothesis. For example, social fragmentation, a property of neighborhoods characterized by a lack of social bonding and cohesion,13 was shown to mediate the risk of urban upbringing on schizophrenia incidence in the Swedish population.13 Recent findings further support this hypothesis, linking urban upbringing in healthy adults to an elevated neural activity under social stress in the perigenual anterior cingulate cortex (pACC), a key brain region for stress and emotion regulation.14

Despite this emerging evidence, possible neuroanatomical correlates of increased risk through urban upbringing remain unstudied. Given that early stress has been shown to impact brain morphology in animals and humans (eg, refs.15-17), and structural abnormalities are present early on in schizophrenia, it appears plausible that an association between urban upbringing and regional reductions in brain GM volume may exist.

A well-validated and robust method to reveal anatomical alterations is voxel-based morphometry (VBM), which we applied in a sample of 110 subjects stratified by early-life urbanicity. In line with the rationale of imaging genetics studies,18 which mostly enroll healthy individuals instead of patient samples to avoid confounding effects of medication, psychopathology, and hospitalization, we investigated healthy subjects who were stratified along an urban-rural gradient with respect to their upbringing. We then tested for voxel-wise association of urban upbringing with the outcome variable of VBM, local GM volume estimates.

We specifically tested for changes in two regions: pACC and hippocampus. These areas were chosen as region of interest (ROI) since prior neuroscience evidence suggest a particular susceptibility to the impact of social stress. As noted above, pACC has been found to be differentially activated under social stress depending on early-life urbanicity. Moreover, GM volumes of the pACC have been shown to be inversely correlated to one’s subjective social standing, a variable likely capturing a stress-related dimension of low socioeconomic status.19 Volume reductions of the pACC are also present in several clinical populations such as children with depressive symptoms,20 patients with bipolar disorder21 and schizophrenia,22 highlighting the role of the pACC in mental health. The hippocampus seems to be affected by chronic stress exposure involving measurable architectural changes like shrinkage of dendrites and disruption of structural connectivity with the prefrontal cortex.23,24 In line with established neuroimaging standards,25 structural effects outside the prehypothesized anatomical regions were only considered significant if they survived stringent multiple comparisons correction across the whole brain, a threshold that does not require a priori regional assumptions.

### Materials and Methods

#### Subjects

We studied 110 healthy, native German participants from the general population residing in communities in and around the city of Mannheim (in the southwestern part of Germany). Characteristics of the sample are presented in table 1. Functional data from a part of this sample have been reported previously.14 The enrolled subjects were “healthy” as defined by the absence of any of the predefined general exclusion criteria, which included a lifetime history of general medical, psychiatric, or neurological illness; psychopharmacological or psychotherapeutical treatment; drug or alcohol abuse; and head trauma. In a subsample of 80 participants, a family history of mood disorders and schizophrenia in first-degree relatives was furthermore explicitly excluded. All participants provided written informed consent for protocols approved by the institutional review board of the University of Heidelberg.

#### Early-life Urbanicity Score

Details about each participant’s place(s) of residence from birth to age 15 were acquired. We chose that time frame based on previous epidemiological work highlighting this period as critical for urban exposure related to illness.
risk. Early-life urbanicity was quantified using categories from the epidemiological literature as described previously. First, residential communities were assigned to 1 of 3 categories depending on the number of inhabitants: 3 = cities with more than 100 000 inhabitants, 2 = towns with 10 000-100 000 inhabitants, 1 = rural areas. Taking into account the finding of a dose-response relationship between schizophrenia risk and both the size of a community and the time of exposure therein in the first 15 years of life, we multiplied the category scores by the number of years spent in the respective category and added the resulting values up to a cumulative proxy score. Final urbanicity scores ranged from 15 to 45, with higher values indicating higher urban exposure during early life.

Data Acquisition
Magnet resonance imaging (MRI) was performed on two identical 3-Tesla Siemens Magnetom Tim Trio (Siemens, Erlangen, Germany) systems, both of which located at the Central Institute of Mental Health, Mannheim. We used a T1-weighted 3D magnetization-prepared rapid gradient-echo (MPRage) sequence with whole-brain coverage, an isotropic spatial resolution of 1 mm^3 and the following sequence specifications: TR = 1570 ms, TE = 2.75 ms, flip angle = 15°, 176 contiguous sagittal slices, 1-mm slice thickness, field of view = 256 mm. For quality assurance (QA) purposes, phantom measurements were conducted on each scanner and data acquisition day according to an established QA protocol.

Image Processing
Images were processed using the VBM toolbox (VBM8, http://dbm.neuro.uni-jena.de/vbm) implemented in SPM8 (http://www.fil.ion.ucl.ac.uk/spm) with default parameters as described in the VBM8 toolbox manual. Briefly, automated image processing included tissue classification into GM, white matter, cerebrospinal fluid, and three other noncerebral tissue classes, normalization to Montreal Neurological Institute (MNI) space with a diffeomorphic image registration algorithm (DARTEL), correction for image intensity nonuniformity, a thorough cleaning up of GM partitions, the application of a hidden Markov random field model and spatial adaptive nonlocal means denoising. The resulting tissue segments were multiplied by the Jacobian determinants of the deformation field to transform the GM density values into volume equivalents. The segmented, normalized, noise-corrected, and modulated GM images were then smoothed with an 8-mm full width at half maximum isotropic Gaussian kernel.

Data Analysis
Processed images were analyzed in SPM8 using a general linear model with whole-brain random-effects group statistics. The effect of early-life urbanicity on GM volume was tested in a multiple regression analysis with early-life urbanicity as covariate of interest. In addition, since male sex has been associated with increased schizophrenia risk, earlier age of onset, and course of illness, adverse interactions with urban upbringing may exist. Thus, potential interaction effects of the variables on GM volume were tested in an ANOVA model with sex as a factor and a sex by early-urbanicity interaction term as covariate of interest. To account for possible confounds, sex, age, the second-degree polynomial expansion of age, years of school education, current urbanicity, total GM volume, and scanner were included in all analyses as nuisance covariates. The second-degree polynomial expansion was included to additionally account for quadratic effects of age on GM volume, which have been reported previously. Because early-life and current urbanicity were moderately intercorrelated (see table 1), current urbanicity was included as covariate of no interest in the model in order to specifically test for the effects of early-life urbanicity in the absence of a possible current urbanicity effect. We obtained the current urbanicity variable by assigning the residence of each subject at the time of study participation to 1 of the 3 categories described above.

We conducted ROI analyses in 2 a priori defined anatomical masks, namely, of the hippocampus and rostral anterior cingulate cortex (ACC) derived from the Wake Forest University (WFU) PickAtlas (http://www.fmri.wfubmc.edu) as defined in previous work. Outside these ROIs, structural effects were only considered significant if they survived stringent multiple comparisons correction across the whole brain, a highly conservative statistical threshold allowing for the reporting of association findings anywhere in the brain and in the absence of a prespecified regional hypothesis. In all analyses, statistical significance was assumed at a threshold of \( P < .05 \) after either ROI or whole-brain family-wise error (FWE) correction for multiple comparisons.

Results
Main Effect of Urban Upbringing
We identified a negative correlation between early-life urbanicity scores until age 15 and GM volumes within the posterior aspects of the right dorsolateral prefrontal cortex (DLPFC) mapping to Brodmann area (BA) 9 (peak voxel at \( x = 45, y = 11, z = 34 \) in MNI space; \( T = 5.24, P = .014; \) FWE-corrected for multiple comparisons across the whole brain, figure 1A). Scatterplots confirmed a linear pattern of association (figure 1B). This effect was relatively specific to this area, as no other brain region was significantly correlated at this threshold. Since we introduced age, its second-degree polynomial expansion, and sex as nuisance covariates in our model, this main effect of early urbanicity on GM volume was not accounted for.
for by either age or sex. Also, no significant pairwise correlation between age and early urbanicity \((r = 0.12, P > .2)\) or sex-dependent group differences in early urbanicity \((t = 0.34, P > .7)\) were detected.

Regarding the pACC, which has been found to be differentially activated under social stress depending on early-life urbanicity (see above), we conducted an ROI analysis with the rostral ACC as ROI and early-life urbanicity as covariate of interest, but no significant main effect of urban upbringing was observed \((T = 2.83, P > .25, \text{FWE-corrected within ROI})\). Similarly, no significant or suggestive result was detected for the hippocampus \((T = 2.27, P > .71, \text{FWE-corrected within ROI})\).

**Urbanicity by Sex Interaction**

Since urban upbringing might affect cortical morphology differentially in men and women, we tested for interaction effects of early-life urbanicity and sex on GM volume. We detected a significant interaction effect within the pACC \((x = 2, y = 45, z = 12, T = 3.85, P = .019, \text{FWE-corrected within ROI})\). Post hoc analyses revealed a significantly negative correlation between early-life urbanicity and pACC GM volume in males \((r = -0.518, P < .001)\), which was absent in females \((r = 0.198, P = .14, \text{see figure 1D for details})\). In contrast, no significant interaction effects were seen in the hippocampus \((T = 2.57, P = .51, \text{FWE-corrected within ROI})\).

**Post hoc Power Analysis in Hippocampus**

Further exploration of the observed lack of association of urban upbringing and hippocampus volume was conducted with the software package G*Power.\(^\text{28}\) The analyses suggested that based on the observed effect sizes in the hippocampus, a sample size of more than 1250 subjects for the main effect of urban upbringing and 337 subjects for the sex by urbanicity interaction would have been necessary to obtain significant associations in the conducted ROI analyses. Considering that the sample sizes of the majority of neuroimaging studies in the field fall far below these numbers and because common concepts assume larger effect sizes of risk associations at the level of the brain (compared to behavior),\(^\text{18,34}\) this outcome argues for a true negative finding and against a lack of power of our hippocampus analysis. However, although the size of

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**Fig. 1.** Early-life urbanicity and GM volume. (A) T-map of negative correlations between GM volume and early-life urbanicity. Significant correlations map to the right posterior DLPFC (BA 9, \(t = 5.24, P = .014, \text{FWE-corrected for the whole brain})\. (B) Scatterplot between GM volumes of the most significantly correlated voxel \((x = 45, y = 11, z = 34)\) and early-life urbanicity scores. Results illustrate a linear relationship between the two variables \((r = -0.48)\). (C) T-map of interaction effects between early-life urbanicity and sex on GM volume. A significant interaction effect is seen in the pACC \((t = 3.85, P = .019, \text{FWE-corrected within ROI})\). (D) Scatterplots of early-life urbanicity and GM volumes in the voxel with the highest interaction effect \((x = 2, y = 45, z = 12)\) illustrate a negative correlation in males and no correlation in females. Coordinates refer to the Montreal Neuroimaging Institute standard space. T-maps are displayed at \(P < .005\) uncorrected for presentation purposes. The color bar represents \(t\)-values. BA, Brodmann area; DLPFC, dorsolateral prefrontal cortex; GM, gray matter; pACC, perigenual anterior cingulate cortex.
the present sample is relatively large for a neuroimaging investigation, we cannot fully exclude the possibility of having underestimated a potentially true effect of early urbanicity on hippocampal volume in the population.

Discussion

In this study, we aimed to identify the brain structural correlates of urban upbringing, a complex but well-established environmental risk factor for schizophrenia that has been linked to stress exposure in early life. Our main findings consisted of an inverse relationship between early-life urbanicity and GM volume in the DLPFC and (in males also) the pACC, indicating reduced GM volume in (male) subjects born and raised in cities. Although the detected associations with a risk variable from psychiatric epidemiology do not imply causality, they are informative for the discussion of the potential mechanisms of environmental risk factors for mental health.

Both the localization and directionality of the detected main effect of urban upbringing are consistent with the existing literature on stress and schizophrenia. The DLPFC is highly sensitive to stress exposure, as indicated by rapid stress-induced impairments of prefrontal cognitive functions and reductions in neural activity. Also, architectural changes of prefrontal dendrites have been repeatedly observed in rodent models exposed to chronic stress, and have been directly linked to stress-induced deficits in cognition. Moreover, reductions in DLPFC volume have been reported in the context of early environmental adversity in humans, eg, in adults who were exposed to harsh punishment during childhood or carriers of plasticity-related genetic risk variants exposed to stressful life events. Likewise, structural alterations of the DLPFC are well-supported findings in schizophrenic patients and certain high-risk individuals, suggesting a link between the observed anatomical effects of early urbanicity on DLPFC volume and schizophrenia risk. Specifically, while prior volumetric studies in medicated patient samples tended to be inconclusive regarding DLPFC involvement, a recent VBM meta-analysis in antipsychotic-naive first-episode patients reported a decrease in GM volume in right DLPFC, in line with an involvement of this region early in the disease process that is independent of the known structural effects of antipsychotic treatment. Moreover, decreases in DLPFC GM volume have been repeatedly associated with reduced cognitive function, which is common in both manifest schizophrenia and individuals at high risk for the illness.

In contrast, no main effect of urban upbringing on pACC volume was detected. This is generally in agreement with our previous functional results, since a main effect of urban upbringing in this region was only found during a stress challenge and was absent in other cognitive paradigms, indicating that the pACC is not globally impaired as a consequence of urban exposure. However, we did detect a significant interaction of urban upbringing and sex on pACC volume consistent with detrimental alterations in males brought up in cities. Notably, the cingulate cortex is one of the best supported regions where structural changes are already evident in schizophrenia at disease onset, and volumetric alterations in this region have been associated with both the genetic liability for the illness and environmental stressors such as social status, hypoxia, and urban violence. Our data extend these prior data by highlighting the possibility that the structural integrity of the pACC may be particularly sensitive to the nonlinear cumulative effects of environmental risk factors. Consistent with our data, male sex is a well-established risk factor for schizophrenia that is associated with an earlier disease onset and poorer outcomes. Moreover, an excess risk in schizophrenia has previously been observed in male individuals born in urban environments. Although the observed interaction effect and the associated structural alterations in pACC are consistent with a more pronounced developmental vulnerability in the context of nonlinear cumulative risk and may also plausibly relate to the sex-specific aspects of the illness, further research is needed to corroborate this speculation.

Notably, we neither observed a main effect of urban upbringing nor sex by urbanicity interaction effect on hippocampus volume, an outcome consistent with our prior functional work on the modulation of neural social stress processing by urban upbringing. Our post hoc power analysis further suggested that these negative findings unlikely relate to a lack of statistical power. Notably, although research in rodent models convincingly demonstrates adverse effects of early stress exposure on hippocampus structure, prior evidence on the effects of psychosocial stress exposure in humans is rather inconsistent, with some studies reporting positive findings (eg, with increased life stress), whereas others failed to demonstrate an association with hippocampus volume (eg, in the context of combat exposure, childhood trauma, adverse caregiving, or childhood maltreatment). The reason for this discrepancy is at present unclear but may relate to a heightened sensitivity of the hippocampus to environmental stressors in certain time windows of neurodevelopment that are insufficiently captured by the employed stress measures, including the urbanicity proxy variable examined here.

This study has several limitations. First, in line with prior research (eg, refs), early urbanicity has been quantified based on the population size of residences. This was done to make our work relatable to the epidemiological evidence base from which the hypothesis originates. It is, however, likely not the population size per se which increases schizophrenia risk, which means that we studied a proxy measure for the risk-increasing attributes of early urban exposure. Future research might aim at exploring and validating more fine-grained measures.

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Second, we did not directly assess early stress exposure. Our hypothesis that urban upbringing might impact volumetric properties of the brain via an elevated early exposure to stress appears plausible but is nevertheless currently still speculative. Third, we examined the effects of urban upbringing in healthy exposed individuals in order to minimize the effects of disorder-related confounds. Since, in theory, urban upbringing might differentially impact brain morphology in those individuals who stay healthy and those who develop schizophrenia later in life, this study should be regarded as hypothesis-generating. Future studies should investigate the associations of urban upbringing and brain morphology also in schizophrenic patients and high-risk samples so that the translation of results to manifest disorder and potential interaction effects with other schizophrenia risk factors can be explicitly addressed. Fourth, to our knowledge, urban exposure during childhood and early adolescence has been exclusively associated with nonaffective psychoses, an observation that may relate to the interference of this risk factor with the remodeling and maturation of brain circuits during this vulnerable period of neurodevelopment. Although the evidence thus suggests that the uncovered brain structural associations of early urbanicity are particularly informative for the risk architecture of schizophrenia, and we corrected our analyses for current urbanicity, it remains possible that the detected structural correlates of urban upbringing may also confer risk to clinical conditions that have been linked to urban exposure during adulthood.56 We hope that the additional data on which brain regions are affected by urbanicity may aid in further parsing such potential associations in neuroepidemiological studies. Fifth, although we tried to control for potential confounds by including several core demographic variables as covariates of no interest, a methodological limitation persists in the possibility that the results may be confounded by variables that we did not ascertain and are correlated to early-life urbanicity. More research is needed to clarify which components of urban exposure could account for the observed structural alteration.

Conclusion

In summary, our data link urban upbringing to anatomical alterations in DLPFC and pACC, regions that have been implicated in the effects of environmental stress and the pathology and risk for schizophrenia. The data further point to a detrimental interaction of urban upbringing and male sex on brain anatomy, suggesting a complex neural mechanism for the association of early urbanicity with schizophrenia risk. We speculate that the described associations of urban upbringing may represent adult outcomes of neurodevelopmental changes caused by early urban exposure, potentially due to increased social stress. Future work should aim to replicate these findings and study specific component features of the urban environment that impact brain structure and function as well as their interaction with other genetic and environmental risk factors.

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