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Full Length Article

Brain structure and habitat: Do the brains of our children tell us where they have been brought up?

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ABSTRACT

Recently many lifestyle factors have been shown to be associated with brain structural alterations. At present we are facing increasing population shifts from rural to urban areas, which considerably change the living environments of human beings. To investigate the association between rural vs. urban upbringing and brain structure we selected 106 14-year old adolescents of whom half were exclusively raised in rural areas and the other half who exclusively lived in cities. Voxel-based morphometry revealed a group difference in left hippocampal formation (Rural > City), which was positively associated with cognitive performance in a spatial processing task. Moreover, significant group differences were observed in spatial processing (Rural > City). A mediation analysis revealed that hippocampal formation accounted for more than half of the association between upbringing and spatial processing. The results are compatible with studies reporting earlier and more intense opportunities for spatial exploration in children brought up in rural areas. The results are interesting in the light of urban planning where spaces enabling spatial exploration for children may deserve more attention.

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Recently there has been a growing interest in variables that shape adult brain structure and thereby elicit so-called neural plasticity (Lövden et al., 2013). Many of these studies have investigated the effects of lifestyle factors such as smoking (Gallinat et al., 2006; Kühn et al., 2010), physical exercise (Hotting and Roder, 2013), or leisure activities such as video gaming (Kühn and Gallinat, 2014a). Recent work has further highlighted the effects on risk (Meyer-Lindenberg and Tost, 2012; Tost et al., 2015) and resilience (Holz et al., 2019) of the living environment of humans, especially urban vs. rural contexts. This is relevant since we are currently facing increasing population shifts from rural to urban areas, which strongly change the living environments of human beings. The United Nations predict that by 2050 68% of the world's population will live in cities (https://population.un.org/wup/, July, 2019). Therefore, evidence on how environmental factors are related to brain integrity is urgently needed. In particular since epidemiological research on mental health has consistently shown that mood as well as anxiety disorders and schizophrenia are more frequent in urban compared to rural regions (Peen and Dekker, 2004; Peen et al., 2010).

Neuroscientific studies point at the importance of brain regions related to stress processing in the context of urbanicity. In an fMRI study current city living has been associated with higher amygdala activity during a stressful mental calculation task (Lederbogen et al., 2011). In the very same study exposure to urbanicity during the first 15 years of life was associated with brain activity in the perigenual anterior cingulate cortex (pACC). This study, focussing on brain function, was followed by five studies focussing on associations between living environment and brain structure. The first reported a negative association between grey matter volume in dorsolateral prefrontal cortex (DLFPC) and pACC (in men only) and urban upbringing, calculated as a cumulative weighted score (Pedersen and Mortensen, 2001) from reported years lived in cities, towns and rural regions for the first 15 years in an adult sample ($n = 110$) (Haddad et al., 2015). A very similar analysis, likewise on a sample of healthy adults ($n = 85$) revealed no association with grey matter volume, but a negative association with cortical thickness in left DLPFC, bilateral medial prefrontal cortex, left superior temporal and parahippocampal cortex with the same urbanicity score (Besteher et al., 2017; Pedersen and Mortensen, 2001). Recently, another study demonstrated a negative association between bilateral DLPFC and right inferior parietal lobe grey matter volume using the very same urbanicity score in healthy adults ($n = 290$) (Lammeyer et al., 2019). A different angle has been taken by a study on older adults ($n = 341$), in which land use categories, such as forest or urban green within a 1 km radius around the home address has been used to characterize the *acute* living environment of participants (Kühn et al., 2017). In a region of interest (ROI) analysis a positive association between amount of forest around the home address and amygdala integrity (a latent factor including grey matter volume, mean diffusivity and magnetisation transfer ratio) has been found. With a similar georeferencing approach, focussing on lifelong exposure to greenness, $n = 253$ children (7–10 years) were examined (Dadvand et al., 2018). This study reported a positive association between a satellite-based vegetation index within 100 m radius around the residential addresses since birth and grey matter volume in prefrontal cortex and in left premotor cortex.

Within the present study we set out to compare extreme groups during adolescence (around 14 years of age) that spent their entire life (until the time of measurement) in rural regions with matched adolescents that spent their entire life in cities. According to previous research, the prevalence of major psychiatric disorders such as schizophrenia has been related to urban upbringing during the first 15 years (Haddad et al., 2015) which makes the focus on brain morphology in adolescence particularly relevant. Based on the previous studies we expected differences between the adolescent extreme groups in prefrontal cortex, temporal/parahippocampal brain regions and the amygdala.

1. MATERIALS and methods

1.1. Participants

We used data of 106 healthy adolescents recruited within the scope of the IMAGEN project ($n \approx 2200$), a European multi-centre genetic-neuroimaging study that started in adolescence (Berlin, Hamburg, Dresden, Mannheim, Nottingham, Dublin, London, Paris) (Schumann et al., 2010). We selected the 106 participants based on their responses to questions about their living environment during the first 15 years of age, which was administered during a follow-up test session when participants were 16–17 years of age. Based on these replies we selected all participants of the IMAGEN sample who indicated that they had lived in rural areas for their first 15 years ("Up until the age of 15, how many years have you spent living in rural areas?"). We included all participants who indicated 15 rural years, since many participants were slightly older then 14 years at the point of MRI data acquisition ($n = 53$) (mean age=14.5, SD=0.52 years; 16 males). During the remainder of the text we will refer to "the last 14 year", since the adolescents were scanned at age 14 years. To these participants who solely lived in rural areas during their lives we matched participants who indicated that they have solely lived in cities ("Up until the age of 15, how many years have you spent living in towns/cities with more than 100.000 inhabitants.") ($n = 53$) (mean age=14.4, SD=0.34 years; 16 males), which were similar in terms of age, sex, total intracranial volume (TIV), MRI-acquisition site and parental education (mother and father). After matching there was no significant difference between parental education between the groups ($t(104)=-0.611$, $p = 0.542$). We tested for group differences in pubertal status, but no differences were observed ($p>0.11$ on all items).

Written informed consent was obtained from all participants as well as from their legal guardians. The adolescents were recruited from secondary schools. The study was approved by the respective local ethics committees and approved by the head teachers of the respective schools. Participants with a medical condition or neurological disorder were excluded.

1.2. Scanning procedure

Structural MRI was performed on 3 Tesla scanners from three manufacturers (Siemens: 5 sites; Philips: 2 sites; and General Electric: 2 sites). The details of the entire MR protocol are described elsewhere (Schumann et al., 2010). In this study, we used the T1-weighted images. These high-resolution anatomical MRIs were obtained using a three-dimensional sequence based on the ADNI protocol (http://adni.loni.usc.edu/methods/documents/mri-protocols/; modified for the IMAGEN study to give a $1.1 \times 1.1 \times 1.1\text{mm}^3$ voxel size).

1.3. Voxel-based morphometry (VBM)

We obtained grey matter volume estimates using CAT12 (v1278) running on SPM12 using default parameters (http://www.neuro.uni-jena.de/cat12/CAT12-Manual.pdf). CAT12 automatically performs intra-subject realignment, bias correction, segmentation, and normalization. Segmentation into three voxel classes (grey matter (GM), white matter (WM), and cerebrospinal fluid (CSF)) was performed using adaptive maximum a posteriori segmentation and partial volume segmentation. The extracted GM maps were smoothed using a FWHM kernel of 8 mm.

Processing included several stages of quality checking: Images were visually inspected for artefacts prior to processing. Then, a statistical

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quality control based on inter-subject homogeneity after segmentation was conducted.

We computed whole-brain analyses contrasting the two groups of interest. We controlled for the covariates: age, sex, TIV, scanner site and parental education. The resulting maps were thresholded with $p<0.001$ and the statistical extent threshold was used to correct for multiple comparisons combined with a non-stationary smoothness correction based on permutation (Hayasaka et al., 2004) as implemented in the CAT12 toolbox.

1.4. Surface-based morphometry analysis

The CAT12 toolbox also contains a processing pipeline for surface-based morphometry, which includes an algorithm for extracting cortical surface (Dahnke et al., 2013).

The T1-weighted images first undergo tissue segmentation to estimate white matter distance. Then local maxima are projected to other grey matter voxels by using a neighbour relationship described by the WM distance (=cortical thickness). All scans are re-sampled and smoothed with a Gaussian kernel of 15 mm (FWHM).

1.5. FreeSurfer subcortical processing

Volume of subcortical structures estimated from the T1 images using FreeSurfer 6.0 software (http://surfer.nmr.mgh.harvard.edu/, (Fischl, 2012)), a set of automated tools for reconstruction of brain cortical surface and subcortical volumes. The segmentation results of FreeSurfer in the hippocampus have been shown to be highly correlated with manual tracings (Morey et al., 2009). In the present analysis we considered right and left hippocampus and amygdala volume segmentations.

1.6. Cognitive performance tasks

Based on the whole brain and ROI findings we selected two tasks assessed within IMAGEN that contain a spatial processing component, namely Block Design from the Wechsler intelligence scale for children (WISC-IV) (Wechsler, 2003) and the Spatial Working Memory Task from the Cambridge Neuropsychological Test Automated Battery (CANTAB) battery. Block Design involves putting together red-and-white blocks in a pattern to match to a displayed model and requires attention, spatial reasoning and response selection. Higher scores indicate higher performance. In the Spatial Working Memory Task an increasing number of boxes is presented. Participants are instructed to search for tokens by opening the boxes. Participants are advised not to return to a box that had already yielded a token. Resulting measures are called between errors (number of times the participant revisits a box where a token has previously been found) and strategy (number of times the participant started a new search by touching a different box).

1.7. Questionnaires

To assess the stage of puberty participants were in, we used the self-report Pubertal Development Scale (PDS) (Petersen et al., 1988).

1.8. Statistical analysis

Although we employed propensity score matching to select the best matching participants, we controlled for the covariates age, sex, TIV, scanner site (9 different scanners, split up into 8 dummy coded variables) and parental education (mean of maternal and paternal education), since these matches are never perfect and in particular MRI volumetric measurements are known to be affected by TIV and acquisition on different MRI scanners. However, the pattern of results is very similar and does not change qualitatively when not controlling for any of the covariates. In order to investigate group differences we ran analyses of covariance (ANCOVAs) (Fisher, 1918) and to investigate relationships we employed partial correlations. The mediation analysis was computed using the PROCESS macro in SPSS (Hayes, 2017).

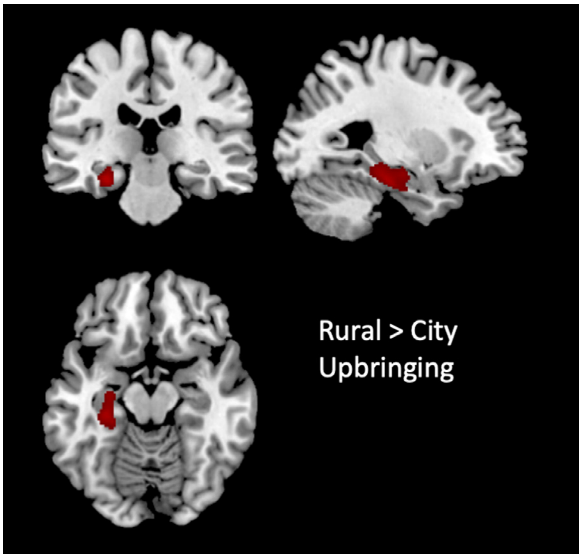

Fig. 1. Results of the voxel-based morphometry whole brain contrast between adolescents exclusively brought up in rural vs. city regions reveals a single cluster with higher grey matter volume in left hippocampal formation (MNI: −30, −22, −20), where adolescents with rural upbringing show higher grey matter volume.

2. RESULTS

In a whole brain analysis on grey matter volume maps exclusive rural upbringing revealed higher grey matter volume in the hippocampal formation, more precisely in left parahippocampal gyrus and left hippocampus (HC) (centre of gravity of the cluster was at the MNI coordinate −30,−22,−20 in mm; $\text{mean}_{\text{city}}= 0.47$, $\text{SD}_{\text{city}}=0.044$, $\text{mean}_{\text{rural}}= 0.51$, $\text{SD}_{\text{rural}}=0.043$, $F(1,92)=34.85$, $p = 0.000000059$; *Fig. 1*) as compared with city upbringing. In order to confirm the localization of the effect with a different methodological approach we conducted a ROI analysis on FreeSurfers' segmentations of HC, observing group differences in both hemispheres (left HC volume: $\text{mean}_{\text{rural}}=4408.11$, $\text{SD}_{\text{rural}}=354.54$, $\text{mean}_{\text{city}}=4181.68$, $\text{SD}_{\text{city}}=422.98$; $F(1,92)=16.24$, $p=0.00012$; right HC volume: $\text{mean}_{\text{rural}}=4492.28$ mm^3, $\text{SD}_{\text{rural}}=371.20$, $\text{mean}_{\text{city}}=4313.70$ mm^3, $\text{SD}_{\text{city}}=407.98$; $F(1,92)=8.495$, $p = 0.004$) (*Fig. 2*). Since we had an a priori hypothesis for amygdala, we compared the groups observing a significant difference for right amygdala, with higher volume in adolescents with rural vs. city upbringing (right amygdala volume: $\text{mean}_{\text{rural}}=1808.35$ mm^3, $\text{SD}_{\text{rural}}=243.21$, $\text{mean}_{\text{city}}=1712.65$ mm^3, $\text{SD}_{\text{city}}=279.15$, $F(1,88)=6.44$, $p = 0.013$; left amygdala volume: $\text{mean}_{\text{rural}}=1669.29$ mm^3, $\text{SD}_{\text{rural}}= 211.06$, $\text{mean}_{\text{city}}=1644.90$ mm^3, $\text{SD}_{\text{city}}= 299.60$, $F(1,88)=0.86$, $p = 0.356$) (*Fig. 2*).

In order to characterize the potential functional involvement of the cluster observed in left hippocampal formation we correlated the extracted mean grey matter volume of all participants with the tasks that were administered that capture spatial processing, namely the Block Design task and the Spatial Working Memory task, since hippocampal formation has been associated with spatial processing. We found a significant positive association between Block Design performance and grey matter volume based on VBM in left hippocampal formation ($r(82)=0.426$, $p<0.001$). Moreover we also observed a group difference in Block Design performance with participants raised in rural regions outperforming those raised in cities ($\text{mean}_{\text{rural}}=10.98$, $\text{mean}_{\text{city}}=9.56$; $F(1,83)=8.06$, $p = 0.006$) (*Fig. 3*). The correlation across all participants was not driven by the mean group difference but present in both groups (rural: $r(48)=0.277$, $p = 0.057$; city: $r(48)=0.424$, $p = 0.003$, without

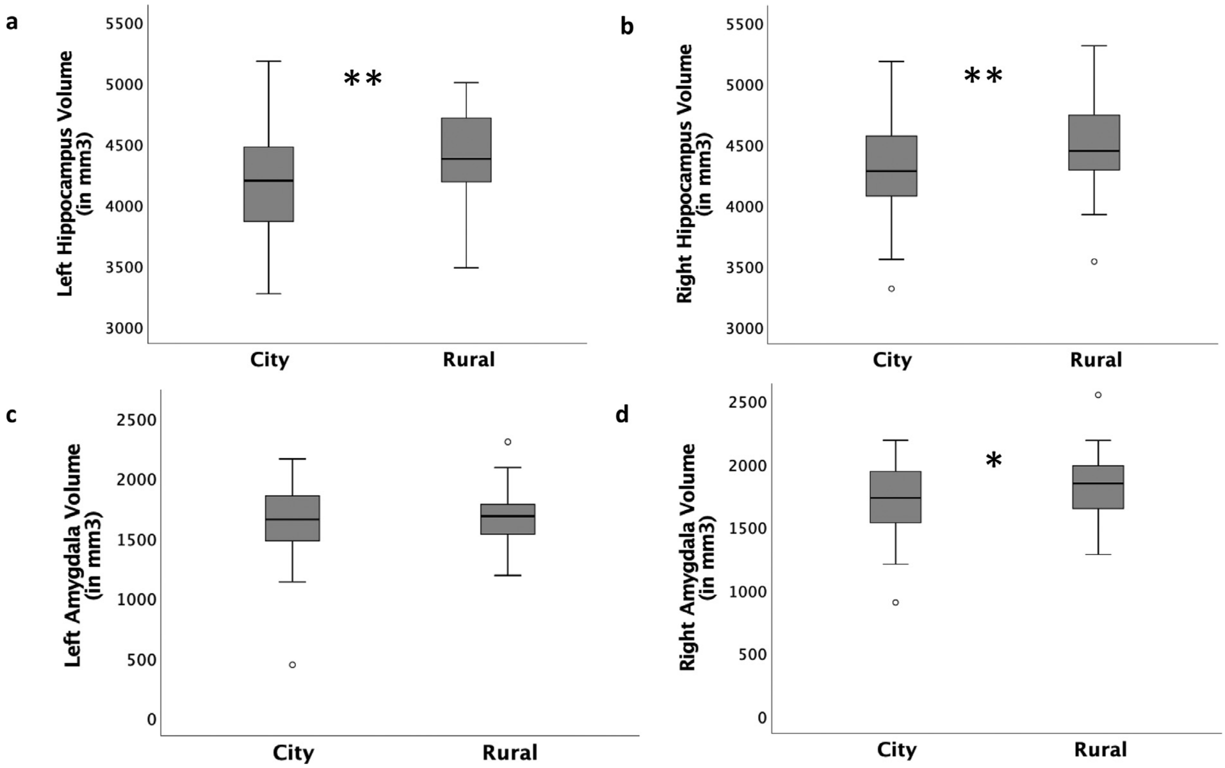

Fig. 2. Box plots displaying group differences in brain structure based on FreeSurfer segmentations of hippocampus (a,b) and amygdala (c,d) between adolescents brought up in rural regions vs. cities for the first 14 years of their life. The data displayed is not corrected for the covariates that have been entered in the statistical analysis reported in the text. The thick line depicts the group-based median, the box illustrates data from the 1st to the 3rd quartile (interquartile range), the whiskers indicate 1.5 times the interquartile range. Circles represent statistical outliers that lie outside the 1.5 interquartile range. * p <0.05, ** p < 0.01.

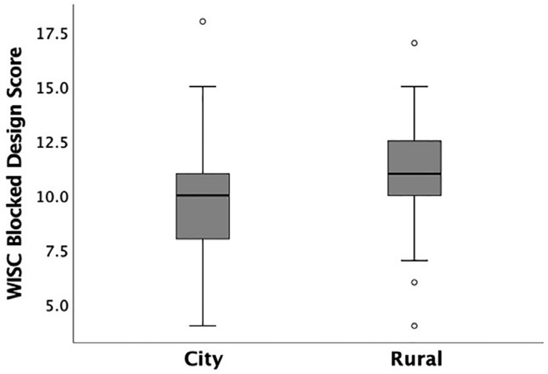

Fig. 3. Box plot displaying behavioural group difference in spatial task between adolescents brought up in rural regions vs. cities for the first 14 years of their life. The data displayed is not corrected for the covariates that have been entered in the statistical analysis reported in the text. (See details about the plot in caption of Fig. 2).

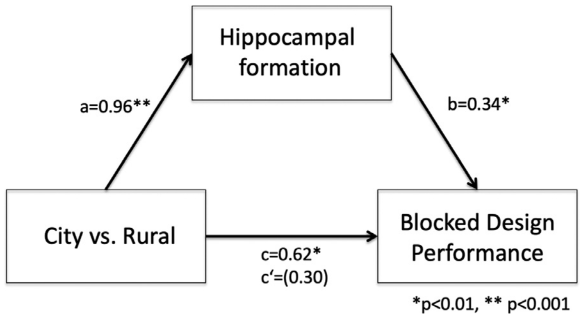

Fig. 4. Results of mediation analysis showing standardized regression coefficients with a*b = indirect effect, c = total effect, and c' = direct effect.

partialling out covariates of no interest otherwise the sample would have been to small). No significant association was found with the error or strategy score of the Spatial Working Memory task (error: $r(88)=-0.001$, p = 0.995; strategy: $r(88)=0.120$, p = 0.259).

Then we tested whether the association between urban vs. rural upbringing and Block Design performance was mediated by grey matter volume in the hippocampal formation. As *Fig. 4* illustrates, the standardized regression coefficients between urban vs. rural upbringing and hippocampal formation were statistically significant, as was the standardized regression coefficient between hippocampal formation and Block Design performance. The standardized indirect effect was (0.96)(0.34)=0.33. The significance of this indirect effect was determined using a bootstrapping procedure (10.000). The 95% confidence interval was computed by determining the indirect effects at the 2.5th and 97.5th percentiles. The bootstrapped unstandardized indirect effect was 0.87 and the 95% confidence interval ranged from 0.064 to 1.64. Thus the indirect effect was statistically significant. Therewith grey matter volume in the hippocampal formation could account for more than half of the total effect (52.6%).

Based on the previous findings of lower prefrontal cortex volume (Haddad et al., 2015; Lammeyer et al., 2019) and cortical thickness (Besteher et al., 2017) associated with urban upbringing and higher prefrontal volume associated with greenness during upbringing in children (Dadvand et al., 2018) we explored the present data before nonstationary smoothness and multiple comparison correction. In the VBM analysis we observed significant larger grey matter volume in a prefrontal cortex cluster (the only additional cluster that reached significance apart from the cluster in the hippocampal formation), namely in right superior frontal gyrus, spanning Brodmann areas 10, 9 and 46 (centre of gravity of the cluster was at the MNI coordinate 22, 54, 40 in mm; $\text{mean}_{\text{city}}$= 0.42, SD_{city}=0.061, $\text{mean}_{\text{rural}}$= 0.45, SD_{rural}=0.057; $F(1,92)=16.74$, $p = 0.000092$) in subjects with exclusive rural upbringing compared to urban controls but only in the absence of correction for non-stationary smoothness and multiple test correction. The grey matter volume in this prefrontal region was positively associated with grey matter volume in the cluster in left hippocampal formation (r(92)=0.372, $p<0.001$ when controlling for scanner site, sex, age, TIV and parental education).

When conducting a whole-brain surface-based morphometric analysis focussing on cortical thickness, as previously done (Besteher et al., 2017), we do not find any significant results. Neither with conventional thresholding nor when using a non-parametrical statistic, namely threshold-free cluster enhancement (TFCE) (Smith and Nichols, 2009).

3. DISCUSSION

The primary goal of the present study was to unravel differences in brain structure associated with exclusive rural vs. exclusive city upbringing during the first 14 years of life. In a whole brain VBM analysis we found that adolescents who were exclusively brought up in rural regions showed higher grey matter volume in the left hippocampal formation compared with adolescents exclusively brought up in cities. These results were confirmed in volumetric measurements derived from FreeSurfer, which also revealed that bilateral hippocampal as well as right amygdala volume was higher in adolescents with exclusive rural upbringing. This finding, in particular the amygdala volume finding fits well to a previous study showing that amygdala integrity was positively associated with amount of forest in a radius of 1 km around the residential address of older healthy adults living in Berlin (Kühn et al., 2017). Due to the fact that hippocampus has been repeatedly shown to be involved in spatial processing and navigation tasks (Kühn and Gallinat, 2014b), we tested for an association between performance in the Block Design task and a Spatial Working Memory task. Grey matter volume in the hippocampal formation that revealed differences between rural and city upbringing was positively associated with performance in the Block Design task. Moreover, adolescents brought up in rural regions outperformed those brought up in cities in the Block Design task. We interpret this correlation and the group difference as an argument in favour of an involvement of the observed grey matter cluster in spatial processing. Further evidence for this comes from a mediation analysis showing that hippocampal formation is indeed a mediator for the relationship between urban vs. rural upbringing and performance in the Block Design task, accounting for more than half of the total effect.

One potential explanation for the higher grey matter volume in the hippocampal formation and the higher spatial processing performance may be the fact that rural areas allow younger children to engage in more spatial exploration. A study conducted in Finland (Kyttä, 1997) found that 8–9 year olds in cities report less journeys outdoors as compared with children in small towns and rural villages. Rural children were more often free to go alone to spend their leisure time, come home from school alone etc. When parents were asked to indicate at what age children were allowed to go out alone this was lowest in rural villages (4.3 years), small towns (4.5 years) and highest in the city (5.6 years). This tendency of higher degrees of freedom, granted by caregivers, to move around for children in lower-density environments compared with children in high-density city environments has been reported multiple times in the literature (Jones, 2000; O'Brien et al., 2000). Since independent mobility is assumed to be essential for the development of cognitive representations of the environment (Neisser, 1980; Piaget and Inhelder, 1956) this may be an important advantage for children raised in rural areas. A study from 1985 has investigated models of the neighbourhoods built by 9–11 years old children. The study found that town children were able to reconstruct the geography of the complete town that was comparable across children from the same town, whereas city children reconstructed smaller areas concentrically around their home address (Dupre and O'Neil-Gilbert, 1985). A recent study on data of 442,195 people, in 38 countries confirmed the observation that individuals who grew up in cities perform worse in a navigation skill task compared to individuals who did not grow up in cities (Coutrot et al., 2019). Interestingly, the authors demonstrate, that the difference is particularly pronounced in countries with cities that have a grid like structure and therewith small Street Network Entropy.

Taken together the association between spatial task performance and grey matter volume in left hippocampal formation, as well as the group difference in spatial performance - given that independent mobility drives spatial skills - may hint at a plasticity interpretation of the observed brain structural differences between groups. This implies that rural upbringing may offer more learning opportunities for spatial skills and therefore may lead to brain structural increases in brain regions such as the hippocampal formation. However, since we only focused on brain data at a single time point a causal inference can not be drawn.

Another limitation is that the IMAGEN test battery did not include any classic semantic memory tests because the hippocampal formation is not only known to be involved in spatial but also in semantic memory processing.

However, a very interesting aspect is the parallel between the observed lower volume in hippocampal formation in adolescents exclusively brought up in cities and previous literature hinting at hippocampal deficits in individuals at risk of mental state (ARMS) (Fusar-Poli et al., 2011; Harrisberger et al., 2016; Smieskova et al., 2010). Likewise, twin studies have revealed differences in hippocampal volume in twins discordant for schizophrenia, suggesting that the hippocampus is subject to modulation by environmental factors (van Erp et al., 2004; van Haren et al., 2004). Therewith the present results of lower hippocampal formation volume in adolescent city dwellers may suggest a causal link between the previous observation of upbringing in cities as a risk factor for the development of psychosis (Pedersen and Mortensen, 2001) and smaller hippocampi which have likewise been identified in ARMS.

Unexpectedly, we did not replicate the previously reported association between city living and lower prefrontal cortex volume (Haddad et al., 2015; Lammeyer et al., 2019), or cortical thickness (Besteher et al., 2017) in adult populations and between greenness and higher prefrontal volume in children (Dadvand et al., 2018). However, before non-stationary smoothness correction we did observe a cluster in right superior frontal gyrus with higher volume in adolescents exclusively raised in rural regions. One possible explanation for the observed differences between studies could be the relatively high percentage of female participants in the present sample (74 vs. 32 males). Haddad and colleagues (Haddad et al., 2015) found the negative association between pACC and the urbanicity score solely in male participants, however the effect reported for DLPFC was present in both sexes. It should also be considered that prefrontal maturation is not complete in early adolescence (Mills et al., 2014), as imaged here, and may in fact be influenced by hippocampal integrity post adolescence (Bertolino et al., 1997). In line with this notion, we observed a positive correlation between hippocampus and prefrontal cortex grey matter volume in the clusters described above. Moreover, the calculation of the urbanicity score (Besteher et al., 2017; Haddad et al., 2015; Pedersen and Mortensen, 2001) assumes a constant gradient between cities with more than 100.000 inhabitants (coded as 3), towns with 10.000–100.000 inhabitants (coded as 2) and rural regions with less than 10.000 inhabitants (coded as 1), since it multiplies its coding with the years participants report to have lived in the respective living environment. This score will probably adequately capture differences in population density, but may obscure categorical differences in rural vs. urban upbringing such as differences in the freedom that children are granted explore their environment and therewith lead to different results than the present study.

A potential limitation of the study is the phrasing of the questionnaire asking for the years in which the children were brought up in "rural regions with less than 10.000 inhabitant", since this may not be an optimal definition or rurality. Moreover, it may vary between countries how borders of municipalities are defined and therefore the number of inhabitants per are may be affected. Therefore, future studies should rather rely on georeferencing data to objectively derive information about rurality of the surrounding of the participants' home address.

Future research may want to focus on investigating differences between the assumption of a continuum from urban to rural environments and a categorical differentiation between these different living contexts. Another important aspect to consider in further investigation is whether the observed associations with brain structure are caused by the *presence* of detrimental exposure in urban context e.g. air pollution, noise pollution, social stress due to population density (Tost et al., 2015) or rather caused by the *absence* of salutogenic influences such as exposure to nature including green spaces, water and free views onto the sky (Bowler et al., 2010; Volker and Kistemann, 2011).

4. CONCLUSION

The present study revealed brain structural differences in left hippocampal formation (as well as in bilateral hippocampus and right amygdala in a ROI-based analysis), with higher volumes in adolescents exclusively brought up in rural regions compared with those exclusively brought up in cities. The grey matter volume in the cluster of the left hippocampal formation was positively correlated to performance in a spatial processing task (Block Design). Moreover, group differences were observed in spatial processing performance (Rural>City). This may suggest that rural regions offer more opportunities for spatial exploration and therefore allow for improvements of spatial skills.

AUTHOR CONTRIBUTIONS

Study design: TB, AB, CB, EBQ, SD, HF, AG, HG, PG, AH, BI, JLM, MLPM, FN, DPO, TP, MNS, RW, GS, JG

Data acquisition: TB, AB, CB, EBQ, SD, HF, AG, HG, PG, AH, BI, JLM, MLPM, FN, DPO, TP, LP, SM, JHF, MNS, HW, RW, GS, JG

Data analysis: SK

Interpretation of results: SK, AML, JG

Manuscript preparation: SK, AML, JG

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