STRESS
2021, VOL. 24, NO. 4, 421–429
https://doi.org/10.1080/10253890.2020.1855420

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

Interaction of *FKBP5* variant rs3800373 and city living alters the neural stress response in the anterior cingulate cortex

Anja Richter[a,b], Mohammad Al-Bayati[c], Foteini Paraskevopoulou[c], Bernd Krämer[a], Jens C. Pruessner[d], Elisabeth B. Binder[e] and Oliver Gruber[a]

[a]Section for Experimental Psychopathology and Neuroimaging, Department of General Psychiatry, Heidelberg University, Heidelberg, Germany; [b]Department of Psychosis Studies, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, United Kingdom; [c]Center for Translational Research in Systems Neuroscience and Psychiatry, Department of Psychiatry and Psychotherapy, University Medical Center Göttingen, Göttingen, Germany; [d]Department of Psychology, University of Constance, Constance, Germany; [e]Department of Translational Psychiatry, Max Planck Institute of Psychiatry, Munich, Germany

ABSTRACT

Psychosocial stress effects of urban living are associated with substantially increased risk for schizophrenia, mood and anxiety disorders, by altering stress-induced activity in the amygdala and pregenual anterior cingulate cortex (ACC). Genetic factors are likely to modulate the impact of city living on stress processing. Growing evidence suggests a key role of *FKBP5*, a co-chaperone regulating the glucocorticoid receptor sensitivity, in the etiology of stress-related disorders. Here we investigated the interaction of city living and genetic variation in *FKBP5* (rs3800373) on neural activity in stress-sensitive brain systems. Functional magnetic resonance imaging was performed in 31 healthy young adults using the Montreal Imaging Stress Task. Subjects were divided into groups depending on the number of inhabitants of their current residency. There was a significant main effect of city living on neural activity in the amygdala-hippocampus complex, replicating prior findings. Moreover, we found an interaction between rs3800373 and city living modulating responses in the bilateral subgenual ACC and right pregenual ACC. Specifically, only city dwellers carrying the *FKBP5* minor risk allele showed increased stress responses in the subgenual and pregenual ACC when compared to those living in small towns. A significant gene-environment interaction on neural stress responses in the amygdala or hippocampus was only found in *FKBP5* major allele carriers. These results point to a potential role of the *FKBP5* rs3800373 minor risk allele in predisposing those who live in bigger cities to changes of functional responsivity in the pre- and subgenual ACC, thereby increasing the risk for developing stress-related mental disorders.

ARTICLE HISTORY
Received 6 July 2020
Accepted 20 November 2020

KEYWORDS
Gene x environment interaction; fMRI; montreal imaging stress task; urban upbringing; psychosocial stress

1. Introduction

Psychosocial stress effects of urban living lead to substantially increased risk for mood and anxiety disorders in city dwellers (Peen et al., 2010), and for schizophrenia in individuals born and raised in cities compared to those living in the countryside (Pedersen & Mortensen, 2001). Although urban living has benefits, e.g. better health care and employment opportunities (Dye, 2008), city dwellers are at greater risk for chronic diseases, e.g. asthma (Cyril et al., 2013), are confronted with a more stressful social environment and greater social isolation (Godfrey & Julien, 2005). In this context, urban living is seen as proxy for psychosocial stress (Lederbogen et al., 2013).

It has been demonstrated that individuals currently living in an urban environment showed increased amygdala activation in response to a cognitive-social stress task, while those individuals born and raised in cities exhibited increased activation in the perigenual anterior cingulate cortex (ACC) (Lederbogen et al., 2011). These brain structures are involved in stress regulation and show structural and functional alterations in patients with schizophrenia (Walton et al., 2018) and depression (Videbech & Ravnkilde, 2004). Chronic or repeated stress exposure has enduring effects on the brain through stimulation of the hypothalamus-pituitary-adrenal cortex (HPA) axis leading to the production of glucocorticoids by the adrenal cortex (Lupien et al., 2009; Ulrich-Lai & Herman, 2009). The hormones involved in the adaptation to acute stress can be pathogenic when the individual is permanently overexposed.

The gene FK506 binding protein 5 (*FKBP5*) is a key regulator of HPA axis responsiveness (Binder, 2009). It is a co-chaperone of the heat shock protein 90 and codes for the protein FK506 binding protein 5–51, which regulates glucocorticoid receptor (GR) sensitivity (Binder, 2009; Denny et al., 2000). Functional genetic variants in *FKBP5* such as rs3800373 have been shown to be associated with an increase in GR-induced *FKBP5* upregulation, which causes a *FKBP5*-mediated intracellular inhibition of GR that interrupt GR-dependent feedback of the HPA axis on a systemic level. In turn, this can lead to HPA axis dysregulation and stress-related phenotypes (Binder

CONTACT Oliver Gruber oliver.gruber@med.uni-heidelberg.de Section for Experimental Psychopathology and Neuroimaging, Department of General Psychiatry, Heidelberg University, Voßstr. 4, 69115 Heidelberg, Germany

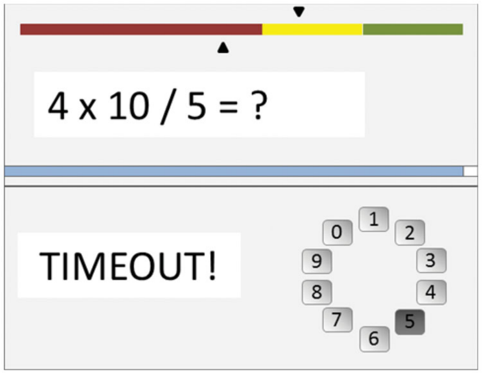

Figure 1. Graphical user interface of the Montreal Imaging Stress Task (MIST). Top: Colored bar shows the performance indicators (top arrow = average performance, bottom arrow = individual subject's performance), the mental arithmetic task, further down the progress bar reflects the imposed time limit. *Bottom:* text field for feedback, and the rotary dial for the response submission (Figure adapted from Dedovic et al., 2005).

et al., 2004). Previous studies showed that carrying the minor risk allele of single nucleotide polymorphism (SNP) rs3800373 is associated with greater risk of developing psychiatric disorders in adulthood when exposed to childhood trauma (Matosin et al., 2018; Zannas et al., 2016), and is further associated with alterations in amygdala, orbitofrontal and hippocampus volume (Hirakawa et al., 2016; Yun et al., 2020). Based on the proven functional relevance, SNP rs3800373 was selected for the final analysis.

So far, no study has investigated the interaction effect of *FKBP5* with city living on brain function. To bridge this gap, we examined the interaction of rs3800373 and urban living on functional activity of brain regions shown to be involved in the stress response, such as the amygdala and ACC (Lederbogen et al., 2011). On the basis that *FKBP5* risk alleles modulate GR sensitivity, we hypothesized increased neural activity patterns in the amygdala, hippocampus and ACC in subjects living in cities and carrying the minor risk allele.

2. Methods

2.1. Participants

The initial sample consisted of 42 healthy subjects who were recruited from the university environment in Goettingen, an university city with around 130.000 inhabitants in Lower Saxony, Germany. Of these, three participants had to be excluded due to excessive head movements (more than 3 mm) and eight participants were excluded because they did not pass MRI data quality control (e.g. image artifacts). FMRI data and *FKBP5* rs3800373 genotype was available for 31 subjects aged 18–31 years (mean age ± SD: 23.39 years ±3.26 years, 16 females). Information on use of nicotine, caffeine and alcohol were assessed in a semi-structured interview two hours before fMRI scanning. All participants were of European ancestry. Information on ancestry were collected by self-reports of each individual and were confirmed by a principal component analysis. Exclusion criteria were past or present neurological or psychiatric disorders according to ICD-10, a positive family history of psychiatric disorders, substance abuse during the last month, cannabis use during the last two weeks, mental retardation, dementia, metabolic diseases and pregnancy in women. The study was carried out in accordance with the latest version of the Declaration of Helsinki and all procedures in this study were approved by the Ethics Committee of the University Medical Center Göttingen. After complete description of all study procedures, participants gave written informed consent.

2.2. Experimental task

To investigate the effects of perceiving and processing psychosocial stress, all subjects performed the Montreal Imaging Stress Task (MIST, Dedovic et al., 2005; see Figure 1) during fMRI scanning (block design). All subjects were trained for three to five minutes before scanning. The MIST consists of a series of computerized mental arithmetic instructions combined with social evaluative threat components that were built into the program or presented by the investigator. The task consisted of three test conditions (rest, control and experimental) allowing the effects of stress and mental arithmetic to be investigated separately. In the rest condition, subjects were instructed to look at a static computer screen on which no tasks were presented. In the control condition, a series of mental arithmetic tasks were displayed on the computer screen, and subjects had to submit their answers by means of a response interface. A feedback appeared after each submitted response (correct, incorrect or timeout). In the experimental condition, difficulty and time limit of each task were manipulated in a way that they exceeded the individual's mental capacity. In addition to the tasks, information about individual and average performance as well as expected performance was displayed. To further increase the social evaluative threat of the situation, a negative feedback was provided after completion of each task. The experiment lasted approximately 45 minutes.

2.3. Urbanicity scores

According to the German Federal Institute for Research on Building, Urban Affairs and Spatial Development, three types of municipal corporations can be distinguished based on the number of inhabitants: big cities (>100k), middle town (>20k <100k), and small town or community (<20k) (Milbert, 2013). Following this classification, the current urbanicity (CU) of participants was determined according to their current residency. Every year of residence was multiplied by the value of related residency (CU score of 1: <20k; CU score of 2: between 20k and 100k; CU score of 3: >100k).

2.4. Genotyping and sample structure

Human DNA was isolated from saliva obtained from all participants and collected into Oragene saliva DNA kits (DNA Genotek) using the Gentra Puregene Blood kit (Qiagen) with standardized protocols. By using 400 ng of DNA, genome-wide SNP genotyping was conducted using Illumina OmniExpress Genotyping BeadChips according to the manufacture's standard protocols.

The SNP rs3800373 in the *FKBP5* gene was identified on the Illumina OmniExpress array. Minor allele (C) frequency of the *FKBP5* SNP rs3800373 was 0.33 in the sample. The observed genotype distribution did not deviate from Hardy-Weinberg equilibrium ($\chi^2=1.97$, $p=0.16$, 1 degree of freedom). To check for population outliers, we conducted a principal component analysis included in EIGENSOFT software (Price et al., 2006; https://www.hsph.harvard.edu/alkes-price/software/). The analysis showed that the subjects included in this study cluster together with HapMap3 European-descent populations. Therefore, it is unlikely that the findings of this study are due to population stratification.

2.5. Group classification

Based on individual genotypes, subjects were divided into two groups following the dominant model: homozygous major allele carriers (A/A; $n=14$) were compared with heterozygous minor allele carriers and homozygous minor allele carriers (TA/C and C/C; $n=17$). The dominant model was chosen, because this defines the extreme in terms of the power to detect the disease allele effect. In addition, all participants were divided into groups depending on the CU score, resulting in a group with CU score 1 and 2 ($n=20$) and a group with CU score 3 ($n=11$). For the factorial analysis, subjects with score 1 and 2 were assigned to the low CU group and with score 3 to the high CU group.

2.6. Statistical analyses

Demographic variables and behavioral data were analyzed using the software package SPSS (IBM SPSS statistics 23.0). Chi-squared tests were applied to determine differences between groups regarding sex, handedness and substance use (nicotine, caffeine, alcohol). Normal distribution of performance data was tested using the Kolmogorov-Smirnov test. To account for possible interaction effects, a repeated measures ANOVA was performed for performance data (percentage of correct responses in the experimental condition as compared to correct responses in the control condition) for each run as well as for overall performance using genotype and CU score as between-subject factor. Independent *t*-tests, Bonferroni-corrected for multiple comparisons, were used to test for differences between groups. The significance threshold was set to $p<0.05$ (two-sided).

2.7. Acquisition and analysis of arousal and stress measures

To assess cardiovascular response to the stress task, heart rate was continuously sampled and blood pressure was recorded before and after the session. Hormonal response was measured by saliva cortisol sampling, with samples taken before and after the stress induction. Subjective stress was measured via numerical rating scale (NRS) before and after the session, with a possible range of 0–10, "0" indicating absence of any stressor and "10" maximum stress intensity (Karvounides et al., 2016; von Baeyer et al., 2009; Debriefing was scheduled after the MRI scanning. Arousal and stress data were analyzed in one-sample *t*-tests to compare the experimental and control conditions using the software package SPSS (IBM SPSS statistics 23.0). The significance threshold was set to $p<0.05$ (two-sided).

2.8. FMRI data acquisition and analyses

The experiment was performed on a 3-Tesla Magnetom TIM Trio Siemens scanner (Siemens Healthcare, Erlangen, Germany) using a standard eight-channel phased-array head coil. First, T1-weighted anatomical data with 1 mm isotropic resolution were acquired. Second, thirty-seven axial slices were acquired in interleaved acquisition order (slice thickness = 3 mm; gap 20%) using a T2*-sensitive echo-planar imaging (EPI) sequence (interscan interval 2000 ms; echo time 30 ms; flip angle 70°; field-of-view 192 mm). In three functional runs, a total of 211 image volumes were acquired. Subjects responded via button presses on a fiber optic computer response device (Current Designs, Philadelphia, Pennsylvania, USA), and stimuli were viewed through goggles (Resonance Technology, Northridge, California, USA).

Functional images were preprocessed and analyzed with SPM 8 (Wellcome Trust Center for Neuroimaging, University College London, London, UK) using a general linear model. Preprocessing comprised coregistration, realignment and unwarping, corrections for slice-time acquisition differences and low-frequency fluctuations, normalization into standard stereotactic space [skull-stripped EPI template by the Montreal Neurological Institute (MNI)], and spatial smoothing with an isotropic Gaussian kernel filter of 9 mm full-width half-maximum. A vector representing the temporal onsets of stimulus presentation was convolved with a canonical hemodynamic response function to produce a predicted hemodynamic response to each experimental condition. Nine regressors went into the single subject analyses: rest, control and experimental condition, each for functional run 1, 2 and 3. Linear t-contrasts were defined for assessing the specific effects of each condition of interest. Single-subject contrast images were taken to the second level to assess group effects with random-effects analyses. Group effects were examined using a full factorial model with the factors "genotype" (2 groups: homozygous major allele carriers versus homo-/heterozygous minor allele carriers), "CU score" (2 groups: CU 1 and 2 versus 3), "task condition" (rest, control, experimental) and "functional run" (1, 2, 3).

A region of interest (ROI) approach was used to investigate group differences in brain regions previously shown to be involved in the neural stress response, namely the amygdala and ACC (Lederbogen et al., 2011). Small volume corrections (SVC) with a 4 mm sphere were applied using these individual ROIs. Coordinates for the subgenual ACC (±12 34 − 8; 8 20 − 8) pregenual ACC (±16 50 0) and amygdala/hippocampus (±24 − 14 − 18) were derived from neuroimaging studies, as determined by a meta-analysis conducted on the neurosynth.org platform (status September 2020). Within these ROIs, statistical inferences were made using a threshold of $p<0.05$ after family-wise error (FWE) correction for multiple comparisons. Results at $p < 0.05$ uncorrected level are reported for completeness.

Marsbar was used to extract mean blood oxygenation level-dependent (BOLD) beta values from 2 mm boxes at the reported maximal activation for each participant (Brett et al., 2002).

3. Results

3.1. Behavioral, demographic and stress data

Thirty-one subjects participated in this study (homozygous major allele A: 14 (8 females, 6 males); homo- and heterozygous minor allele C: 17 (7 females, 10 males)). Demographic characteristics of the sample are presented in Table 1. To preclude that differences across groups would be driven by age, sex, years of education and task performance instead of genotype and urbanicity score, we tested for statistical differences in these parameters (Table 1). Analyses of percentage of correct responses in the experimental condition compared to the control condition for each run separately revealed no significant main effect of genotype or urbanicity score ($p > 0.05$). However, analysis of overall performance (i.e. over all three experimental sessions) in the experimental condition vs. control condition showed an effect of urbanicity score ($F_{(1,30)}$=6.94, $p = 0.01$, partial η^2=0.39), indicating that only the analysis of performance over all three runs is sensitive enough to uncover an effect of urbanicity score.

Post hoc t-tests revealed better arithmetic performance in subjects with high CU score (85.94 ± 16.18) when compared to those with low CU score (73.99 ± 12.11) ($t_{(30)}$=2.33, $p = 0.03$, Hedges' g = 0.81). However, this finding did not survive correction for multiple comparisons which would correspond to $p < 0.0125$.

Statistical analysis of the online and offline stress measures revealed significantly elevated heart rate ($t_{(30)}$=8.45, $p < 0.001$, Hedges' g = 0.74), systolic and diastolic blood pressure ($t_{(30)}$= −3.90, $p = 0.001$, Hedges' g = 0.67; $t_{(30)}$= −3.00, $p = 0.005$, Hedges' g = 0.40), and subjective stress intensity ($t_{(30)}$= −5.24, $p < 0.001$, Hedges' g = 0.79) after stress induction in all participants. However, saliva cortisol levels did not significantly change over time ($t_{(30)}$= −0.45, $p = 0.66$, Hedges' g = 0.09) (see Table 2).

3.2. FMRI data

ROI analyses revealed a significant main effect of city living on BOLD response bilaterally in the amygdala-hippocampus complex during arithmetical calculations inducing social stress when compared to the control condition (right, $p = 0.014$; xyz = 26 − 16 − 20, Z = 2.20, k = 15, Hedges' g = 0.644; left, $p = 0.030$; xyz= −20 − 10 − 18, Z = 1.88, k = 6, Hedges' g = 0.529) and in the subgenual ACC (right, $p = 0.024$; xyz = 14 32 − 12, Z = 1.97, k = 15, Hedges' g = 0.533; left, $p = 0.014$; xyz= −16 30 − 10, Z = 2.19, k = 12, Hedges' g = 0.548; Table 3, Figure 2). Activation in these brain regions significantly increased with higher urbanicity level.

Furthermore, we found a significant G x E interaction of the *FKBP5* minor risk allele (C) with city living on neural stress responses in the subgenual and pregenual ACC. More specifically, only city dwellers carrying the *FKBP5* minor risk allele exhibited increased stress responses in the bilateral subgenual ACC (right, pFWE = 0.036; xyz = 14 36 − 10, Z = 2.44, k = 25, Hedges' g = 0.789; left, pFWE = 0.033; xyz= −16 34 − 8, Z = 2.47, k = 19, Hedges' g = 0.719) and right pregenual ACC ($p = 0.029$; xyz = 14 52 − 2, Z = 1.90, k = 15, Hedges' g = 0.636) when compared to minor allele carriers living in small towns (high CU > low CU), whereas this urbanicity-related effect was not present in *FKBP5* major allele carriers (Table 4, Figure 3).

There was an increase of stress-related activation in the bilateral amygdala-hippocampus complex in major allele carriers (A/A) living in bigger cities compared to those living in rural areas (high CU > low CU, right, pFWE = 0.041; xyz = 26 − 16 − 16, Z = 2.38, k = 55, Hedges' g = 1.026; left, pFWE = 0.036; xyz= −22 − 10 − 20, Z = 2.71, k = 68, Hedges' g = 0.902; Table 4), confirming the significant main effect of city living on amygdala-hippocampus responsivity observed in prior studies (Lederbogen et al., 2011). We only detected a significant G x E interaction effect of the *FKBP5* minor risk allele with city living on amygdala-hippocampus complex activity on a subthreshold level.

4. Discussion

The present study investigated the impact of urban environment and genetic variation in the *FKBP5* gene on functional characteristics of stress-sensitive brain regions. We found a significant main effect of city living on functional responses in the amygdala-hippocampus complex and subgenual ACC, which replicates prior results indicating increasing activity with higher CU (Lederbogen et al., 2011). More importantly, we demonstrated a significant interaction of the *FKBP5* rs3800373 genotype and city living with respect to functional responsivity of bilateral subgenual ACC and right pregenual ACC. Specifically, only *FKBP5* rs3800373 minor allele carriers living in bigger cities - when compared to those living in small towns - showed increased activity in the bilateral subgenual and right pregenual ACC in response to cognitive stress (MIST, Dedovic et al., 2005). In major allele carriers, stress-related activity increased with the level of CU in the amygdala-hippocampus complex.

The finding of elevated amygdala-hippocampus and subgenual ACC activity with increasing CU in response to social stress during arithmetical calculation replicates a previous study using the MIST (Lederbogen et al., 2011), and a study investigating the effect of urban environment on stress- and reward-related activity in the mesolimbic system (Krämer et al., 2017). Both amygdala and hippocampus play a key role in regulating the response to stress. Exposure to stress leads to an increase of cortisol levels stimulating the amygdala that exerts positive feedback over the HPA axis. The hippocampus and prefrontal cortex, in turn, provide negative feedback to the HPA axis enabling self-regulation of the stress response (Dedovic, Duchesne, et al., 2009; Herman

Table 1. Demographic data, current urbanicity scores and task performance of all subjects ($n = 31$).

	All participants ($n = 31$)	A/A carriers ($n = 14$)	A/C and C/C carriers ($n = 17$)	T or $\chi 2$	p value	g or φ
Age (in years)	23.39 ± 3.21	24.36 ± 3.32	22.59 ± 2.98	1.56	0.13[a]	0.55
Sex, N	15 F/16 M	8 F/6 M	7 F/10 M	0.78	0.38[b]	0.16
Education (in years)	15.52 ± 2.26	16.07 ± 2.50	15.06 ± 2.01	1.25	0.22[a]	0.44
Handedness, N	29 R/2 L	13 R/1 L	17 R/1 L	0.02	0.89[b]	0.03
Nicotine, N^+	2	0	2	1.76	0.19[b]	0.24
Caffeine, N^+	3	0	3	2.74	0.10[b]	0.30
Alcohol, N^+	0	0	0	-	1.00[b]	–
CU $1 + 2/3$	20/11	7/7	13/4	2.35	0.13[b]	0.28
Overall task performance (in %)	78.23 ± 14.62	76.58 ± 16.54	79.58 ± 13.21	1.89	0.58[a]	0.20

Abbreviations: CU: Current urbanicity score; F: female; g or φ: Hedges' g or phi; L: left; M: male; R: right.
Data are presented as mean ± standard deviation.
[a]p values derived from t-tests between groups.
[b]Chi-squared tests were performed between groups.
$^+$Nicotine, caffeine or alcohol use during the last two hours before fMRI scanning.

Table 2. Effect of stress induction on study participants.

	Before stress	Stress induction	T	p value[a]	g or φ
Heart rate (beats per minute)	84.04 ± 12.37	94.59 ± 15.00	8.45	<0.001	0.74
Blood pressure (mmHg)					
Systolic	132.26 ± 13.57	141.29 ± 14.82	-3.90	0.001	0.67
Diastolic	75.10 ± 10.00	79.74 ± 10.97	-3.00	0.005	0.40
Saliva cortisol concentration (ng/ml)	3.87 ± 1.50	4.08 ± 2.99	-0.45	0.66	0.09
Subjective stress intensity	4.00 ± 2.11	5.92 ± 2.70	-5.24	<0.001	0.79

Abbreviations: g or φ , Hedges' g or phi.
Data are presented as mean ± standard deviation.
[a]p values derived from t-tests before and after stress induction.

Table 3. Main effect of city living on neural stress responses in *a priori* regions of interest (experimental condition compared to the control condition).

	MNI coordinates						
Brain area	x	y	z	k	T	Z	Voxel-wise p value
	Main effect of city living						
R amygdala-hippocampus complex	26	-16	-20	15	2.21	2.20	0.014
L amygdala-hippocampus complex	-20	-10	-18	6	1.88	1.88	0.030
R subgenual ACC	14	32	-12	15	1.98	1.97	0.024
L subgenual ACC	-16	30	-10	12	2.20	2.19	0.014

Abbreviations: ACC: anterior cingulate cortex; l: left; r: right.
$p < 0.05$, uncorrected.

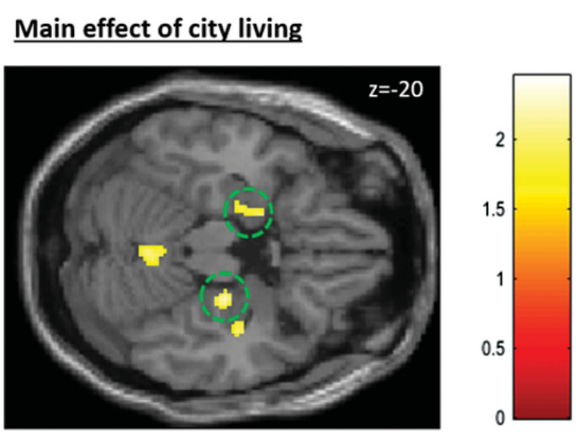

Figure 2. Main effect of city living on neural stress responses. Increasing activation in the amygdala-hippocampus complex with increasing urbanicity score (experimental condition > control condition). Activation was thresholded at $p < 0.05$, uncorrected for illustration purposes.

et al., 2003; Ulrich-Lai & Herman, 2009). In vivo animal studies have provided evidence that chronic stress exposure alters the morphology (Joels et al., 2007) and enhances the excitability of amygdala neurons (Rosenkranz et al., 2010). Increased amygdala activation has commonly been observed in stress-related disorders, such as depression (Drevets et al., 2008). Greater hippocampal activity has been linked to inhibition of the emotional response to stress (Dedovic, Rexroth, et al., 2009), suggesting a central role of both brain regions in stress vulnerability.

In addition, we found a significant interaction effect of the *FKBP5* minor risk allele and city living in the pre- and subgenual ACC during arithmetical calculations. It is assumed that rs3800373 minor allele carriers exhibit a higher expression of *FKBP5* and, thus, a higher cortisol level due to slower recovery after stress (Binder et al., 2004; Linnstaedt et al., 2018). In line with this assumption, comparison of rs3800373 risk allele carriers living in big cities to those living in small towns demonstrated increased reactivity in the right pregenual ACC as well as bilaterally in the subgenual ACC. The subgenual ACC plays a central role in regulating emotion and arousal (Bush et al., 2000; Rudebeck et al., 2014), and has been implicated in the neurobiology of depression and other affective disorders (Davidson et al., 2002). Gotlib et al. (2005) demonstrated greater activation in the subgenual ACC to both negative and positive emotional stimuli in patients with major depressive disorder when compared to nondepressed controls. Furthermore, both subgenual and pregenual ACC have been

Table 4. Brain activations in *a priori* regions of interest modulated by genotype and current urbanicity in the experimental condition compared to the control condition.

Brain area	MNI coordinates x	y	z	k	T	Z	Voxel-wise p value
	C carriers High CU > Low CU						
R pregenual ACC	14	52	−2	15	1.90	1.90	0.029
R subgenual ACC	14	36	−10	25	2.46	2.44	0.036*
L subgenual ACC	−16	34	−8	19	2.49	2.47	0.033*
	C carriers High CU < Low CU						
Not significant							
	A/A carriers High CU > Low CU						
R amygdala-hippocampus complex	26	−16	−16	55	2.40	2.38	0.041*
L amygdala-hippocampus complex	−22	−10	−20	68	2.71	2.69	0.036*
	A/A carriers High CU < Low CU						
Not significant							

Abbreviations: ACC: anterior cingulate cortex; l: left; r: right.
$p < 0.05$, uncorrected; *$p < 0.05$, FWE-corrected for small volume.

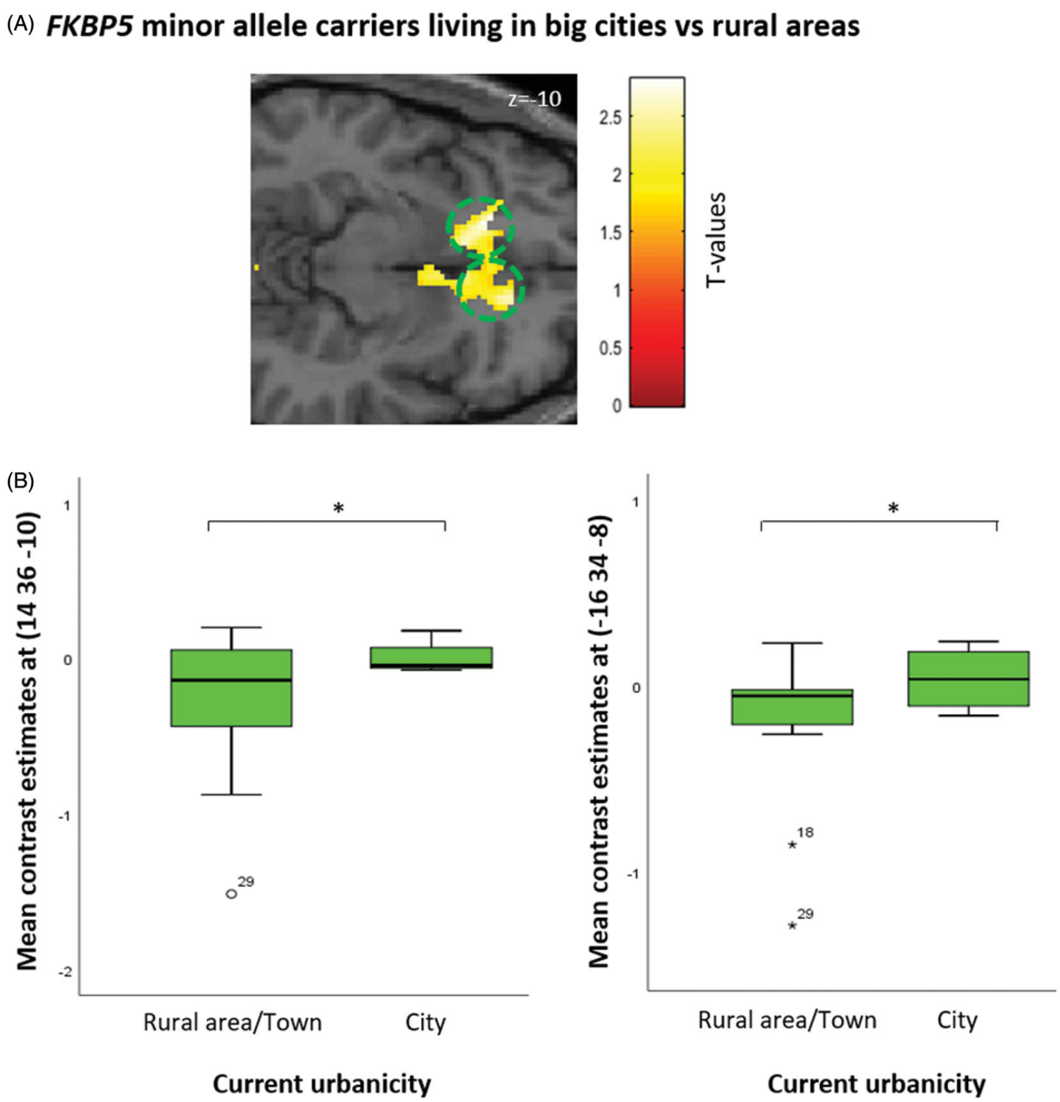

Figure 3. Interaction effect of *FKBP5* minor risk allele carriers and city living on neural stress responses. (A) Minor allele carriers: subjects living in big cities compared to subjects living in rural areas/towns showed an increased activation in the pregenual ACC and subgenual ACC (experimental condition > control condition). Activation was thresholded at $p < 0.05$, uncorrected for illustration purposes. (B) Box plots show mean contrast estimates at the local activation maxima in the subgenual ACC in minor allele carriers (right: x = 14, y = 36, z = −10; left: x = 16, y = 34, z = −8) for the experimental compared to the control condition. (*$p < 0.05$).

shown to be a significant regulatory region for amygdala activity (LeDoux, 2000), and may thereby modulate HPA activation via the amygdala (Herman et al., 2003). The pregenual ACC is further involved in processing chronic social stressors such as social defeat. Abnormalities in connectivity between the pregenual ACC and amygdala during processing of affectively negative stimuli have been shown in patients with schizophrenia, but not in individuals at genetic risk for psychosis (Rasetti et al., 2009), indicating a link to environmental factors. A previous study reported that urban upbringing was associated with reduced functional connectivity between pregenual ACC and amygdala, while CU showed no effect (Lederbogen et al., 2011). This may indicate that exposure to urban environment early in life exclusively has an effect on these functionally close brain regions which needs to be addressed in future studies. The present findings provide evidence that carrying the *FKBP5* minor risk allele, associated with higher cortisol levels, together with exposure to social stress, as expressed by higher urbanicity scores, may collectively increase functional responsivity in brain regions regulating the HPA axis. Taken together, the increased functional activity in the pre- and subgenual ACC with increasing urbanicity score may represent, in part, *FKBP5*-mediated sensitization of neural systems to stress.

Finally, we showed increased activation in the amygdala-hippocampus complex both in *FKBP5* major allele carriers and – although at a subthreshold level – also in minor risk allele carriers living in big cities when compared to the respective control groups living in rural environments. The weak interaction effect between minor risk allele carriers living in an urban environment on amygdala-hippocampus activation might partly be explained by the limited number of participants, and needs to be addressed in future studies with larger genotype group sizes. These findings, however, confirm the significant main effect of city living on amygdala-hippocampus responsivity observed in previous studies (Krämer et al., 2017; Lederbogen et al., 2011).

The MIST (Dedovic et al., 2005) has been proven to induce stress-related effects on brain activity and physiological measures (Lederbogen et al., 2011). Indeed, we found altered activity in the amygdala-hippocampus complex and ACC after stress induction, as well as increased heart rate, blood pressure and subjective experiences of stress. Although participants showed a nominally increase in saliva cortisol concentration, the difference before and after stress induction was not statistically significant. This might partly be explained by the well-known interindividual variability in HPA axis stress responses (Kudielka et al., 2009). In addition, a weak cortisol response does not necessarily exclude a prior robust central stress response, as we found a significant cardiovascular and subjective stress response. Cortisol response to stress is an important validation criterion, but it might be that the observed weak endocrine response is related to HPA axis anticipation effects associated with the upcoming, unfamiliar MRI procedure. Indeed, another fMRI study also reported modest endocrine responses to stress using the MIST (Pruessner et al., 2008).

The moderate sample size and its limited statistical power are major limitations of the present study. Consequently, we decided to test only a single gene variant and effects in ROIs defined *a priori*. Therefore, all reported findings can only be regarded as preliminary and warrants confirmation in future studies with much bigger samples. However, some aspects of our study may at least partly counterbalance this limitation. We used a well-established experimental stress task (MIST, Dedovic et al., 2005) and a promising intermediate phenotype approach. It has been shown that intermediate phenotypes are important tools to target brain mechanisms of psychopathology even in limited samples (Mier et al., 2010). In line with this, a previous study with a comparable sample size ($n = 42$) found an interaction between a functional variant in the neuropeptide S receptor 1 gene and urban living in the amygdala (Streit et al., 2014). In addition, even with this moderate sample size we successfully replicated the finding of increased activity in the amygdala/hippocampus with increasing CU (Krämer et al., 2017; Lederbogen et al., 2011). As we only investigated one genetic variant, we are not able to estimate the overall size of the genetic effect of *FKBP5* on our phenotypes. Nevertheless, considering the functional characteristics of rs3800373 and its proven link to inadequate normalization of cortisol secretion in healthy individuals after psychosocial stress (Ising et al., 2008; White et al., 2012), it is assumable that differences in *FKBP5*-induced signaling modulate the link between urban upbringing and stress processing in the ACC.

Urbanicity, as defined by population size, represents a nonspecific factor with effects on brain function that may be modified by other influences such as socioeconomic status or exposure to stress and adversity in childhood. However, the current sample consists of relatively high functioning university students and all participants were screened for any history of mental illness. Although we cannot entirely preclude that the observed effects were not confounded by other environmental influences early in life, epidemiological studies provided evidence for urbanicity effects to be independent of socioeconomic factors and selective migration (Krabbendam & Van Os, 2005). In future research we aim to clarify whether the observed changes of brain function related to HPA axis genetic variation and urbanicity predict later psychopathology (Binder et al., 2004; 2010).

5. Conclusion

The presented findings not only confirm a previously reported effect of city living on increased brain responses in the amygdala-hippocampus complex, but more importantly also provide new evidence for a G x E interaction between *FKBP5* genotype and CU in modulating stress-related responses in the pregenual and subgenual ACC, emphasizing the importance of these brain regions in understanding stress-related psychopathology. A significant G x E interaction on responses in the amygdala-hippocampus complex was only found in FKBP5 major allele carriers. These results suggest that genetic variation leading to differences in HPA axis function may increase individual's vulnerability to urbanicity-related potentiation of stress-related functional responses in the pre- and subgenual ACC, which in turn may promote future psychopathology when exposed to further stress.

Acknowledgements

We thank Peter Dechent and the staff of the unit "MR-Research in Neurology and Psychiatry" at the University Medical Center Göttingen (Germany) for their valuable work with MRI data acquisition. We are very thankful to all study participants.

Disclosure statement

The authors report no conflicts of interest.

References

Binder, E. B. (2009). The role of FKBP5, a co-chaperone of the glucocorticoid receptor in the pathogenesis and therapy of affective and anxiety disorders. Psychoneuroendocrinology, *34*(Suppl 1), S186–S195. https://doi.org/10.1016/j.psyneuen.2009.05.021

Binder, E. B., Owens, M. J., Liu, W., Deveau, T. C., Rush, A. J., Trivedi, M. H., Fava, M., Bradley, B., Ressler, K. J., & Nemeroff, C. B. (2010). Association of polymorphisms in genes regulating the corticotropin-releasing factor system with antidepressant treatment response. *Archives of General Psychiatry*, *67*(4), 369–379. https://doi.org/10.1001/archgenpsychiatry.2010.18

Binder, E. B., Salyakina, D., Lichtner, P., Wochnik, G. M., Ising, M., Pütz, B., Papiol, S., Seaman, S., Lucae, S., Kohli, M. A., Nickel, T., Künzel, H. E., Fuchs, B., Majer, M., Pfennig, A., Kern, N., Brunner, J., Modell, S., Baghai, T., … Muller-Myhsok, B. (2004). Polymorphisms in FKBP5 are associated with increased recurrence of depressive episodes and rapid response to antidepressant treatment. *Nature Genetics*, *36*(12), 1319–1325. https://doi.org/10.1038/ng1479

Brett, M., Anton, J., Valabregue, R., & Poline, J. B. (2002). Region of interest analysis using an SPMtoolbox. *Neuroimage*, *16*(Suppl 1), 1141.

Bush, G., Luu, P., & Posner, M. I. (2000). Cognitive and emotional influences in anterior cingulate cortex. *Trends in Cognitive Sciences*, *4*(6), 215–222. https://doi.org/10.1016/S1364-6613(00)01483-2

Cyril, S., Oldroyd, J. C., & Renzaho, A. (2013). Urbanisation, urbanicity, and health: A systematic review of the reliability and validity of urbanicity scales. *BMC Public Health*, *13*, 513. https://doi.org/10.1186/1471-2458-13-513

Davidson, W. C., Pizzagalli, D., Nitschke, J. B., & Putnam, K. (2002). Depression: Perspectives from affective neuroscience. *Annual Review of Psychology*, *53*, 545–574. https://doi.org/10.1146/annurev.psych.53.100901.135148

Dedovic, K., Duchesne, A., Andrews, J., Engert, V., & Pruessner, J. C. (2009). The brain and the stress axis: The neural correlates of cortisol regulation in response to stress. NeuroImage, *47*(3), 864–871. https://doi.org/10.1016/j.neuroimage.2009.05.074

Dedovic, K., Renwick, R., Mahani, N., Khalili, E., Veronika, L., Sonia, J., & Pruessner, J. C. (2005). The Montreal Imaging Stress Task: Using functional imaging to investigate the effects of perceiving and processing psychosocial stress in the human brain. *Journal of Psychiatry & Neuroscience: Jpn*, *30*(5), 319–325.

Dedovic, K., Rexroth, M., Wolff, E., Duchesne, A., Scherling, C., Beaudry, T., Lue, S. D., Lord, C., Engert, V., & Pruessner, J. C. (2009). Neural correlates of processing stressful information: An event-related fMRI study. *Brain Research*, *1293*, 49–60. https://doi.org/10.1016/j.brainres.2009.06.044

Denny, W. B., Valentine, D. L., Reynolds, P. D., Smith, D. F., & Scammel, J. G. (2000). Squirrel monkey immunophilin FKBP51 is a potent inhibitor of glucocorticoid receptor binding. Endocrinology, *141*(11), 4107–4113. https://doi.org/10.1210/endo.141.11.7785

Drevets, W. C., Price, J. L., & Furey, M. L. (2008). Brain structural and functional abnormalities in mood disorders: Implications for neurocircuitry models of depression. *Brain Structure & Function*, *213*(1–2), 93–118. https://doi.org/10.1007/s00429-008-0189-x

Dye, C. (2008). Health and urban living. *Science (New York, N.Y.)*, *319*(5864), 766–769. https://doi.org/10.1126/science.1150198

Godfrey, R., & Julien, M. (2005). Urbanisation and health. *Clinical Medicine (London, England)*, *5*(2), 137–141. https://doi.org/10.7861/clinmedicine.5-2-137

Gotlib, I. H., Sivers, H., Gabrieli, J. D. E., Whitfield-Gabrieli, S., Goldin, P., Minor, K. L., & Canli, T. (2005). Subgenual anterior cingulate activation to valenced emotional stimuli in major depression. *Neuroreport*, *7*(16), 1731–1734.

Herman, J. P., Figueiredo, H., Mueller, N. K., Ulrich-Lai, Y., Ostrander, M. M., Choi, D. C., & Cullinan, W. E. (2003). Central mechanisms of stress integration: Hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. *Frontiers in Neuroendocrinology*, *24*(3), 151–180. https://doi.org/10.1016/j.yfrne.2003.07.001

Hirakawa, H., Akiyoshi, J., Muronaga, M., Tanaka, Y., Ishitobi, Y., Inoue, A., Oshita, H., Aizawa, S., Masuda, K., Higuma, H., Kanehisa, M., Ninomiya, T., & Kawano, Y. (2016). FKBP5 is associated with amygdala volume in the human brain and mood state: A voxel-based morphometry (VBM) study. *International Journal of Psychiatry in Clinical Practice*, *20*(2), 106–115. https://doi.org/10.3109/13651501.2016.1144772

Ising, M., Depping, A.-M., Siebertz, A., Lucae, S., Unschuld, P. G., Kloiber, S., Horstmann, S., Uhr, M., Müller-Myhsok, B., & Holsboer, F. (2008). Polymorphisms in the FKBP5 gene region modulate recovery from psychosocial stress in healthy controls. *The European Journal of Neuroscience*, *28*(2), 389–398. https://doi.org/10.1111/j.1460-9568.2008.06332.x

Joels, M., Karst, H., Krugers, H. J., & Lucassen, P. J. (2007). Chronic stress: Implications for neuronal morphology, function and neurogenesis. *Frontiers in Neuroendocrinology*, *28*(2–3), 72–96. https://doi.org/10.1016/j.yfrne.2007.04.001

Karvounides, D., Simpson, P. M., Davies, W. H., Khan, K. A., Weisman, S. J., & Hainsworth, K. R. (2016). Three studies supporting the initial validation of the stress numerical rating scale-11 (Stress NRS-11): A single item measure of momentary stress for adolescents and adults. *Pediatric Dimensions*, *1*(4), 105–109. https://doi.org/10.15761/PD.1000124

Krabbendam, L. & Van Os, J.(2005). Schizophrenia and urbanicity: A major environmental influence–conditional on genetic risk. *Schizophrenia Bulletin*, *31*(4), 795–799. https://doi.org/10.1093/schbul/sbi060

Krämer, B., Diekhof, E. K., & Gruber, O. (2017). Effects of city living on the mesolimbic reward system-An fmri study. *Human Brain Mapping*, *38*(7), 3444–3453. https://doi.org/10.1002/hbm.23600

Kudielka, B. M., Hellhammer, D. H., & Wüst, S. (2009). Why do we respond so differently? Reviewing determinants of human salivary cortisol responses to challenge. Psychoneuroendocrinology, *34*(1), 2–18. https://doi.org/10.1016/j.psyneuen.2008.10.004

Lederbogen, F., Haddad, L., & Meyer-Lindenberg, A. (2013). Urban social stress-risk factor for mental disorders. The case of schizophrenia. *Environmental Pollution (Barking, Essex: 1987)*, *183*, 2–6. https://doi.org/10.1016/j.envpol.2013.05.046

Lederbogen, F., Kirsch, P., Haddad, L., Streit, F., Tost, H., Schuch, P., Wüst, S., Pruessner, J. C., Rietschel, M., Deuschle, M., & Meyer-Lindenberg, A. (2011). City living and urban upbringing affect neural social stress processing in humans. Nature, *474*(7352), 498–501. https://doi.org/10.1038/nature10190

LeDoux, J. E. (2000). Emotion circuits in the brain. *Annual Review of Neuroscience*, *23*, 155–184. https://doi.org/10.1146/annurev.neuro.23.1.155

Linnstaedt, S. D., Riker, K. D., Rueckeis, C. A., Kutchko, K. M., Lackey, L., McCarthy, K. R., Tsai, Y.-H., Parker, J. S., Kurz, M. C., Hendry, P. L., Lewandowski, C., Datner, E., Pearson, C., O'Neil, B., Domeier, R., Kaushik, S., Laederach, A., & McLean, S. A. (2018). A functional riboSNitch in the 3' Untranslated Region of FKBP5 Alters MicroRNA-320a Binding Efficiency and Mediates Vulnerability to Chronic Post-Traumatic Pain. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *38*(39), 8407–8420. https://doi.org/10.1523/JNEUROSCI.3458-17.2018

Lupien, S. J., McEwen, B. S., Gunnar, M. R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behavior and cognition. *Nature Reviews. Neuroscience*, *10*(6), 434–445. https://doi.org/10.1038/nrn2639

Matosin, N., Halldorsdottir, T., & Binder, E. B. (2018). Understanding the molecular mechanisms underpinning gene by environment interactions in psychiatric disorders: The FKBP5 model. *Biological Psychiatry*, *83*(10), 821–830. https://doi.org/10.1016/j.biopsych.2018.01.021

Mier, D., Kirsch, P., & Meyer-Lindenberg, A. (2010). Neural substrates of pleiotropic action of genetic variation in COMT: A meta-analysis. *Molecular Psychiatry*, *15*(9), 918–927. https://doi.org/10.1038/mp.2009.36

Milbert, A. (2013). *BBSR homepage – Stadt- und Gemeindetyp. Stadt- und Gemeindetypen in Deutschland*. http://www.bbsr.bund.de/BBSR/DE/

Raumbeobachtung/Raumabgrenzungen/StadtGemeindetyp/StadtGemeindetyp_node.html

Pedersen, C. B., & Mortensen, P. B. (2001). Evidence of a dose-response relationship between urbanicity during upbringing and schizophrenia risk. *Archives of General Psychiatry*, *58*(11), 1039–1046. https://doi.org/10.1001/archpsyc.58.11.1039

Peen, J., Schoevers, R. A., Beekman, A. T., & Dekker, J. (2010). The current status of urban-rural differences in psychiatric disorders. *Acta Psychiatrica Scandinavica*, *121*(2), 84–93. https://doi.org/10.1111/j.1600-0447.2009.01438.x

Price, A. L., Patterson, N. J., Plenge, R. M., Weinblatt, M. E., Shadick, N. A., & Reich, D. (2006). Principal components analysis corrects for stratification in genome-wide association studies . *Nature Genetics*, *38*(8), 904–909. https://doi.org/10.1038/ng1847

Pruessner, J. C., Dedovic, K., Khalili-Mahani, N., Engert, V., Pruessner, M., Buss, C., Renwick, R., Dagher, A., Meaney, M. J., & Lupien, S. (2008). Deactivation of the limbic system during acute psychosocial stress: Evidence from positron emission tomography and functional magnetic resonance imaging studies. *Biological Psychiatry*, *63*(2), 234–240. https://doi.org/10.1016/j.biopsych.2007.04.041

Rasetti, R., Mattay, V. S., Wiedholz, L. M., Kolachana, B. S., Hariri, A. R., Callicott, J. H., Meyer-Lindenberg, A., & Weinberger, D. R. (2009). Evidence that altered amygdala activity in schizophrenia is related to clinical state and not genetic risk. *The American Journal of Psychiatry*, *166*(2), 216–225. https://doi.org/10.1176/appi.ajp.2008.08020261

Rosenkranz, J. A., Venheim, E. R., & Padival, M. (2010). Chronic stress causes amygdala hyperexcitability in rodents. *Biological Psychiatry*, *67*(12), 1128–1136. https://doi.org/10.1016/j.biopsych.2010.02.008

Rudebeck, P. A., Putnam, P. T., Daniels, T. E., Yang, T., Mitz, A. R., Rhodes, S. E. V., & Murray, E. A. (2014). A role for primate subgenual cingulate cortex in sustaining autonomic arousal. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(14), 5391–5396. https://doi.org/10.1073/pnas.1317695111

Streit, F., Haddad, L., Paul, T., Frank, J., Schäfer, A., Nikitopoulos, J., Akdeniz, C., Lederbogen, F., Treutlein, J., Witt, S., Meyer-Lindenberg, A., Rietschel, M., Kirsch, P., & Wüst, S. (2014). A functional variant in the neuropeptide S receptor 1 gene moderates the influence of urban upbringing on stress processing in the amygdala. *Stress (Amsterdam, Netherlands)*, *17*(4), 352–361. https://doi.org/10.3109/10253890.2014.921903

Ulrich-Lai, Y. M., & Herman, J. P. (2009). Neural regulation of endocrine and autonomic stress responses. *Nature Reviews. Neuroscience*, *10*(6), 397–409. https://doi.org/10.1038/nrn2647

Videbech, P., & Ravnkilde, B. (2004). Hippocampal volume and depression: A meta-analysis of MRI studies. *The American Journal of Psychiatry*, *161*(11), 1957–1966. https://doi.org/10.1176/appi.ajp.161.11.1957

von Baeyer, C. L., Spagrud, L. J., McCormick, J. C., Choo, E., & Neville, K. (2009). Three new datasets supporting use of the numerical rating scale (NRS-11) for children's self-reports of pain intensity. *Pain*, *143*, 223–227. https://doi.org/10.1016/j.pain.2009.03.002

Walton, E., Hibar, D. P., van Erp, T. G. M., Potkin, S. G., Roiz-Santiañez, R., Crespo-Facorro, B., Suarez-Pinilla, P., van Haren, N. E. M., de Zwarte, S. M. C., Kahn, R. S., Cahn, W., Doan, N. T., Jørgensen, K. N., Gurholt, T. P., Agartz, I., Andreassen, O. A., Westlye, L. T., Melle, I., Berg, A. O., … Ehrlich, S. (2018). Prefrontal cortical thinning links to negative symptoms in schizophrenia via the ENIGMA consortium. *Psychological Medicine*, *48*(1), 82–94. https://doi.org/10.1017/S0033291717001283

White, M. G., Bogdan, R., Fisher, P. M., Munoz, K., Williamson, D. E., & Hariri, A. R. (2012). FKBP5 and emotional neglect interact to predict individual differences in amygdala reactivity. *Genes, Brain, and Behavior*, *11*(7), 869–878. https://doi.org/10.1111/j.1601-183X.2012.00837.x

Yun, J.-Y., Jin, M. J., Kim, S., & Lee, S.-H. (2020). Stress-related cognitive style is related to volumetric change of the hippocampus and FK506 binding protein 5 polymorphism in post-traumatic stress disorder. *Psychological Medicine*, 1–12. https://doi.org/10.1017/S0033291720002949

Zannas, A. S., Wiechmann, T., Gassen, N. C., & Binder, E. B. (2016). Gene-stress-epigenetic regulation of FKBP5: Clinical and translational implications. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *41*(1), 261–274. https://doi.org/10.1038/npp.2015.235