Stress and the City: Impact of Urban Upbringing on the (re)Activity of the Hypothalamus-Pituitary-Adrenal Axis

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Objective: Urbanization is a major challenge for the 21st century with a significant impact on health; mental health, in particular, can be negatively affected. The mechanisms linking urban living to psychopathology, however, remain unclear. We tested the hypothesis that urban upbringing may alter the activity of the hypothalamus-pituitary-adrenal (HPA) axis, one of the body's major stress response systems. **Methods:** In three independent experiments ($n = 248$ in total), we measured the changes in cortisol, the end-product of the HPA axis, in response to different stress tasks (memory recall with critical social evaluation [Experiment 1] or Socially Evaluated Cold Pressor Test [Experiment 2] and to awakening in participants raised in cities or more rural areas. **Results:** Urban upbringing was associated with elevated cortisol responses to acute stress (task × time point of measurement × urbanicity interaction: $F(2,132) = 3.10$ [$p = .048$] in Experiment 1 and $F(2,112) = 3.29$ [$p = .041$] in Experiment 2) but with a blunted cortisol awakening response (time point of measurement × urbanicity interaction: $F(1,114) = 4.00$, $p = .048$). The autonomic stress response, as indicated by blood pressure measurements, was not affected by urban upbringing. Moreover, current city living was not associated with any changes in the physiological responses to stress or awakening. **Conclusions:** Our findings suggest that urban upbringing changes the (re)activity of the HPA axis. Given that changes in HPA axis regulation have been associated with several psychiatric disorders, this may represent a mechanism that contributes to the increased risk for psychopathology in urban populations. **Key words:** stress, HPA axis, glucocorticoids, cortisol, cortisol awakening response, urbanization.

HPA = hypothalamus-pituitary-adrenal; **BMI** = body mass index.

INTRODUCTION

Urbanization is one of the major global trends in the 21st century. Thirty years ago, approximately 40% of the world's population was living in cities, but by 2050, this percentage will grow to 70% (1). This trend toward urbanization is accompanied by social, economic, and environmental challenges and has a significant impact on health. Although urban living provides some health benefits (2), it is also associated with health risks. In particular, mental health may be negatively affected by urbanization. For instance, the prevalence of mood and anxiety disorders is significantly higher in city dwellers than in rural dwellers (3,4). The risk for schizophrenia is even doubled in people who grew up in a city (5), and there is evidence that this association may be causal (6). Which psychobiological mechanisms mediate the impact of urban living on mental health is largely unknown.

Recent evidence shows that city living and urban upbringing may alter the neural processing of stressful experiences (7). More specifically, it has been shown that current city living increased amygdala activity during stress and that urban upbringing was associated with increased stress-related activity in the anterior cingulate cortex. However, whether current or early life urbanicity may also alter the physiological response to stress is still unclear. The two major stress response systems of the body are the rapidly acting autonomic nervous system and the slower hypothalamus-pituitary-adrenal (HPA) axis, which leads via intermediate steps to the release of glucocorticoids (mainly cortisol in humans) from the adrenal cortex (8). In particular, the activity of the HPA axis has been related to mental health and disease (9). For example, HPA axis dysregulation is often observed in depressive patients (10,11) and HPA axis activity is a predictor of relapse to depressive symptoms (12). Alterations of HPA axis activity have also been reported in schizophrenia (13) and anxiety disorders (14,15). Thus, if city living or urban upbringing changed the (re)activity of the HPA axis, this could be a link between urbanization and psychiatric disorders.

Given that the regulation of the HPA axis can be "pre-programmed" by the early life environment (16,17), we tested in the present series of experiments the hypothesis that urban upbringing affects the (re)activity of the HPA axis. In the first two experiments, we assessed the cortisol response to two different stressors in participants who were raised in cities or in more rural areas. Based on the finding that early life urbanicity increased neural stress processing (7), we expected that urban upbringing would also increase the cortisol response to stress. In a third experiment, we examined whether urban upbringing alters also the cortisol awakening response, a 50% to 75% increase in cortisol within the 30 minutes after awakening that can be used as an indicator of HPA axis regulation (18).

METHODS

We tested 248 healthy, normal-weight participants in three experiments. In all experiments, exclusion criteria were checked in a standardized interview. The exclusion criteria were current illness or medication intake, smoking, drug abuse, prior experience with a laboratory stressor, and lifetime history of any neurological or psychiatric disorders (including clinical and subclinical depression). Data collection took place between October 2012 and March 2013.

Experiment 1
Participants

Seventy-two healthy, normal-weight university students participated in this experiment (36 men, 36 women; age: mean [M; standard error of the mean {SEM}] = 23.2 [= 0.4] years; body mass index [BMI]: M [SEM] = 22.4 [0.3[kg/m²). All participants provided written informed consent for participation in the study, which was approved by the ethics committee of the German Psychological Society.

Stress Protocol and Physiological Measurements

Because of the diurnal rhythm of the stress hormone cortisol, all testing was carried out in the afternoon between 1 PM and 6 PM. Upon their arrival at the laboratory, all participants were asked to rest for approximately 10 minutes, before baseline measurements of blood pressure and the first saliva sample were taken (see later). Participants were then randomly assigned to the stress or control condition ($n = 36$ per group). In the stress condition, participants underwent a modified version of the Trier Social Stress Test (19) that was designed to mimic an academic oral examination. Participants were informed that they should recall a list of 50 previously learned items in front of an audience and that they would be videotaped during this test for subsequent facial expression analysis. The audience consisted of a man and a woman, both dressed in a white coat and sitting at a table opposite to the (standing) participant. Before participants had to recall the learned material, they were videotaped for 1 minute while standing in front of the rather cold and nonreinforcing panel (preparation phase). All in all, the stress manipulation took 5 minutes. In the control condition, participants were asked by the experimenter to recall the previously learned material. There was no panel and participants in the control condition were not videotaped. The stress and control groups differed only in the experimental manipulation; apart from that, the experimental procedure was exactly the same for all participants.

We used in this experiment a modified version of the Trier Social Stress Test that resembled an oral examination because a) an oral examination is a highly relevant "real-life" stressor, in particular in a student population, and b) we aimed to test in this experiment also memory performance under stress. Please note, however, that the data related to memory performance are beyond the scope of the present article and have been reported elsewhere (20).

To assess the physiological stress response, we took blood pressure measurements and saliva samples at several time points across the experiment. Blood pressure was measured with a Dinamap system (Critikon, Tampa, FL) at the left upper arm before, during, and immediately after the stress task. In addition, participants collected saliva samples immediately before and after the task as well as 25 minutes after the task, when peak cortisol concentrations were expected (19). From saliva, we measured cortisol concentrations using an immunoassay (IBL, Hamburg, Germany). Interassay and intra-assay coefficients of variance were less than 10%.

Experiment 2
Participants

Sixty healthy, normal-weight university students participated in the second experiment (30 men, 30 women; age: M [SEM] = 22.7 [0.4] years; BMI: M [SEM] = 22.0 [SEM] = 0.3 kg/m^2), none of which had participated in the first experiment. All participants provided written informed consent for participation in the study, which was approved by the ethics committee of the German Psychological Society.

Questionnaire Data

To control for potential differences in depressive mood, social support, or personality between participants raised in cities versus rural areas, participants completed the German version of the Beck Depression Inventory (21), a German social support scale (22), and the German version of the NEO-Five-Factor Inventory (23) at the beginning of the experiment.

Stress Protocol and Physiological Measurements

Consistent with the procedure of Experiment 1, participants were asked to rest for approximately 10 minutes after their arrival in the laboratory, before baseline blood pressure measurements were taken and the first saliva sample was collected. After completing the questionnaires, participants in the stress condition ($n = 30$) were exposed to the Socially Evaluated Cold Pressor Test, a standardized laboratory stressor that combines physiological and psychosocial stress elements (24). Briefly, participants immersed their right hand up to and including the wrist for 3 minutes (or until they could not tolerate it any more) into ice water (2°C–5°C). During hand immersion, they were videotaped and monitored by a rather cold and unsociable experimenter. Participants in the

control condition ($n = 30$) submerged their right hand up to and including the wrist for 3 minutes into warm water (35°C–37°C); they were neither videotaped nor monitored. Apart from the different experimental manipulation, the experimental procedure was exactly the same for all participants.

To assess the physiological stress response, we measured participants' blood pressure before, during, and immediately after the stress task, and participants collected saliva samples for subsequent cortisol measurements before, immediately after, and 25 minutes after the task in the same manner as in Experiment 1. In addition to these physiological measurements, participants indicated immediately after the stressor/control manipulation on a scale from 0 ("not at all") to 100 ("very") how stressful, painful, and unpleasant they had experienced the previous situation. Consistent with the procedure of Experiment 1, testing took place in the afternoon between 1 PM and 6 PM.

Experiment 3
Participants

One hundred sixteen healthy, normal-weight men and women participated in the third experiment (55 men, 61 women; age: M [SEM] = 24.9 [4.2] years; BMI: M [SEM] = 22.8 [3.3] kg/m^2), none of which had participated in Experiment 1 or 2. All participants provided written informed consent for participation in the study, which was approved by the ethics committee of the Faculty of Psychology of the Ruhr University Bochum.

Measurement of the Cortisol Awakening Response

To measure the cortisol awakening response, saliva samples were collected by the participants at home on a weekday using Salivette collection devices (Sarstedt, Germany). Participants were instructed to collect a first saliva sample immediately after awakening and a second sample exactly 30 minutes later. Moreover, they were asked not to brush their teeth and abstain from eating or drinking until the second saliva sample was collected. They were also asked to rate sleep quality and duration and to indicate at what time they woke up. We decided to collect only two saliva samples to reduce participant burden. Previous studies have shown that the cortisol increase between 0 and 30 minutes after awakening is highly sensitive to group differences (25,26).

Quantification of Urban Upbringing and Data Analyses

Urban upbringing was quantified retrospectively in accordance with previous studies ((7,27); but see Ref. (28) for an elegant example of more objectively defined early life experiences) and in exactly the same way in the three experiments. At the beginning of each experiment, participants were asked to provide details on their place of birth and living environment in their first 15 years of life. Urbanization was scored in the categories "city with more than 100,000 inhabitants" (category score 3), "town with 10,000 to 100,000 inhabitants" (category score 2), and "place with less than 10,000 inhabitants" (category score 1). The urbanicity score was calculated by multiplying the number of years living in these areas until age 15 years with the respective category score and adding up the scores across categories. Thus, the urbanicity score could vary between 15 (15 years in a town with <10,000 inhabitants) and 45 (15 years in a city with <100,000 inhabitants).

Based on their individual urbanicity score, participants were classified with a median split into "low urbanicity" and "high urbanicity" groups. Between 72% and 93% of the low urbanicity participants in the three experiments had never lived in a city. Excluding those who had lived for a short time in a city did not significantly change our findings; thus, we decided to leave these participants in the analyses. Between 82% and 93% of the participants in the high urbanicity groups in the three experiments had lived at least 10 of the first 15 years of life in a city. At the time of testing, most participants (between 80% and 88% in the three experiments) were living in a city.

Statistical Analyses

Blood pressure and salivary cortisol data of the first two experiments were analyzed by mixed-design analyses of variance (ANOVAs) with the between-participant factors task (control versus stress) and urbanicity (high versus low) and the within-participant factor time point of measurement. The cortisol data

of Experiment 3 were subjected to an urbanicity × time point of measurement ANOVA. All analyses were calculated with SPSS 20 (IBM). All reported p values are two tailed.

RESULTS

Experiment 1: Urban Upbringing Increases the Cortisol Response to a Psychosocial Stressor

Salivary cortisol and blood pressure increased significantly in response to the psychosocial stressor. To assess whether urban upbringing affected the physiological stress response, we quantified urban upbringing in line with previous studies (7,27) and subdivided our sample in participants who were raised mainly in cities (high urbanicity group) and those who were raised in small towns or rural areas (low urbanicity group; see "Methods"). These groups did not differ in age, BMI, or sex distribution (all $p > .15$; see Table S1, Supplemental Digital Content, http://links.lww.com/PSYMED/A163). There were 15 participants who were raised in rural areas and 21 who were brought up in cities in the control group; in the stress group, there were 21 participants who were raised in rural areas and 15 who were brought up in cities ($\chi^2(1) = 2.00$, $p = .16$).

A task × time point of measurement × urbanicity ANOVA yielded, in addition to a significant task × time point of measurement effect ($F(2,132) = 17.58$, $p < .001$, $\eta^2 = 0.21$), a significant task × time point of measurement × urbanicity in-

teraction ($F(2,132) = 3.10$, $p = .048$, $\eta^2 = 0.05$). Although the exposure to the stressor led to a significant cortisol increase in both the low and the high urbanicity groups (task × time point of measurement interactions: both $F > 3.30$, both $p < .044$, both $\eta^2 > 0.09$), participants who were raised in a city showed a significantly more pronounced cortisol response to the stressor (Fig. 1A). Follow-up tests indicated that the two urbanicity groups differed in their cortisol response to the stressor (urbanicity × time point of measurement interaction: $F(2,66) = 4.95$, $p = .010$, $\eta^2 = 0.13$) but not in their response to the control manipulation ($F(2,66) = 0.01$, $p = .99$). The cortisol response (defined as difference between the peak and baseline cortisol concentrations) was increased by 167% in the high compared with the low urbanicity group. To rule out an influence of current city living on the cortisol response to the stressor, we performed an analysis of covariance (ANCOVA) with current urbanicity as a covariate. This analysis showed that current city living did not affect the cortisol response to stress ($p = .53$) and that the modulatory influence of urban upbringing remained when we controlled for current city living ($F(2,132) = 3.08$, $p = .049$, $\eta^2 = 0.05$). Interestingly, we obtained for participants in the stress group also a significant correlation between the individual urbanicity score and the cortisol response to the stressor ($r = 0.35$, $p = .037$; Fig. 1B).

Figure 1. Physiological response to psychosocial stress in Experiment 1. A, Although salivary cortisol increased in response to the stressor both in participants raised in rural areas and in those raised in a city, this increase was more pronounced in participants who grew up in an urban environment. Time point 0 denotes the beginning of the stress/control task. B, Moreover, the individual urbanicity score correlated with the cortisol response to the stressor. C, Systolic and diastolic blood pressures increased in response to the stressor; this increase, however, was not affected by urban upbringing. Groups differed during the stress task but neither before (pre) or immediately thereafter (post). Error bars represent SEM. SEM = standard error of the mean.

Systolic and diastolic blood pressures were also elevated by the psychosocial stressor (task × time point of measurement interactions: both $F > 8.51$, both $p < .001$, both $\eta^2 > 0.11$; Fig. 1C). However, the blood pressure response was not modulated by urban upbringing or current city living (all main or interaction effects: all $F < 1.5$, all $p > .23$).

Experiment 2: Urban Upbringing Increases the Cortisol Response to the Socially Evaluated Cold Pressor Test

To replicate the findings of Experiment 1 and to extend these findings to another stressor, participants underwent the Socially Evaluated Cold Pressor Test (or a control manipulation) in a second experiment. Participants were again classified into groups that were raised in cities versus small towns or rural areas. These groups did not differ in age, BMI, sex distribution, depressive mood, social support, or personality, except a higher openness score in participants raised in rural areas ($t(58) = 2.96$, $p = .020$; all other $p > .10$; see Table S2, Supplemental Digital Content, http://links.lww.com/PSYMED/A163). In the control group, there were 16 participants who were raised in rural areas and 14 who were brought up in cities; in the stress group, there were 16 participants who were raised in rural areas and 14 who were brought up in cities ($\chi^2(1) = 0.27$, $p = .61$).

The Socially Evaluated Cold Pressor Test resulted in significant elevations in salivary cortisol and blood pressure (Fig. 2). In line with the findings of Experiment 1, salivary cortisol increased in the high and low urbanicity groups in response to the stressor (both $F(2,56) > 9.74$, both $p < .001$, both $\eta^2 > 0.25$); however, this increase was significantly stronger in participants who grew up in a city compared with participants raised in small towns or rural areas (task × time point of measurement × urbanicity interaction: $F(2,112) = 3.29$, $p = .041$, $\eta^2 = 0.06$; task × time point of measurement effect: $F(2,112) = 24.38$, $p < .001$, $\eta^2 = 0.30$). Again, the low and high urbanicity groups differed specifically in their cortisol response to the stressor (urbanicity × time point of measurement: $F(2,56) = 2.55$, $p = .067$, $\eta^2 = 0.08$), whereas there were no differences in the control condition ($F(2,56) = 0.88$, $p = .42$, $\eta^2 = 0.03$). Compared with the low urbanicity group, the cortisol response was increased by 115% in the high urbanicity group. Controlling for the influence of current city living in an ANCOVA did not alter this effect of urban upbringing ($F(2,106) = 3.55$, $p = .032$, $\eta^2 = 0.06$). In addition to the significant differences between the low and high urbanicity groups, for stressed participants, there was also a significant correlation between the individual urbanicity score and the cortisol response to the stressor ($r = 0.37$, $p = .044$; Fig. 2B).
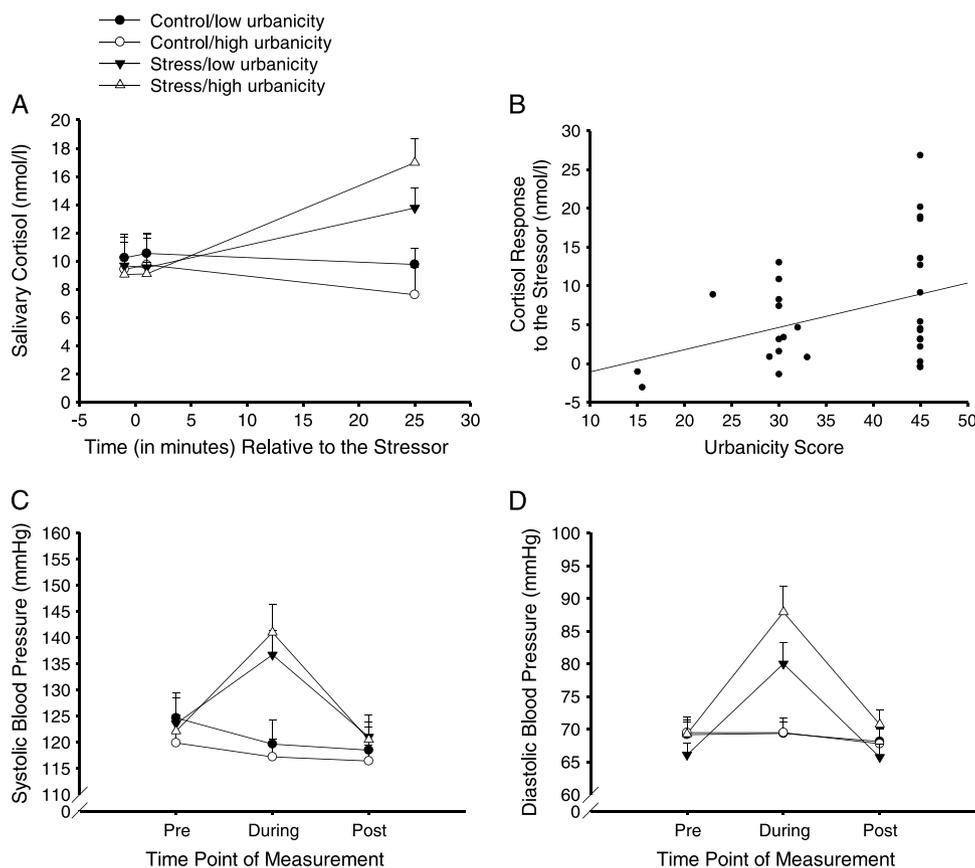
Figure 2. Physiological response to the Socially Evaluated Cold Pressor Test in Experiment 2. The salivary cortisol response to the stressor (time point 0 denotes the beginning of the stress/control task) was more pronounced in participants who were brought up in a city than in participants raised in small towns or rural areas (A) and significantly correlated with the individual urbanicity score (B). C, Systolic and diastolic blood pressures were significantly elevated during but not immediately before (pre) or after (post) the Socially Evaluated Cold Pressor Test, without any significant differences between participants raised in rural versus urban areas. Error bars represent SEM. SEM = standard error of the mean.

As expected, systolic and diastolic blood pressures were also significantly elevated by the Socially Evaluated Cold Pressor Test (task × time point of measurement interactions: both $F > 30.68$, both $p < .001$, both $\eta^2 > 0.35$; Fig. 2C). This autonomic stress response, however, was not modulated by urban upbringing or current city living (all $F < 2.56$, all $p > .082$). Moreover, participants in the stress condition assessed the task as significantly more stressful (mean [SEM] = 44.83 [5.20] versus 0.67 [0.67]), unpleasant (63.45 [4.36] versus 2.67 [0.95]), and painful (62.41 [4.05] versus 0.67 [0.67]) than did participants in the control condition (all $F > 70$, all $p < .001$), without any significant influence of urban upbringing or current city living (all $p > .12$).

Experiment 3: Urban Upbringing Reduces the Cortisol Awakening Response

In the third experiment, we tested whether urban upbringing changes also the cortisol awakening response. Corroborating several previous reports ((18); for a review, see Ref. (29)), cortisol concentrations increased significantly within the 30 minutes after awakening (Fig. 3). As in the first two experiments, participants were subdivided into those who were raised in a city and those who were brought up in a more rural area. The low and high urbanicity groups did not differ in age or BMI, nor did they differ in sleep quality, sleep duration, or time of awakening (all $p > .33$; Table S3, Supplemental Digital Content, http://links.lww.com/PSYMED/A163). The distribution of men and women tended to be different between groups, yet this difference did not reach statistical significance ($\chi^2(1) = 2.80$, $p = .094$). A time point of measurement × urbanicity ANOVA on the cortisol response to awakening, however, showed, in addition to a main effect of time ($F(1,114) = 193.74$, $p < .001$, $\eta^2 = 0.63$), a significant time point of measurement × urbanicity interaction ($F(1,114) = 4.00$, $p = .048$, $\eta^2 = 0.04$), indicating that the
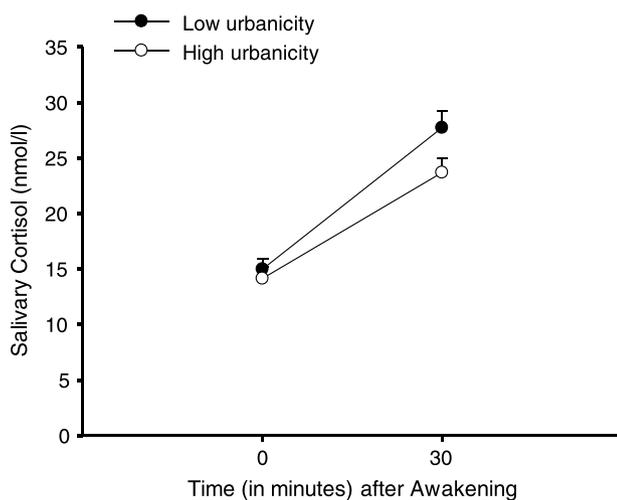
Figure 3. Cortisol awakening response in participants raised in rural versus urban environments. Salivary cortisol increased significantly within the first 30 minutes after awakening. This cortisol awakening response, however, was reduced in participants who were brought up in a city compared with those who were raised in small towns or rural areas. Error bars represent SEM. SEM = standard error of the mean.

cortisol awakening response was reduced in participants who grew up in a city. Although the two groups did not differ in their cortisol concentrations immediately after awakening ($p = .57$), participants who were raised in a city had a significantly lower cortisol concentration 30 minutes later ($p = .041$). An ANCOVA with current city living as a covariate showed that current city living did not alter the effect of urban upbringing on the cortisol awakening response ($F(1,100) = 4.95$, $p = .028$, $\eta^2 = 0.05$). Likewise, an ANCOVA with participants' sex as a covariate revealed that the effect of urban upbringing remained when we controlled for the (nonsignificant) difference in sex distribution in the two groups ($F(1,113) = 4.33$, $p = .040$, $\eta^2 = 0.04$).

DISCUSSION

The present series of experiments aimed to investigate whether urban upbringing changes the activity of the HPA axis, one of the organisms' major stress response systems, in healthy adults. To this end, we contrasted the cortisol responses to different stressors and to awakening in participants who were raised in a city with those of participants who were brought up in more rural areas. We observed increased cortisol responses to stress and a blunted cortisol awakening response in participants raised in a city compared with those who grew up in small towns or rural areas, suggesting that early life urbanicity may indeed alter HPA axis activity later in life.

There is abundant evidence from animal and human studies demonstrating that the early life environment can influence the activity of the HPA axis later in life (30,31). In rodents and nonhuman primates, postnatal maternal separation or social deprivation leads mainly to increased glucocorticoid responses to stress (32,33). Likewise, adverse experiences during childhood promote subsequent HPA axis hyperactivity in humans (34,35). Living in cities is associated with many environmental and social stressors, ranging from road traffic, noise, pollution, low-quality housing, and restricted space to lack of social cohesion, social conflicts, and a higher prevalence of criminality (36–38). The present study is, to the best of our knowledge, the first showing that growing up in the relatively stressful environment of a city may affect the (re)activity of the HPA axis. Previous research showed an influence of urban upbringing on neural stress processing but failed to show an association with cortisol responses (7), which might be due to differences in the used stress protocol or the statistical power. Notably, the size of the effect of urban upbringing on the cortisol response to the stressors in the present study was comparable to those reported previously for the impact of childhood trauma and/or depression (39,40).

At first glance, the finding that urban upbringing increased the cortisol response to stress but decreased the cortisol response to awakening might be viewed as contradictory. It is, however, important to note that the cortisol increase in response to acute stress and the cortisol awakening response reflect different aspects of HPA axis functioning. Whereas the HPA axis response to acute (psychosocial) stress is triggered by the individuals' interpretation of a current event, the cortisol awakening response is a phenomenon superimposing the diurnal

cortisol rhythm (41). Accordingly, there are virtually no correlations between cortisol responses to acute stress and awakening (42). Moreover, factors that have a significant impact on the HPA axis response to stress leave the cortisol awakening response largely unaffected (43). Together, the early urbanicity-related changes in the cortisol response to stress and the cortisol response to awakening suggest that the HPA axis is differentially regulated in people who were brought up in cities relative to those raised in more rural areas. Future studies that combine pharmacological challenge tests with neuroimaging could help to elucidate the neuroendocrine processes contributing to the impact of urban upbringing on the regulation of the HPA axis.

Recent evidence indicates that participants raised in a city show enhanced activity of the anterior cingulate cortex during social stress (7), suggesting that this region may also play a part in the effects of urban upbringing that we observed in the present experiments. Indeed, the anterior cingulate cortex has been shown to be critically implicated in the regulation of HPA axis activity (44). However, another brain area that plays most likely an important role in the observed effects of early urbanicity is the hippocampus. The hippocampus is one of the primary targets of stress hormones (45), and it is particularly sensitive to stress during infancy and childhood (46). For example, stressful conditions during childhood reduce the density of glucocorticoid receptors and the expression of genes for NMDA receptor subunits in the hippocampus (33,47), which may impair the negative feedback control of the HPA axis (48). During stressful episodes, the hippocampus is deactivated and the degree of hippocampal deactivation correlates with the cortisol response to stress (49). In addition to its role in the acute stress response, the hippocampus has also been implicated in the regulation of the cortisol awakening response. Bilateral or unilateral hippocampus damage abolishes the cortisol response to awakening (50). Similarly, reduced hippocampal volumes that are related to depressive mood are associated with a blunted cortisol awakening response (51). Thus, the opposite effects of urban upbringing on the cortisol response to acute stress and to awakening that we observed here would be well in line with the idea that the influence of urban upbringing on HPA axis (re)activity is related to a reduced hippocampal volume in consequence of a relatively stressful (urban) environment during a critical period of brain development. Interestingly, reduced hippocampal volume is also a hallmark feature of psychiatric disorders such as depression or posttraumatic stress disorder (52,53), and there is evidence that these reductions in hippocampal volume are not necessarily a consequence of the disorder but may be a risk factor that could be genetic or rooted early in life (54). Although these data suggest that the hippocampus might be one locus mediating the influence of urban upbringing on HPA axis functioning, it is to be noted that the only study that assessed the effect of urban upbringing on stress processing in the brain did not find alterations in hippocampal activity in participants who were raised in cities (7). The absence of any changes in hippocampal activity might be due to the functional magnetic resonance imaging methodology and the specific contrast that was analyzed. However, future volumetric

and functional magnetic resonance imaging studies are needed to examine the potential role of the hippocampus in the effects of urban upbringing on HPA axis responses to stress.

In contrast to urban upbringing, current urbanicity had no effect on the cortisol responses to stress and awakening. Thus, although current city living may alter the neural processing of stressful experiences (7), the present results suggest that it does not influence the (re)activity of the HPA axis. This finding is in line with the view that the regulation of the HPA axis is preprogrammed relatively early in life (55). Moreover, our findings suggest that urban upbringing affected specifically the functioning of the HPA axis because the autonomic response to stress, as indicated by the blood pressure data, remained unaffected by where the participants grew up. There is, however, evidence showing that adverse early life environments may also affect the subsequent autonomic stress response (39,56). Whether urban upbringing affects indeed selectively the functioning of the HPA axis or whether there are also effects on the reactivity of the autonomic system remains to be tested in further studies that should include more parameters of the autonomic nervous system (e.g., heart rate, skin conductance, or α-amylase).

Finally, some limitations of this series of experiments have to be noted. First, we focused on the urban versus rural environment during the first 15 years of life, in line with a recent study on the influence of urban upbringing on neural stress processings (7). Future studies, however, might equally well focus on the first 12 or 18 years of life (beginning of puberty and adulthood, respectively). Second, we measured cortisol only at relatively few time points after stress and awakening. Measuring cortisol concentrations more often would allow a more fine-grained analysis of the differential HPA axis responses in individuals who were raised in urban versus rural areas. Third, we measured the cortisol awakening response only on a single weekday. Given that the cortisol awakening response seems to be stronger on weekdays compared with weekends (57,58), it would be interesting to test whether urban upbringing modulates the cortisol response to awakening also on the weekend. Fourth, based on our quasi-experimental design, we can hardly draw conclusions regarding causal effects of urban upbringing on the HPA axis. There are certainly other factors such as childhood trauma (16,35), early childhood adversity (59), and, in particular, parental socioeconomic status (60,61) that can affect the activity of the HPA axis and may be related to participants' urbanicity score. Future studies are required to include such factors and to test whether these factors moderate the effect of urban upbringing. Thus, although the present findings have, in our view, relevant implications, they represent only a first step and further research is needed to shed light on the time course of the differences in HPA axis activity in individuals who grew up in cities versus rural areas and on the factors that mediate or moderate such differences.

In sum, our findings show that growing up in a city may alter the (re)activity of the HPA axis. Given that a dysregulated HPA axis constitutes a risk factor for several psychiatric disorders (9), the observed changes in HPA axis regulation may be a mechanism linking urbanization and psychopathology. In

addition, elevated HPA axis responsiveness may also adversely affect biological processes in a wide range of medical conditions, including diabetes, cardiovascular disease, and cancer (62). Although the observed alterations in HPA axis (re)activity could contribute to the increased risk for several disorders in urban areas, one might speculate whether the altered stress responses have also some beneficial effects. The altered stress response might increase vigilance, attention, or learning when living in a dangerous neighborhood of a city. For example, it has been shown that offspring of low-caring mothers shows enhanced learning and neural plasticity compared with offspring of high-caring mothers in periods in highly stressful environments (63).

Compared with rural areas, cities are associated with many stressors. It remains unclear which of these factors is critical for the altered stress response observed in participants who were brought up in a city, although it has been proposed that social stressors play a key role for the stress level associated with urban environments (7). It is also important to note that we have tested healthy participants who grew up in Germany, a very prosperous country. Differences between urban and rural environments are most likely bigger in countries with lower living standards, and we would expect that the influence of urban upbringing is more pronounced in these countries. This is particularly important because urban growth is highest in these developing countries (64). Although urbanization can hardly be stopped or reversed, our findings underline that improving the urban living conditions is essential.

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