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PII: S0306-4530(22)00001-4

DOI: <https://doi.org/10.1016/j.psyneuen.2022.105660>

Reference: PNEC105660

To appear in: *Psychoneuroendocrinology*

Received date: 22 June 2021

Revised date: 5 December 2021

Accepted date: 3 January 2022

Please cite this article as: Pauline S. Effenberger, Tabea S. Send, Maria Gilles, Isabell A.-C. Wolf, Josef Frank, Stephan Bongard, Robert Kumsta, Stephanie H. Witt, Marcella Rietschel, Michael Deuschle and Fabian Streit, Urbanicity, behavior problems and HPA axis regulation in preschoolers, *Psychoneuroendocrinology*, (2021) doi:<https://doi.org/10.1016/j.psyneuen.2022.105660>

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## Urbanicity, behavior problems and HPA axis regulation in preschoolers

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### Abstract

Growing up in cities is associated with increased risk for developing mental health problems. Stress exposure and altered stress regulation have been proposed as mechanisms linking urbanicity and psychopathology, with most research conducted in adult populations. Here, we focus on early childhood, and investigate urbanicity, behavior problems and the regulation of the hypothalamus-pituitary-adrenal (HPA) axis, a central circuit of the stress system, in a sample of  $N = 399$  preschoolers aged 45 months. Urbanicity was coded dichotomously distinguishing between residences with more or less than 100,000 inhabitants. Behavior problems were measured using the Child Behavior Checklist (CBCL) 1½ - 5. Cortisol stress reactivity was assessed using an age-appropriated game-like stress task, and cortisol in the first morning urine was measured to assess nocturnal HPA axis activity. Urbanicity was not associated with behavior problems, urinary cortisol or the cortisol stress response. Neither urinary cortisol nor salivary cortisol response after stress exposure were identified as mediators of the relationship between urbanicity and behavior problems. The findings

suggest no strong association of urbanicity with behavior problems and HPA axis regulation in preschool age. To our knowledge, this is the youngest sample to date studying the relationship between urbanicity and behavior problems as well as HPA axis regulation. Future research should examine at which age associations can first be identified and which mechanisms contribute to these relationships.

**Keywords:** Urbanicity, behavior problems, HPA axis reactivity, nocturnal HPA axis activity, cortisol, children

### Highlights

- Urbanicity in early life is a well established risk factor for psychopathology
- Investigation of urbanicity with behavior problems and HPA axis regulation
- Investigation in homogenous sample of  $N = 399$  45-months-old preschoolers
- Urbanicity was not associated with behavior problems
- Urbanicity was not associated with cortisol reactivity or cortisol in morning urine

### 1. Introduction

Living in cities is a rising trend: From 1950 to 2018 the quota of people living in cities worldwide increased from 29.6% to 55.3% — a trend that is expected to increase up to 68.4% until 2050 (United Nations, Department of Economic and Social Affairs, Population Division, 2018). Critically, urbanicity has been identified as a risk factor for mental health problems, as urban living during adulthood (Peen et al., 2010) as well as urban upbringing are associated with psychopathology (Evans et al., 2018; Newbury et al., 2016). This relationship is especially well established for schizophrenia (Pedersen & Mortensen, 2001). Most studies have focused on mental health during adulthood or adolescence and later childhood. Less is known about the link between urbanicity and mental health in early childhood. Furthermore, the potential mechanisms linking urbanicity with psychopathology risk have not yet been sufficiently explored. One established hypothesis is that the relationship

between urbanicity and mental health can be explained by increased exposure to social stress in cities (Lederbogen et al., 2013). Stress is an established risk factor for mental disorders (Agorastos & Chrousos, 2021; de Kloet et al., 2005; Mah et al., 2016; Tessner et al., 2011), and dysregulation of the hypothalamus-pituitary-adrenal (HPA) axis, an important circuit of the human stress system, is associated with mental disorders in adults (Agorastos & Chrousos, 2021; Handwerger, 2009; Zorn et al., 2017) and behavior problems in children, but not consistently (Alink et al., 2008; Badanes et al., 2011; Essex et al., 2002; Wesarg et al., 2020). The HPA axis controls the release of cortisol, which follows a diurnal rhythm and is secreted in response to physiological or psychological challenges to the organism. Nocturnal HPA axis activity can be measured by cortisol concentration in the first morning urine, and HPA axis reactivity is typically assessed through cortisol concentration in saliva after stress exposure.

Urban upbringing was found associated with cortisol reactivity in response to psychosocial stressor tasks in adulthood and adolescence, but different directions of this association were shown: In adolescents, current urbanicity was associated with lower cortisol reactivity in response to psychosocial stressor tasks (Evans et al., 2013; Evans, Huizink et al., 2020), whereas urban upbringing in adults was associated with higher cortisol reactivity in response to psychosocial stressor tasks but with reduced cortisol awakening response (Steinheuser et al., 2014). Noise exposure from road and rail traffic, common in urban environments, was associated with increased cortisol levels in morning urine of children (Evans et al., 2001). Furthermore, brain activation patterns following stress exposure in the fMRI environment further support the notion that urban upbringing is associated with distinct brain regions involved in stress regulation (Lederbogen et al., 2011).

So far, two studies examined urbanicity, behavior problems, and HPA axis regulation in youth. No direct association between urbanicity and internalizing and externalizing problems was shown, and no mediating role of HPA axis regulation operationalized through diurnal cortisol secretion and cortisol reactivity in response to psychosocial stressor tasks was identified in children (Evans, van der Ende et al., 2020) and in adolescents (Evans, Huizink et al., 2020).

To date, no study has examined urbanicity and stress reactivity in early childhood. We thus set out to investigate the relationship between urbanicity, HPA axis regulation and mental health in preschoolers.

The aim of this study was to investigate the following hypotheses: H1. Urbanicity is positively associated with behavior problems. H2. Urbanicity is associated with cortisol concentration in morning urine. H3. Urbanicity is associated with salivary cortisol response after stress exposure. H4a. The association between urbanicity and behavior problems is mediated by cortisol levels in morning urine. H4b. The association between urbanicity and behavior problems is mediated by salivary cortisol response after stress exposure. The hypotheses, methods and statistical analyses were preregistered at Open Science Framework (Effenberger et al., 2020).

## **2. Methods**

### **2.1 Sample**

This work is part of the ongoing longitudinal study “POSEIDON” (Pre-, Peri- and Postnatal Stress: Epigenetic Impact on Depression) examining early stress and development of children. 410 pregnant women (age: 16-45 years) were recruited for the first study wave (T1) from October 2010 to March 2013. Exclusion criteria were: hepatitis B, hepatitis C or human immunodeficiency virus, a current psychiatric disorder requiring inpatient treatment, a history or current diagnosis of bipolar disorder or schizophrenia, and substance dependency besides nicotine during pregnancy. Additional exclusion criteria at birth were: gestational age under 30 weeks and birth weight under 1,500g. Data was collected at four waves: third trimester of pregnancy (T1), in the days after childbirth (T2), and six (T3) and 45 months (T4) postpartum. The present work uses data from study wave T4 when children were 45 months old. 302 families and their children of the original cohort participated again at T4. To replace dropouts, 101 new parents with their 45-months-old children were included in the study at T4. This new sample contains four twin pairs whereas multiple birth was an exclusion criterion for the original cohort. Details on the recruiting process, inclusion criteria and sample characteristics have been described previously (Send et al., 2017; Send, Bardtke, Gilles, Wolf, Sütterlin, Kirschbaum et al., 2019; Wolf et al., 2017). In total, the sample at T4 included 403 mothers

with their 45-months-old children. This study was conducted following the Declaration of Helsinki. The Ethics Committee of the Medical Faculty Mannheim of the University of Heidelberg approved the study. Before participation, all families provided written informed consent.

## **2.2 Procedure**

Testing at T4 took place at the Central Institute of Mental Health in Mannheim, either at 10 am or 2 pm. Mothers collected the first morning urine of the child at home in a provided container, stored it in a refrigerator and brought it to the laboratory. In some cases, the urine sample was sent by post instead ( $n = 36$ ). During the visit, children underwent a stress test designed for this particular age group (Kryski et al., 2011; Lewis & Ramsay, 2002). See Send, Bardtke, Gilles, Wolf, Sütterlin, Kirschbaum et al. (2019) for details of the procedure. Briefly, a game in which the children experienced repeated failures and obtained negative feedback was carried out. Children were instructed they could win a sticker if they successfully completed the task. During the task, the children had to attach magnets of two colors to corresponding animals on a game board. The task was performed under time pressure, which was signaled by a remote controlled stoplight. The available time was adapted so that all children failed. The children performed three attempts on the task, or until they refused to continue the task. During the task, the researcher used non-reinforcing language and provided negative feedback on the children's performance between the attempts. Immediately after the task, the child was debriefed and praised for its performance, and rewarded with the sticker. Saliva samples of the child were taken before the test as well as 10, 30 and 40 minutes after the test using salivettes (Sarstedt, Nümbrecht, Germany). During the testing of the child, a structured interview was conducted with the mother in the same room to obtain demographic information.

## **2.3 Measures**

### **2.3.1 Urbanicity**

Urbanicity of the child was operationalized by the number of inhabitants of the current residence of the mother at T4. The number of inhabitants of the municipality was coded using data from the federal statistical office of Germany (Statistisches Bundesamt [Destatis], 2020). In the current sample, the number of inhabitants was not normally distributed with most participants either

living in municipalities with less than 50,000 inhabitants or in larger cities of more than 150,000 inhabitants. Corresponding to the German definition of a larger city having at least 100,000 inhabitants (Bundesinstitut für Bau-, Stadt- und Raumforschung im Bundesamt für Bauwesen und Raumordnung, 2017), two categories were created: (1) less than 100,000 inhabitants, and (2) equal or more than 100,000 inhabitants.

### **2.3.2 Behavior problems**

The German version of the Child Behavior Checklist (CBCL) 1½ - 5 by Achenbach and Rescorla (2000) was completed by the main caregiver in a paper-pencil-format at home and brought to the visit at T4. Using 100 items, the CBCL assesses different types of behavior problems. It covers internalizing problems by combining the scales emotionally reactive, anxious/depressed, somatic complaints, and withdrawn as well as externalizing problems by combining the scales attention problems and aggressive behavior. A total problem score can be calculated by combining internalizing problems, externalizing problems and sleep problems as well as one open item asking for problems not yet mentioned. This total problem score was used in the present study. In this sample, Cronbach's alpha of the total problem score was .94.

### **2.3.3 HPA axis measures**

The nocturnal activity of the HPA axis was operationalized by the cortisol concentration in the morning urine of the child. Additionally, creatinine values were assessed. The laboratory analyses and preparation of urinary cortisol data is described elsewhere (Send, Bardtke, Gilles, Wolf, Sütterlin, Wudy et al., 2019). Briefly, cortisol concentration in urine was measured by online turbulent flow chromatography in combination with high performance liquid chromatography-tandem mass spectrometry (TFC-HPLC-MS/MS). Urinary cortisol concentrations were corrected for urinary creatinine concentration by dividing cortisol by creatinine. To reduce skewness, these values were log<sub>10</sub>-transformed.

Stress reactivity was assessed through cortisol concentration in children's saliva before and 10, 30 and 40 minutes after finishing the stress test. Salivary samples were analyzed using a chemiluminescence immunoassay, details are described elsewhere (Send, Bardtke, Gilles, Wolf, Sütterlin, Kirschbaum et al., 2019). Briefly, within- and between-assay coefficients of variation for

salivary cortisol were less than 8%. Salivary cortisol concentrations were log<sub>10</sub>-transformed to reduce skewness as described previously (Send, Bardtke, Gilles, Wolf, Sütterlin, Kirschbaum et al., 2019).

## 2.4 Statistical analysis

Outliers above or below 3 standard deviations (SD) were winsorized to the closest value within 3 SD: 1 for log<sub>10</sub>-transformed and creatinine corrected urinary cortisol, 3 for urine sampling time, 7 for salivary cortisol (3 of the baseline sample, 2 of the 10 minutes sample, 1 of the 30 minutes sample and the 40 minutes sample), 3 for the AUC<sub>i</sub>, 2 for the total problem score, and 7 for household income. The area under the curve with respect to increase (AUC<sub>i</sub>) according to Pruessner et al. (2003) was calculated as an index for the course of salivary cortisol levels using the log<sub>10</sub>-transformed salivary cortisol data and the time distances  $t_{1-2} = 20$ ,  $t_{2-3} = 20$  and  $t_{3-4} = 10$  (see hypothesis 4b). As a sensitivity analysis, AUC<sub>i</sub> was calculated on untransformed values (not preregistered; 6 resulting values winsorized).

For hypotheses 1 and 2, linear regressions were performed with urbanicity as independent variable (IV) and CBCL total score respectively urinary cortisol as dependent variable (DV). Covariates were sex, household income, and maternal education as well as for H2 additionally urine sampling time and wearing a night diaper. Since the assumption of homoscedasticity of the residuals was violated in the context of H2, bootstrapping was conducted using 1,000 random draws of the present sample to estimate the regression parameters for H2.

For hypothesis 3, an analysis of variance (ANOVA) with repeated measures was calculated, with urbanicity as grouping factor, time of measurement as repeated measurement factor, and salivary cortisol as DV. As effects of interest, a main effect of urbanicity and an urbanicity x time interaction effect were tested. Covariates were sex, household income, maternal education, and time of baseline saliva sample. Since the assumption of sphericity was violated, Greenhouse-Geisser corrections were applied.

In the context of hypotheses 4a and 4b, two models were calculated for each mediation: A linear regression predicting the HPA axis measure by urbanicity and covariates as well as a linear regression predicting CBCL total score by the HPA axis measure, urbanicity, and covariates. In both



models, the same set of covariates was used. Based on these two models, the mediation analysis was performed using the mediation package in R following a quasi-Bayesian approach and using 10,000 simulations (Tingley et al., 2014). H4a tested whether urinary cortisol mediates an association between urbanicity and behavior problems. Covariates were the same as in H2. H4b tested whether stress-associated salivary cortisol release which was operationalized as AUC<sub>i</sub> mediates the association between urbanicity and behavior problems. Covariates were the same as in H3. Time of baseline saliva sample was used as a covariate in all models analyzing salivary cortisol. Additionally, as a sensitivity analysis, the mediation analysis for H4b was repeated using the AUC<sub>i</sub> calculated on untransformed values (not preregistered).

The significance level for the hypotheses tests was Bonferroni-adjusted for the number of tested effects (association between urbanicity and behavior problems, association between urbanicity and urinary cortisol, main effect of urbanicity for salivary cortisol, and interaction effect of urbanicity x time for salivary cortisol) and therefore set to  $p < .05/4 = .0125$ . The hypotheses were tested two-tailed.

The power to test the primary hypothesis 1 (effect of urbanicity on behavior problems) was calculated with GPower 3.1.9.7. (Faul et al., 2009) using the zero order Pearson correlation of  $r = .24$  observed by Evans et al. (2018) (personal communication), the available sample size of  $n = 394$ , and a two-side alpha level of  $\alpha = .0125$ , and revealed a power of  $\beta = .975$  (power analysis not pre-registered).

### 3. Results

From a total of  $N = 403$  mother-child-dyads,  $n = 3$  were excluded from the analyses because of missing values regarding household income. Furthermore,  $n = 1$  subject whose current residence at assessment was not in Germany was excluded. In total,  $N = 399$  children were included in the analyses. Mean age of the children at T4 was  $M = 45.05$  ( $SD = 1.02$ ) months. 53.6% of the children were female. For the specific analyses including behavior problems,  $n = 5$  mother-child-dyads were excluded who did not complete the CBCL questionnaire. In case of eight and less missing items of the CBCL, these items were counted as 0. According to Send, Bardtke, Gilles, Wolf, Sütterlin, Wudy et al. (2019), participants with urine samples taken after 11 am ( $n = 9$ ), without recorded time ( $n = 2$ ) or

who did not provide an urine sample ( $n = 20$ ) were excluded from the specific analyses regarding urine. Following Send, Bardtke, Gilles, Wolf, Sütterlin, Kirschbaum et al. (2019), participants were excluded from the specific analyses regarding saliva if the child refused the baseline saliva sample ( $n = 31$ ) or at least one saliva sample after the test ( $n = 11$ ), did not provide sufficient saliva in at least one sample ( $n = 3$ ), did not participate in the stress test due to postal participation without local appointment ( $n = 11$ ), refused to participate in the test ( $n = 1$ ), had problems in understanding the instruction of the test ( $n = 4$ ) or if a saliva sample was lost ( $n = 1$ ).

Means and standard deviations respectively frequencies of all used variables are shown in supplementary table S1. Correlations of these variables are presented in supplementary table S2. Variables were visually inspected for normal distribution. If normal distribution was missing, Spearman-Rank-correlation was used.

The model predicting CBCL total score with urbanicity, sex, household income, and maternal education explained 11% of the variance of CBCL total score ( $F(4, 389) = 12.10, p < .001, R^2 = .11$ ). Regression parameters as well as the results of the bootstrapping process are shown in table 1. Urbanicity did not significantly predict CBCL total score ( $\beta = 0.02, p = .76$ ) which did not vary with bootstrapping ( $B = 0.56, p = .77$ ).

Table 1 *Parameter estimation to predict CBCL total score*

	Bootstrapping								
	<i>B</i>	<i>SE B</i>	$\beta$	<i>t</i>	<i>p</i>	<i>SE B</i>	LL	UL	<i>p</i>
Constant	46.88	6.78		6.92	< .001	6.64	34.06	60.85	.001
Urbanicity <sup>a</sup>	0.56	1.82	0.02	0.31	.76	1.77	-3.22	3.68	.77
Sex <sup>b</sup>	3.38	1.75	0.09	1.93	.05	1.75	-0.16	6.87	.06
Household income	-0.002	< .001	-0.26	-4.7	< .001	< 0.001	-0.003	-0.001	.001

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Maternal education	-0.69	0.44	-0.09	-1.58	.11	0.43	-1.54	0.18	.12
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*Notes.*  $n = 394$ . LL = Lower limit of 95% Confidence interval. UL = Upper limit of 95% Confidence interval.

<sup>a</sup> less than 100,000 inhabitants was coded as 1. <sup>b</sup> female was coded as 0.

The model predicting cortisol concentration in morning urine with urbanicity, sex, household income, maternal education, wearing a night diaper, and time of urine sample explained 7% of the variance of urinary cortisol ( $F(6, 361) = 4.44, p < .001, R^2 = .07$ ). Regression parameters are shown in table 2. Urbanicity did not significantly predict urinary cortisol ( $\beta = -0.06, p = .22$ ).

Table 2 *Parameter estimation to predict log10-transformed urinary cortisol*

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	<i>B</i>	<i>SE B</i>	$\beta$	<i>t</i>	<i>p</i>
Constant	-1.13	0.23		-5.01	< .001
Urbanicity <sup>a</sup>	-0.05	0.04	-0.06	-1.23	.22
Sex <sup>b</sup>	0.06	0.04	0.08	1.5	.14
Household income	0.02	< .001	0.03	0.55	.58
Maternal education	0.02	0.01	0.13	2.12	.04
Wearing a night diaper <sup>c</sup>	0.26	0.09	0.14	2.76	.006
Time of urine sample	0.01	< .001	0.12	2.33	.02

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*Notes.*  $n = 368$ .

<sup>a</sup> less than 100,000 inhabitants was coded as 1. <sup>b</sup> female was coded as 0. <sup>c</sup> not wearing a night diaper was coded as 0.

Means and standard deviations of cortisol concentration in saliva at different times of measurement for both urbanicity groups are shown in table 3. Descriptive mean differences can be identified with children in the high urbanicity group tending to show higher salivary cortisol.

Table 3 Means and standard deviations of salivary cortisol for both urbanicity groups

	Not transformed values <sup>a</sup>		Log10-transformed values	
	Low	High	Low	High
	Urbanicity <sup>b</sup>	Urbanicity <sup>c</sup>	Urbanicity <sup>b</sup>	Urbanicity <sup>c</sup>
Baseline sample before stress test	1.7 (1.28)	2.02 (1.95)	0.14 (0.26)	0.21 (0.25)
10 minutes after stress test	1.96 (1.72)	2.14 (1.97)	0.18 (0.3)	0.22 (0.29)
30 minutes after stress test	2.35 (2.09)	2.39 (1.95)	0.24 (0.33)	0.27 (0.31)
40 minutes after stress test	2.46 (2.26)	2.59 (2.62)	0.25 (0.35)	0.29 (0.32)

Notes. Standard deviations in brackets. Salivary cortisol concentration in nmol/l.

<sup>a</sup> before winsorizing. <sup>b</sup>  $n = 123$ . <sup>c</sup>  $n = 214$ .

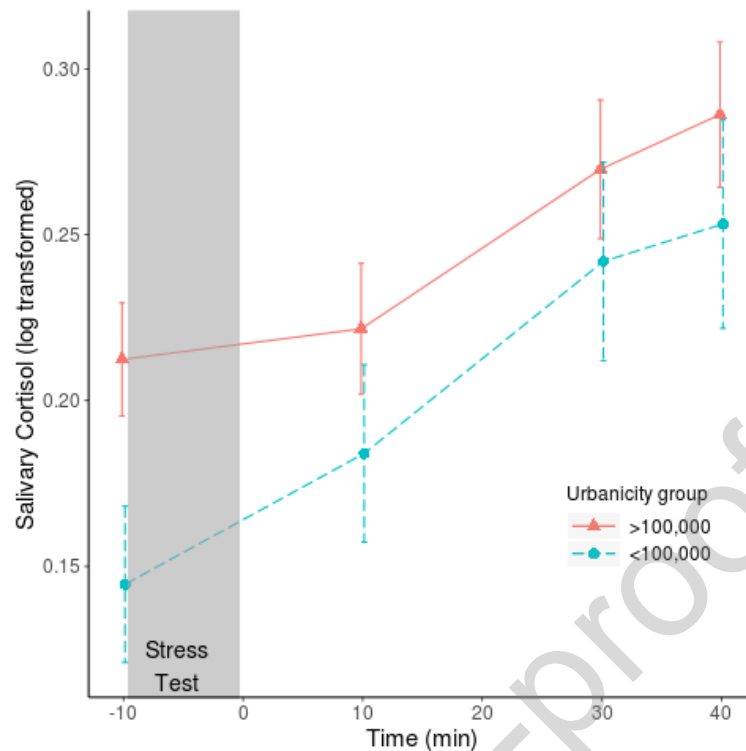
Results of the ANOVA with repeated measures are presented in table 4. No interaction effect of urbanicity and time of measurement on salivary cortisol was identified ( $F(1.8, 595.93) = 0.26, p = .75$ ). Descriptively, higher salivary cortisol in children with high urbanicity were observed before and after the stress test, but the main effect of urbanicity on salivary cortisol was not significant ( $F(1, 331) = 3.66, p = .06$ ). Figure 3 shows salivary cortisol in both urbanicity groups.

Table 4 ANOVA with repeated measurements of log<sub>10</sub>-transformed salivary cortisol concentrations

	<i>df</i>	<i>SS</i> <sup>a</sup>	<i>MS</i>	<i>F</i>	<i>p</i>	$\eta^2_p$
Urbanicity	1	0.87	0.87	3.66	.06	.01
Time	1.8	0.13	0.07	1.58	.21	.01
Urbanicity x time	1.8	0.02	0.01	0.26	.75	.001
Time of baseline saliva sample	1	13.38	13.38	56.2	< .001	.15
Time of baseline saliva sample x time	1.8	0.24	0.13	3.02	.06	.01
Sex	1	0.26	0.26	1.08	.30	.003
Sex x time	1.8	0.25	0.14	3.17	.048	.01
Maternal education	1	0.05	0.05	0.19	.67	.001
Maternal education x time	1.8	0.2	0.11	2.51	.09	.01
Household income	1	0.07	0.07	0.29	.59	.001
Household income x time	1.8	0.03	0.02	0.43	.63	.001
Error	331	78.81	0.24			
Error (time)	595.93	26.37	0.04			

Notes.  $n = 337$ . Greenhouse-Geisser corrected values were reported for the inner-subject as the assumption of sphericity was violated. Time = time of measurement.

<sup>a</sup> Sum of squares type III.



*Figure 1.* Mean levels of log<sub>10</sub>-transformed salivary cortisol (nmol/l) over the course of the experiment are depicted separately for the group with higher (> 100,000 inhabitants) and the group with lower (< 100,000 inhabitants) urbanicity. The error bars represent the standard error of the mean.

The model with urbanicity, urinary cortisol concentration, and the covariates sex, household income, maternal education, wearing a night diaper, and time of urine sample explained 12% of the variance of CBCL total score ( $F(7, 356) = 6.77, p < .001, R^2 = .12$ ). Urinary cortisol was no significant mediator of the relationship between urbanicity and CBCL total score as no average causal mediation effect of urbanicity on CBCL total score mediated by urinary cortisol was shown ( $B = 0.09, 95\% \text{ CI } [-0.25, 0.56], p = .63$ ). Supplementary figure S3 and supplementary tables S3 and S4 provide the results of this mediation analysis.

The model with urbanicity, salivary cortisol response after stress exposure, and the covariates sex, household income, maternal education, and time of baseline saliva sample explained 11% of the variance of CBCL total score ( $F(6, 326) = 6.51, p < .001, R^2 = .11$ ). Salivary cortisol response after stress exposure was no significant mediator of the relationship between urbanicity and CBCL total score as no average causal mediation effect of urbanicity on CBCL total score mediated by salivary

cortisol response was observed ( $B = -0.01$ , 95% CI  $[-0.33, 0.28]$ ,  $p = .95$ ). The results of this mediation analysis are shown in supplementary figure S4 and supplementary tables S3 and S4. The sensitivity analysis indicated a correlation of  $r = 0.86$  ( $n = 337$ ) between the AUC<sub>i</sub> based on the log transformed values, and the AUC<sub>i</sub> based on the untransformed cortisol values, and the mediation analysis did not differ substantially (results shown in supplementary figure S9 and supplementary tables S10 and S11).

#### 4. Discussion

The present study investigated the relationship between urbanicity and mental health in preschoolers as well as the role of HPA axis regulation in this context. Urbanicity did not significantly predict behavior problems. Furthermore, urbanicity did not significantly predict urinary cortisol, and was not associated with salivary cortisol reactivity after stress exposure. Descriptively, higher salivary cortisol values before and after the stress test were observed in children living in cities, an effect that fell short of statistical significance. Mediation analyses showed that neither urinary cortisol nor salivary cortisol reaction after stress exposure were identified as mediators of a relationship between urbanicity and behavior problems. Therefore, all hypotheses have to be rejected.

The results are in line with other studies showing no relationship of urbanicity and behavior problems in children of older age (Evans, van der Ende et al., 2020) and no direct relationship of urbanicity and behavior problems in adolescents (Evans, Huizink et al., 2020). Associations between urbanicity and mental health might be difficult to detect in childhood and become more visible in adulthood. The age of the present sample of 45 months might be too young for this relationship to be already established. Our results suggest no increased risk of mental health problems in preschoolers growing up in cities.

As previous research links urban upbringing to diurnal HPA axis regulation, especially to HPA axis reactivity, during adolescence and adulthood (Evans et al., 2013; Steinheuser et al., 2014), our findings might suggest that nocturnal HPA axis activity could be influenced by other factors compared to diurnal HPA axis activity. However, as already described, the young age could have also led to not identifying any relationships. Consistent with our results of no relationship between

urbanicity and HPA axis reactivity, other studies also demonstrate no relationship in childhood (Evans et al., 2013; Evans, van der Ende et al., 2020). This suggests that the relationship might only become apparent from adolescence and adulthood on and that growing up in an urban environment does not seem to influence HPA axis regulation as early as in preschool age. In line with our findings, other studies did not identify HPA axis regulation as mediator of a relationship between urbanicity and behavior problems in childhood and adolescence (Evans, Huizink et al., 2020; Evans, van der Ende et al., 2020).

The present study has several limitations: First, recruitment for the present study took place in two German cities, and generalizability of the results might therefore be limited. Second, due to the distribution of the number of inhabitants, two urbanicity groups were created resulting in a loss of information. Additionally, current urbanicity at T4 was analyzed, and it was not considered how long participants had been living at their current residence. However, a dose-response relationship has been demonstrated for the effects of urbanicity on mental health (Pedersen & Mortensen, 2001). Future studies might benefit from assessing urbanicity characteristics and associated risk factors in more detail. For example, in the POSEIDON sample, we do not observe associations of urbanicity with HPA axis regulation, but found associations of prenatal maternal stress with stress reactivity (Send, Bardtke, Gilles, Wolf, Sütterlin, Kirschbaum et al., 2019) and cortisol levels in morning urine (Send, Bardtke, Gilles, Wolf, Sütterlin, Wudy et al., 2019). Third, we adjusted the analyses for education and income to control for major influence factors related to socioeconomic status. We did not observe significant effects of urbanicity on the assessed HPA axis measures. However, we cannot exclude that more subtle differences between the urbanicity groups not captured by this approach might have masked urbanicity effects on the HPA axis regulation. For example, we did not analyze the effects of parental stress on the association of urbanicity and the HPA axis measures in the present study, and can thus not exclude moderating effects of perceived stress by parents on children's HPA axis measures. Fourth, the degree of behavior problems in the present sample might not have been sufficient to detect relationships. It is possible that relationships could be identified in samples with more behavior problems – a research question that should be addressed by future studies. Fifth, assessment of behavior problems was based on estimation of the main care giver as only source.



Interestingly, other studies using parental assessment of behavior problems did also not identify relationships with urbanicity in childhood (Evans, van der Ende et al., 2020), while studies using teacher assessment of behavior problems show associations with urbanicity in childhood (Evans et al., 2018; Rutter et al., 1975). Therefore, future studies should examine different assessments of behavior problems in relation with urbanicity.

To our knowledge, this study is the first study to investigate cortisol concentration in morning urine in relation to urbanicity. It suggests that nocturnal HPA axis activity is not related to urbanicity in preschool age. To our knowledge, the present sample is the youngest sample so far in which urbanicity, behavior problems and HPA axis regulation were investigated. Therefore, this study contributes to assessing urban environment as living space for young children and to understanding of factors influencing mental health and HPA axis regulation in early childhood. The homogenous age of the present sample allows precise conclusions about the investigated associations at the age of 45 months. Our findings suggest that either stress exposure in urban environments is not increased in preschool age or effects of increased stress in cities are not yet reflected in HPA axis regulation and mental health of that age. In order to verify this, subsequent study waves should investigate urbanicity, HPA axis regulation and mental health in this sample at a later stage. Future research should examine at which age relationships can be first identified and clarify the mechanisms of the relationship between urbanicity and mental health.

### **Acknowledgement**

We thank all parents and children for taking part in this study and our student employees and interns for their support with data acquisition and data entry. Furthermore, we would like to thank Prof. Dr. Manfred Laucht for his helpful advice.

### **Funding and Disclosure**

This work was supported by an Era-Net Neuron grant to M.D. and M.R., the German Federal Ministry of Education and Research (BMBF) through the Integrated Network IntegraMent (Integrated Understanding of Causes and Mechanisms in Mental Disorders), under the auspices of the e:Med Programme (grant 01ZX1614G to M.R.), through ERA-NET NEURON “Impact of Early life

MetaBolic and psychosocial strEss on susceptibility to mental Disorders; from converging epigenetic signatures to novel targets for therapeutic intervention” (01EW1904 to M.R.) ERA-NET NEURON “SynSchiz - Linking synaptic dysfunction to disease mechanisms in schizophrenia—a multilevel investigation.” (01EW1810 to M.R.), and by a grant of the Dietmar Hopp Foundation. The study was supported by the German Research Foundation (DFG; grant FOR2107; RI908/11-2 to M.R.; WI3429/3-2 to S.H.W.).

Conflict of interest: The authors declare that they have no conflict of interest.

### Author Contributions

Substantial contributions to the conception or design of the study, or the acquisition of data, or analysis and interpretation of data: P.S.E., T.S.S., M.G., I.A.C.W., J.F., R.K., S.H.W., M.R., M.D., F.S.

Drafting the article or revising it critically for important intellectual content: P.S.E., T.S.S., S.B., R.K., F.S.

Final approval of the version to be submitted: P.S.E., T.S.S., M.G., I.A.C.W., J.F., S.B., R.K., S.H.W., M.R., M.D., F.S.

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## Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

## Highlights

- Urbanicity in early life is a well established risk factor for psychopathology
- Investigation of urbanicity with behavior problems and HPA axis regulation
- Investigation in homogenous sample of  $N = 399$  45-months-old preschoolers
- Urbanicity was not associated with behavior problems
- Urbanicity was not associated with cortisol reactivity or cortisol in morning urine