Psychoneuroendocrinology 102 (2019) 53–57

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen

Short Communication

Childhood urbanicity and hair steroid hormone levels in ten-year-old children

B.E. Evans[a,b,*], R. Beijers[b], C. Hagquist[a], C. de Weerth[b]

[a] Centre for Research on Child and Adolescent Mental Health, Karlstad University, Universitetsgatan 2, 651 88, Karlstad, Sweden
[b] Behavioural Science Institute, Radboud University, Montessorilaan 3, 6525 HR, Nijmegen, the Netherlands

ARTICLE INFO

Keywords:
Steroid hormones
Hair
Urbanicity
HPA axis
Socioeconomic status
Children

ABSTRACT

Background: Research suggests that it may be more stressful for children to grow up in an urban area than in a rural area. Urbanicity may affect physiological stress system functioning as well as the timing of sexual maturation. The purpose of the current study was to investigate whether moderate urbanicity (current and childhood, ranging from rural areas to small cities) was associated with indices of long-term hypothalamic-pituitary-adrenal and hypothalamic-pituitary-gonadal axis functioning (cortisol, cortisone, dehydroepiandrosterone and progesterone levels) and whether sex moderated any associations.

Method: Children ($N = 92$) were all 10 years old and from the Dutch general population. Hair samples were collected and single segments (the three cm most proximal to the scalp) were assayed for concentrations of steroid hormones (LCMS/MS method). Neighborhood-level urbanicity and socioeconomic status were measured from birth through age ten years. Analyses were controlled for neighborhood- and family socioeconomic status, body mass index and season of sampling.

Results: The results from multivariate analyses of variance showed no associations between current or childhood moderate urbanicity and hair steroid hormone concentrations. Interaction terms between moderate urbanicity and sex were not statistically significant.

Conclusions: Associations between urbanicity and steroid hormone levels may only be detectable in highly urban areas and/or during later stages of adolescence. Alternatively, our findings may have been due to most children being from families with a higher socioeconomic status.

1. Introduction

Urban areas may be stressful living environments for humans (Mizrahi, 2016). They are characterized by high population density, encounters with strangers and unclear dominance order, factors that may increase the threat of social evaluation and thus social stress (Van Os et al., 2000). Living in a socially stressful environment is considered to be a major contributor to dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis (Champagne, 2013) and has been linked to earlier sexual maturation in females (i.e. earlier increased activation of the hypothalamic-pituitary-gonadal; HPG axis; Belsky, 2012). Thus, youth living in more stressful environments may manifest different hormonal profiles compared to those living in less stressful environments.

There is some evidence that living in a more urban area is linked to blunted HPA axis reactivity (Evans et al., 2013) and earlier sexual maturation (Keizer-Schrama and Mul, 2001) in adolescents. Previous research focused mainly on urban versus rural environments (Peen et al., 2010) and little is known about moderately urban areas. In the same sample as the current study, urbanicity was not associated with salivary cortisol levels when children were six years (Evans et al., under review). However, indices of long-term HPA axis functioning may be more appropriate regarding characteristics of the broader, long-term living environment. For example, recent work in children showed that lower socioeconomic living conditions were associated with higher hair cortisol concentrations (e.g. Vaghri et al., 2013; Vliegenthart et al., 2016). In this study we investigated whether moderate urbanicity (current and childhood, ranging from rural areas to small cities) was associated with indices of long-term HPA and HPG axis functioning (cortisol, cortisone, dehydroepiandrosterone; DHEA, progesterone).

* Corresponding author at: Centre for Research on Child and Adolescent Mental Health, Karlstad University, Room 1D 349A, Universitetsgatan 2, 651 88, Karlstad, Sweden.
E-mail addresses: brittany.evans@kau.se (B.E. Evans), r.beijers@psych.ru.nl (R. Beijers), curt.hagquist@kau.se (C. Hagquist), C.deWeerth@psych.ru.nl (C. de Weerth).

https://doi.org/10.1016/j.psyneuen.2018.11.039
Received 2 July 2018; Received in revised form 27 November 2018; Accepted 28 November 2018

Considering evidence that girls and boys may be differently sensitive to socio-environmental stress (Belsky, 2012; Cowan and Richardson, 2018; McCormick and Mathews, 2007) we examined whether sex moderated any associations. We hypothesized that urbanicity would be positively associated with progesterone and DHEA. Given the inconsistent evidence regarding the direction of effects between stressful living environments and HPA axis functioning (e.g. Evans et al., 2013; Vliegenthart et al., 2016), we did not hypothesize the direction of association between urbanicity and cortisol and cortisone levels.

2. Materials and methods

2.1. Participants and procedure

Participants were followed from birth through age ten years in a longitudinal study examining early caregiving, environmental factors and children's development (BIBO study; $N = 193$; Beijers et al., 2011). The Faculty Ethics Committee of Radboud University approved the study (ECG300107; SW2017-1303-497; SW2017-1303-498), and all mothers gave written informed consent, prior to its initiation. All participants were born between January 2006 and July 2007 in and around two eastern Dutch cities and were of Dutch ethnicity. When children were ten years old, they were asked to provide a hair sample to assess hormone concentrations. Of the 158 children that participated in home visits at age ten ($M = 10$ years and 19 days, $SD = 122$ days), hormone concentrations were available for 93. Missingness was mainly due to too short hair in boys. One child lived in Germany, therefore information on urbanicity was not available and they were excluded (final sample $N = 92$).

2.2. Measures

2.2.1. Hair hormone concentrations

Hair samples were collected by the second author and trained research assistants. At least three mm (thickness) of hair was cut from the back of the head near the scalp. Samples were stored in closed envelopes in a cabinet and collectively sent to the laboratory (Dresden LAB Service GmbH). Concentrations of cortisol, cortisone, DHEA, progesterone and testosterone[1] were simultaneously assayed in one segment of the three cm of hair most proximal to the scalp (LCMS/MS). Non-detectable levels (< 0.2 pg/mg; for cortisol: 30%, cortisone: 0%, DHEA: 11%, progesterone: 25%) were set to a value of 0.1 pg/mg. One outlier (> 100 pg/mg) was excluded, four others (> 3 SD above the mean but < 100 pg/mg) were winsorized[2]. Cortisol values were natural log transformed and DHEA values were square root transformed to correct skewness and kurtosis.

2.2.2. Urbanicity

Urbanicity was measured at the neighborhood level. Children were assessed almost yearly during the study, therefore their home address was known from birth through age ten. Using these addresses, we extracted data from Statistics Netherlands on the neighborhood the children lived in from birth through age ten. Urbanicity is calculated by Statistics Netherlands using the surrounding address density (SAD) and coded on a scale from 0 (very rural; SAD $<$ 500 addresses per km^2) to 4 (very urban; $\geq$ 2500 addresses per km^2). The urbanicity score was aggregated across children's first ten years as a measure of childhood urbanicity. Current urbanicity was indicated by urbanicity at age ten.

2.2.3. Other variables

Children's sex was mother-reported after birth. Neighborhood-level socioeconomic status (SES) was indicated by 13 characteristics of the neighborhood reported by Statistics Netherlands (e.g. average value of housing, proportion of employed persons). For 2008, 2011 and 2015, most income-related variables were missing, and data from 2016 and 2017 were not yet available in the Statistics Netherlands database, therefore we did not use data from those years. A principal components analysis of these variables was carried out for each year (2006–2014, except 2008 and 2011) in all participants for whom address information was available for the given years. For each year, this resulted in three components: employment, income, and multi-ethnicity, explaining 79%–85% of the variance ($M = 81\%$, $SD = 2\%$). The factor scores on each of these components were aggregated across the study years for each participant. Family SES was based on the higher education level of either parent (parent-reported when children were 30 months and four years), categorized into low/average and high SES. Height and weight were measured by the research assistant during the age ten home visit and used to calculate body mass index (BMI). Season of sampling was categorized as spring/summer and autumn/winter. Tanner stage (Marshall and Tanner, 1970) was self-reported at age ten.

2.3. Analysis plan

All variables were centered and scaled prior to analysis. Because some of the children lived in the same neighborhood (92 participants lived in 79 neighborhoods), we examined whether it was necessary to execute multilevel analyses. We calculated intraclass correlations (ICC) using empty models for the outcomes of each of the hair hormone concentrations (using the R, 2017, package 'Multilevel'; Bliese, 2013). Neighborhood explained very little variance in the hormone levels (ICC1: .00–.37) and had very low reliability (ICC2: .00–.39), therefore we tested a single-level model. The main analysis (using R) consisted of two multivariate analyses of variance (MANOVA), each with four outcomes (cortisol, cortisone, DHEA, progesterone) and the main predictor being either current urbanicity or childhood urbanicity. Main effects for sex and interactions between sex and urbanicity were included in both models, as well as the control variables neighborhood- and family-level SES, BMI and season. Follow-up ANOVAs were executed and results from these models were considered significant at $p < .02$ (Bonferroni correction).

3. Results

Table 1 shows the descriptive statistics. Cortisol and cortisone levels were highly correlated ($r = 0.86$), therefore, we omitted cortisone from the main analyses in order to avoid multicollinearity[3]. The results of the MANOVA analyses (Table 2) showed that neither current nor childhood moderate urbanicity were associated with any of the hair hormone concentrations[4]. Sex was not associated with any of the hormones, and there were no significant interactions between sex and moderate urbanicity[5]. Family SES was a significant covariate in the analysis regarding childhood moderate urbanicity and follow-up ANOVAs showed that it was positively associated with DHEA levels.

[1] Testosterone levels were not analyzed in the current study due to non-detectable levels in the majority (91%) of the sample.

[2] We also ran the models excluding all five outliers and this did not change our results.

[3] We re-ran the analyses omitting cortisol instead of cortisone, and the results were not different.

[4] As a sensitivity analysis, we reran the analysis differentiating between early (birth-five years) and late (six-ten years) childhood urbanicity and the results remained the same.

[5] We also tested whether the results were similar in girls only, because of high missingness of hair samples for boys, and they were.

Table 1
Descriptive statistics of and correlations between all variables.

	N	*M*(*SD*) or *F*(%)	Range	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1.Current urbanicity	74	1.54(1.36)	0.00-4.00	–																
2.Childhood urbanicity	92	1.53(1.15)	0.00-4.00	**.92**	–															
3.Cortisol	91	2.99(4.51)	0.10-29.30	.14	.02	–														
4.Cortisone	92	10.08(12.03)	0.98-47.76	.09	.00	**.86**	–													
5.DHEA	92	7.01(4.19)	0.10-22.24	−.07	.02	**−.29**	**−.31**	–												
6.Progesterone	92	0.95(0.55)	0.10-2.15	.11	.01	**.38**	**.38**	−.02	–											
7.Sex	92	32/68		−.19	−.16	**−.21**	−.19	−.16	**−.23**	–										
8.Current SES1	73	−0.06(1.03)	−3.65-2.15	**−.49**	**−.45**	−.13	−.12	.11	−.07	.16	–									
9.Childhood SES1	92	−0.04(0.93)	−3.29-1.79	**−.43**	**−.50**	−.09	−.10	.02	−.09	**.22**	**.82**	–								
10.Current SES2	73	0.06(1.02)	−2.11-2.98	.02	−.02	.04	.06	**−.30**	.02	.12	.05	.00	–							
11.Childhood SES2	92	0.04(0.85)	−1.60-2.93	.12	−.01	.01	−.03	**−.21**	−.01	.09	.07	**.22**	**.83**	–						
12.Current SES3	73	0.14(0.93)	−1.85-2.17	**.65**	**.70**	−.06	−.17	.07	.01	.01	−.19	**−.24**	−.13	−.07	–					
13.Childhood SES3	92	0.13(0.91)	−1.78-1.94	**.59**	**.68**	−.04	−.10	.02	−.03	.00	−.20	**−.23**	−.12	**−.22**	**.82**	–				
14.Family SES	90	9/91		.01	.04	−.10	−.11	**.21**	.04	.04	.05	−.03	.22	.16	.03	−.04	–			
15.Body mass index	90	17.29(2.14)	13.96-26.58	−.11	−.04	.01	−.01	.12	−.13	.03	.02	−.03	−.06	−.09	−.03	−.00	−.05	–		
16.Season	92	44/56		.02	.02	−.13	−.08	.14	−.08	.07	−.05	−.09	−.17	−.13	−.01	.01	−.04	.02	–	
17. Tanner stage	82	51/33/15/1		.13	.11	.09	.01	.05	.11	.00	−.05	.01	**−.28**	**−.26**	.20	.16	−.00	.22	−.11	–

Note. **Bold** indicates $p < .05$; DHEA = dehydroepiandrosterone; SES1 = socioeconomic status employment component; SES2 = socioeconomic status income component; SES3 = socioeconomic status multi-ethnicity component; BMI = body mass index. Raw values of cortisol and DHEA were used in the mean, SD and range statistics, transformed values of cortisol and DHEA were used in the correlation statistics; urbanicity and SES variables were measured at the neighborhood level. Categorical variables were coded as follows: sex (0 = boy, 1 = girl), family SES (0 = low/average, 1 = high), season (0 = spring/summer, 1 = autumn/winter), Tanner stage (1 = stage 1, 2 = stage 2, 3 = stage 3, 4 = stage 4).

Table 2
Statistics for multivariate- and follow-up univariate analyses of variance.

	Multivariate analysis of variance			Univariate analyses of variance					
				Cortisol		Progesterone		DHEA	
	Pillai	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Current (*N* = 70)									
Main effects									
Urbanicity	0.02	0.39	.76	0.87	.36	0.32	.57	0.03	.87
Sex	0.07	1.45	.24	1.45	.23	2.48	.12	0.53	.47
SES1	0.01	0.27	.85	0.01	.94	0.00	.98	0.78	.38
SES2	0.08	1.74	.17	0.77	.38	0.21	.65	5.36	.02
SES3	0.03	0.71	.55	0.84	.36	0.03	.86	1.68	.20
Family SES	0.08	1.68	.18	0.06	.81	0.00	.97	4.90	.03
BMI	0.12	2.75	.05	0.38	.54	4.13	.05	1.65	.20
Season	0.06	1.31	.28	0.39	.53	0.87	.36	3.50	.07
Interaction effects									
Sex X urbanicity	0.02	0.49	.69	1.10	.30	0.89	.35	0.18	.67
Childhood (*N* = 87)									
Main effects									
Urbanicity	0.02	0.55	.65	0.25	.62	0.25	.62	0.83	.37
Sex	0.08	2.23	.09	2.49	.12	4.44	.04	0.45	.50
SES1	0.03	0.68	.57	0.22	.64	0.92	.34	1.33	.25
SES2	0.02	0.48	.69	0.12	.73	0.29	.59	1.27	.26
SES3	0.02	0.50	.69	0.00	.95	0.17	.68	1.23	.27
Family SES	**0.13**	**3.77**	**.01**	0.81	.37	0.11	.74	**11.09**	**.001**
BMI	0.08	2.24	.09	1.08	.30	3.26	.07	0.05	.82
Season	0.08	2.09	.11	1.27	.26	0.70	.40	6.11	.02
Interaction effects									
Sex X urbanicity	0.02	0.64	.59	0.30	.59	1.51	.22	0.62	.43

Note. **Bold** indicates $p < .05$ in the MANOVA results; significance levels for the ANOVA results were Bonferroni corrected and considered significant at $p < .02$. Main effects are reported from the model not including the interaction term. DHEA = dehydroepiandrosterone; SES1 = socioeconomic status employment component; SES2 = socioeconomic status income component; SES3 = socioeconomic status multi-ethnicity component; BMI = body mass index; urbanicity and SES variables were measured at the neighborhood level.

4. Discussion

To our knowledge, this is the first study to examine associations between moderate urbanicity (current and childhood) and concentrations of hair steroid hormones in ten-year-old children. At this age, children are at the critical developmental junction between childhood and adolescence. Indeed, half of the children in our sample were prepubertal (51%) and half had experienced the onset of puberty (Tanner stages 2–4). Steroid hormones begin to increase around age seven with adrenarche (de Peretti and Forest, 1978) and further with gonadarche

some years later (Terasawa and Fernandez, 2001). Cortisol, cortisone, DHEA and progesterone are secreted by the adrenal glands during childhood and adolescence, and progesterone additionally by the ovaries in peri- and post-pubertal females. As adolescence is considered a sensitive period in the development of both the HPA (Lupien et al., 2009) and HPG (Marceau et al., 2015) axes, we were interested in potential effects of the broader social environment during this particular period. We found that moderate urbanicity was not associated with cortisol, cortisone, DHEA or progesterone levels. That we did not find evidence for an association between urbanicity and steroid hormones might indicate that such effects are only detectable in highly urban areas and/or during later stages of adolescence.

Alternatively, the lack of associations between moderate urbanicity and steroid hormone levels may have to do with the majority of the children in our sample (91%) coming from high SES families. Higher family SES may act as a protective factor for the effects of stress through higher quality housing (e.g. less crowding inside the house, better insolation against noise), residency in neighborhoods with more green areas, or higher quality parenting. Parents with higher educational levels may tend to exhibit higher quality caregiving (Baumrind, 1966). Thus, the majority of children in our sample may have been buffered by advantages that come with having a higher family SES.

Children with a higher family SES exhibited higher DHEA levels. Given the small number of children with a lower family SES ($n = 8$), this result must be interpreted cautiously. The finding is in contrast to predictions from life history theory that sexual maturation will be delayed in children growing up in financially secure families (Belsky, 2012). It is more consistent with studies showing that DHEA may protect against the neurotoxic effects of stress (Kimonides et al., 1998). Descriptive statistics from our study showed that lower neighborhood income levels were correlated with higher Tanner pubertal stages, which is in line with life history theory. Clearly, more research is needed to resolve the inconsistencies in the current literature regarding socioeconomic conditions and development.

Strengths of our study include the longitudinal design and the measurement of four steroid hormones. We measured urbanicity and socioeconomic status at the neighborhood level to index children's proximal environment from birth through age ten. Of course, the day-to-day living experience in even the more urban neighborhoods of the small cities in our study is distinct from that of major metropolitan areas. Additionally, we lacked information on perceived psychosocial stress related to urbanicity (e.g. due to high population density, encounters with strangers), which would be interesting to consider in future studies. Further limitations are that we had no information on hair color and there were high percentages of undetectable levels of cortisol and progesterone. Also, the children in our sample were mostly from higher SES families, which limits the generalizability of our findings. Finally, we did not examine urbanicity in relation to more complex hormone measures such as ratios between hormones, although this would be a relevant avenue for future studies.

5. Conclusion

In this preliminary study, we did not find any associations between current and childhood moderate urbanicity and steroid hormone levels in a sample of ten-year-old children. These associations may only be detectable in highly urban areas and/or during later stages of adolescence. Alternatively, our findings may have been due to most children being from families with a higher socioeconomic status.

Conflicts of interest

All authors declare to have no conflicts of interest.

Role of the funding source

The funding source had no role in any part of the manuscript.

Author contributions

BEE contributed to the funding of the BIBO wave 10 data collection, conceived the research questions, analyzed the data and interpreted the findings, and drafted and revised the manuscript. RB contributed to the design and funding and coordinated the data collection of the BIBO study, co-conceived the research questions, collaborated in the interpretation of the data, and made critical revisions to the manuscript. CH collaborated in the interpretation of the data and made critical revisions to the manuscript. CdW designed and secured funding for the BIBO study, collaborated in the interpretation of the data and made critical revisions to the manuscript. All authors have approved the final manuscript.

Acknowledgements

We would like to thank all participants of the BIBO study and their parents, as well as the students that helped collect the data. This research was supported by a Netherlands Organization for Scientific Research (NWO) Vidi grant awarded to CdW (grant 452-04-320), a Jacobs Foundation Advanced Fellowship to CdW, a Sara van Dam Project Grant of the Royal Netherlands Academy of Arts and Sciences to RB and a Behavioural Science Institute Fellowship awarded to BEE.

References

Baumrind, D., 1966. Effects of authoritative parental control on child behavior. Child Dev. 37, 887–907.
Beijers, R., Jansen, J., Riksen-Walraven, M., de Weerth, C., 2011. Attachment and infant night waking: a longitudinal study from birth through the first year of life. J. Dev. Behav. Pediatr. 32, 635–643.
Belsky, J., 2012. The development of human reproductive strategies: progress and prospects. Curr. Dir. Psychol. Sci. 21, 310–316.
Bliese, P., 2013. Multilevel: Multilevel Functions. R Package version 2.5.
Champagne, F.A., 2013. Early environments, glucocorticoid receptors, and behavioral epigenetics. Behav. Neurosci. 127, 628–636.
Cowan, C.S., Richardson, R., 2018. Early-life stress leads to sex-dependent changes in pubertal timing in rats that are reversed by a probiotic formulation. Dev. Psychobiol. https://onlinelibrary.wiley.com/doi/full/10.1002/dev.21765.
Evans, B.E., Greaves-Lord, K., Euser, A.S., Tulen, J.H.M., Franken, I.H.A., Huizink, A.C., 2013. Determinants of physiological and perceived physiological stress reactivity in children and adolescents. PLoS One 8, e61724.
Evans, B.E., van der Ende, J., Greaves-Lord, K., Huizink, A.C., Beijers, R., de Weerth, C., under review. Urbanicity, hypothalamic-pituitary-adrenal axis functioning, and behavioral and emotional problems in children. Int. J. Psychol.
Keizer-Schrama, S., Mul, D., 2001. Trends in pubertal development in Europe. Hum. Reprod. Update 107, 287–291.
Kimonides, V.G., Khatibi, N.H., Svendsen, C.N., Sofroniew, M.V., Herbert, J., 1998. Dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEAS) protect hippocampal neurons against excitatory amino acid-induced neurotoxicity. Proc. Natl. Acad. Sci. U. S. A. 95, 1852–1857.
Lupien, S.J., McEwen, B.S., Gunnar, M.R., Heim, C., 2009. Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nat. Rev. Neurosci. 10, 434–445.
Marceau, K., Ruttle, P.L., Shirtcliff, E.A., Essex, M.J., Susman, E.J., 2015. Developmental and contextual considerations for adrenal and gonadal hormone functioning during adolescence: implications for adolescent mental health. Dev. Psychobiol. 57, 742–768.
Marshall, W.A., Tanner, J.M., 1970. Variations in the pattern of pubertal changes in boys. Arch. Dis. Child. 45, 13–23.
McCormick, C.M., Mathews, I.Z., 2007. HPA function in adolescence: role of sex hormones in its regulation and the enduring consequences of exposure to stressors. Pharmacol. Biochem. Behav. 86, 220–233.
Mizrahi, R., 2016. Social stress and psychosis risk: Common neurochemical substrates? Neuropsychopharmacol. 41, 666–674.
Peen, J., Schoevers, R.A., Beekman, A.T., Dekker, J., 2010. The current status of urban-rural differences in psychiatric disorders. Acta Psychiatr. Scand. 121, 84–93.
de Peretti, E., Forest, M.G., 1978. Pattern of plasma dehydroepiandrosterone sulfate levels in humans from birth to adulthood: evidence for testicular production. J. Clin. Endocrinol. Metab. 47, 572–577.
R Core Development Team, 2017. R: A language and environment for statistical computing. . R Foundation for Statistical Computing, Vienna, Australia.
Terasawa, E., Fernandez, D.L., 2001. Neurobiological mechanisms of the onset of puberty

in primates. Endocr. Rev. 22, 111–151.
Vaghri, Z., Guhn, M., Weinberg, J., Grunau, R.E., Yu, W., Hertzman, C., 2013. Hair cortisol reflects socio-economic factors and hair zinc in preschoolers. Psychoneuroendocrinol. 38, 331–340.
Van Os, J., Driessen, G., Gunther, N., Delespaul, P., 2000. Neighbourhood variation in incidence of schizophrenia - Evidence for person-environment interaction. Brit. J. Psychiatr. 176, 243–248.
Vliegenthart, J., Noppe, G., Van Rossum, E., Koper, J., Raat, H., Van den Akker, E., 2016. Socioeconomic status in children is associated with hair cortisol levels as a biological measure of chronic stress. Psychoneuroendocrinol. 65, 9–14.