# Supporting Information

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### SI Materials and Methods

**Recruiting.** A commuting accident insurance was installed for participating volunteers. Experimenters were covered by the employer's public liability insurance. For the actual experiment, all participants were asked to abstain from caffeine, any kind of drugs (e.g., analgesics, sleep-inducing drugs, dietary supplements), exercise, alcohol, and nicotine for a minimum of 3 d. Furthermore, participants were told to sleep at least 8 h during the night before the experiment and to drink at least 1 L of water on the experimental day itself. In cases of unforeseen illness, test persons were told to delay the experiment. Data were collected between October 2016 and April 2017.

**Experimental Procedure (Fig. S1).** On the test day itself, participants were told to arrive at the laboratory at 1 PM, and immediately afterward their current health status was determined and sociodemographic features were assessed (Table S1). Only if no signs of illness were reported, the venous catheter (nondominant arm) as well as the blood pressure and heart rate monitor (dominant arm) were placed (−60-min time point) in a room adjacent to the TSST room. Immediately before catheterization, all participants had been informed about possible side effects of the catheterization and TSST procedure by the principal investigators and gave informed consent to participate in the current study, while afterward, basal physical and emotional health statuses of the participants were assessed, employing validated questionnaires. Although PBMC ex vivo culturing and plasma sampling were done and samples stored (−80 °C) at each time point (−5, 5, 15, 60, 90, and 120 min), plasma cytokine concentrations were measured only at −5, 60, 90, and 120 min and cytokine concentrations in the supernatants only at −5 and 120 min.

**TSST.** Briefly, the test consisted of a 3-min preparation phase for a simulated job interview, followed by completion of the Primary Appraisal Secondary Appraisal Scale (PASA) (~2 min), a 5-min public-speaking task, and a 5-min arithmetic task. At the beginning of the test, the experimenter guided the participant into the TSST room and positioned him in the center of the room, facing a video camera and a jury consisting of two judges sitting behind a table and wearing white laboratory coats. The judges were told to maintain a neutral evaluative facial expression during the whole test procedure. The experimenter introduced the participant to a standardized job advertisement, for which he wanted him to apply later during the test by explaining why his personality made him ideally qualified for this dream job. After this brief familiarization with the test setting, the experimenter guided the test person back into the adjacent room, allowing him to prepare for the simulated job interview for 3 min. Before the test person was brought back to the test room, he was asked to complete the PASA, which, on average, took about 2 min. Back in the test room, the participant was asked to start with the public speech, without any information about the intended duration of this speech. In cases of more than 20 s of silence, the jury started to ask neutral and standardized questions on potential job qualifications of the participant. After 5 min, the experimenter came back and explained the now-imminent arithmetic task, consisting of counting backward from 3,079 by subtraction of 17, again not providing information of the intended duration of this task. Whenever failing, the participant was asked to start again at 3,079. After 5 min, the TSST was finished.

**Blood Pressure and Heart Rate.** The cuff placed around the dominant arm at the −60-min time point stayed in place until the last measurement was performed at the 120-min time point; the connection between the cuff and the device was released after each measurement. During measurement of blood pressure and heart rate, the participant was sitting on a chair, placing the arm in a slightly bent position on a table.

**PBMC Isolation and Stimulation.** Nine milliliters of blood were transferred from lithium-heparin–coated monovettes into Leucosep tubes (Greiner Bio-One), which were prepared with Ficoll Paque (GE Healthcare Life Sciences) according to the manufacturer's instructions beforehand. The remaining volume was filled up to 50 mL with PBS and then centrifuged for 10 min at room temperature (1,000 × *g*, no brake). The buffy coat layer containing PBMCs was transferred into another 50-mL Falcon tube and washed with RPMI medium containing 10% FCS and 1% penicillin/streptomycin (323 × *g*, 10 min, room temperature). The number of viable (trypan blue) cells was then determined using an automated cell counter (TC20 Automated Cell Counter; Bio-Rad Laboratories), before cells were centrifuged again (323 × *g*, 10 min, room temperature) and adjusted to a final concentration of $2.5 \times 10^6$ cells per mL. A total of $2.5 \times 10^5$ cells was then cultured in 96-well plates, either under basal conditions (100 μL of RPMI were added to a final volume of 200 μL per well) or in the presence of Con A (final concentration in 200-μL volume was 2.5 μg/mL) or lipopolysaccharide (LPS) (final concentration in 200-μL volume was 1 μg/mL) at 37 °C and 5% $CO_2$ for 24 h. Supernatants were collected afterward and stored at −80 °C until further analysis.

**ELISA.** Plasma samples were analyzed using commercially available ELISA kits for interleukin-6 (IL-6) (lowest standard, 0.16 pg/mL; Quantikine HS ELISA; R&D Systems Europe) and IL-10 (lowest standard, 0.78 pg/mL; Quantikine HS ELISA; R&D Systems Europe) and cortisol (lowest standard, 20 ng/mL; IBL International) according to the manufacturers' instructions. Of note, plasma IL-10 concentrations of all participants were under the detection limit of the employed high-sensitive ELISA with the lowest standard being 0.78 pg/mL (Quantikine HS ELISA; R&D Systems). Supernatants from PBMC stimulations were analyzed using commercially available ELISA kits (Human DuoSet ELISA, 5 Plate; R&D Systems Europe) for IL-6 (lowest standard, 9.38 pg/mL) and IL-10 (lowest standard, 31.3 pg/mL) according to the manufacturer's instructions. Of note, basal ex vivo IL-10 concentrations of all participants were under the detection limit of the employed ELISA with a lowest standard of 9.38 pg/mL (Human IL-6 DuoSet ELISA; R&D Systems).

**Determination of Salivary α-Amylase Concentrations.** Salivary α-amylase as a surrogate marker of sympathetic nervous system activity was measured as described earlier (1). In detail, saliva was processed on a FLUENT liquid handling system (Tecan). Saliva was diluted at 1:625 with ultrapure water by the liquid handling system. Twenty microliters of diluted saliva and standard were then transferred into 96-well polystyrol microplates (Roth). Standard was prepared from "Calibrator f.a.s." solution (Roche Diagnostics) with concentrations of 326, 163, 81.5, 40.75, 20.38, 10.19, and 5.01 U/L α-amylase, respectively, and ultrapure water as zero standard. Afterward, 50 μL of substrate reagent (α-amylase EPS Sys; Roche Diagnostics) was pipetted into each well. The microplate containing sample and substrate was then

heated to 37 °C in a Thermomixer (Eppendorf). Immediately afterward, a first interference measurement was obtained at a wavelength of 405 nm using a standard absorbance reader (Infinite M200; Tecan). The plate was then incubated for another 5 min at 37 °C, before a second measurement at 405 nm was taken. Increases of absorbance in samples were transformed to α-amylase concentrations using a linear regression computed against the standard curve on each microplate. Interassay and intraassay variation was below 10%.

**Statistics.** Extreme outliers were identified using Grubbs' test and excluded from further analysis [PBMC counts: $n = 1$ (urban); plasma IL-6: $n = 2$ (rural), $n = 1$ (urban); ex vivo PBMC stimulation: IL-6 basal, $n = 1$ (rural), $n = 1$ (urban); IL-6 Con A, $n = 2$ (rural), $n = 2$ (urban); IL-10 LPS, $n = 1$ (rural); plasma cortisol: $n = 3$ (rural), $n = 2$ (urban); α-amylase: $n = 1$ (rural), $n = 1$ (urban); mean arterial pressure: $n = 1$ (urban)]. Datasets were subsequently analyzed using $\chi^2$ test (nominal scaled data), parametric Student's $t$ test (one factor, two independent samples) or a linear mixed model approach. A linear mixed model analysis was used because it has several advantages over the repeated-measures ANOVA when analyzing repeated measures data, including (*i*) the accommodation of multiple missing data values, (*ii*) the ability to more effectively estimate model parameters in unbalanced experimental designs, (*iii*) more flexibility in model fitting through the objective selection of covariance structures that better fit the correlations between data points, and (*iv*) the ability to model nonlinear changes in a dependent variable across time and treatment (2–5). The latter was followed, when a significant main effect for one factor or an interaction between the two factors was found, by post hoc analysis using Bonferroni pairwise comparison. Data are presented as mean + or ± SEM. The level of significance was set at $P \leq 0.05$.


1. Thoma MV, Kirschbaum C, Wolf JM, Rohleder N (2012) Acute stress responses in salivary alpha-amylase predict increases of plasma norepinephrine. *Biol Psychol* 91: 342–348.
2. Krueger C, Tian L (2004) A comparison of the general linear mixed model and repeated measures ANOVA using a dataset with multiple missing data points. *Biol Res Nurs* 6: 151–157.
3. Cnaan A, Laird NM, Slasor P (1997) Using the general linear mixed model to analyse unbalanced repeated measures and longitudinal data. *Stat Med* 16:2349–2380.
4. Duricki DA, Soleman S, Moon LDF (2016) Analysis of longitudinal data from animals with missing values using SPSS. *Nat Protoc* 11:1112–1129.
5. Judd CM, Westfall J, Kenny DA (2012) Treating stimuli as a random factor in social psychology: A new and comprehensive solution to a pervasive but largely ignored problem. *J Pers Soc Psychol* 103:54–69.


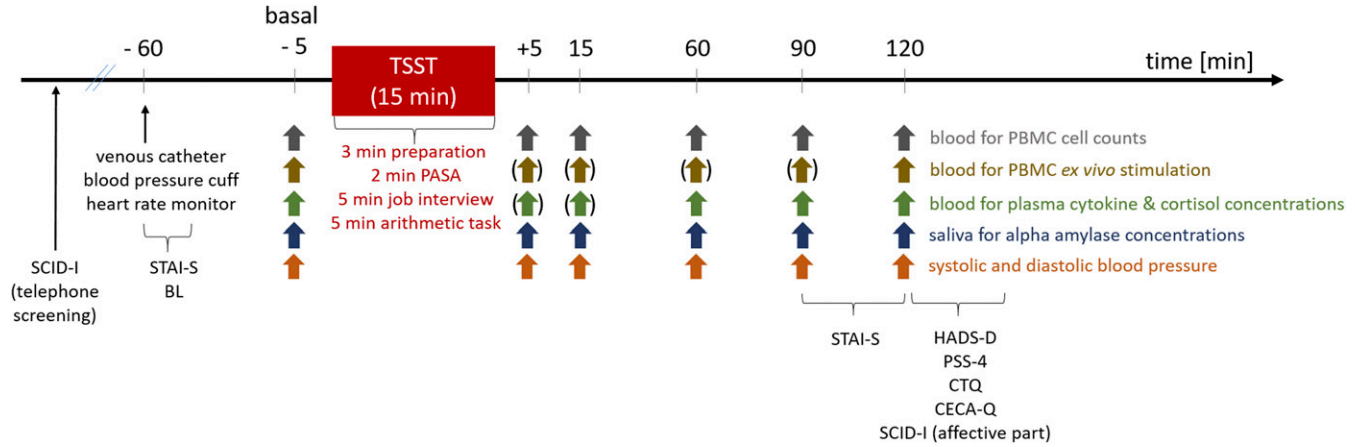


**Fig. S1.** Diagrammatic illustration of the experimental procedure. BL, list of complaints for quantitative analysis of current bodily and general complaints; CECA-Q, Childhood Experience of Care and Abuse Questionnaire; CTQ, Childhood Trauma Questionnaire; HADS-D, Hospital Anxiety and Depression Scale–German Version; PASA, Primary Appraisal Secondary Appraisal Scale; PBMC, peripheral blood mononuclear cell; PSS-4, Perceived Stress Scale-4; SCID-I, Structured Clinical Interview for DSM-IV Axis I Disorders; STAI-S, State–Trait Anxiety Inventory; TSST, Trier social stress test. (↑) indicates that supernatants from ex vivo PBMC cultures and plasma samples have been collected and stored at −80 °C at the respective time points, but cytokine concentrations have not been measured in the present study.

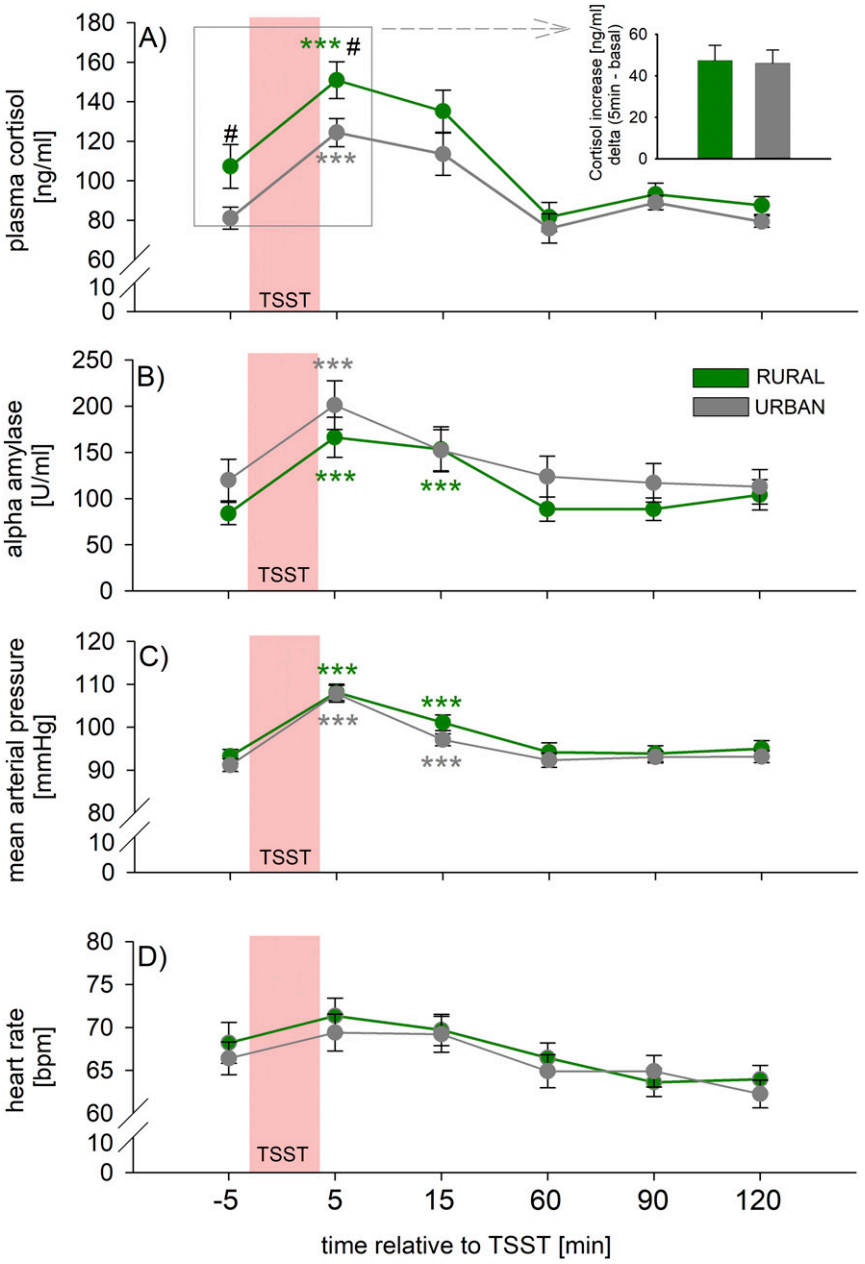


**Fig. S2.** Effects of urban vs. rural upbringing in the absence or presence of animals, respectively, on basal and Trier social stress test (TSST)-induced hypothalamic–pituitary–adrenal (HPA) axis and cardiovascular (re)activity. Urban and rural upbringing in the absence or presence of animals, respectively, was associated with a comparable HPA axis and autonomic nervous/cardiovascular system activation in response to the TSST, indicated by comparable increases in (*A*) plasma cortisol, (*B*) salivary α-amylase, (*C*) mean arterial blood pressure, and (*D*) heart rate. Initial HPA axis activity (*A*) was increased in rural vs. urban participants. Data are presented as mean ± SEM. ***$P \leq 0.001$ vs. respective basal (−5-min) group; $^{\#}P \leq 0.05$ vs. respective rural group.

**Table S1. Sociodemographic features of experimental groups**

| Parameter | Rural | Urban | *P* value (*t* test; $\chi^2$) |
|---|---|---|---|
| Age, y, mean ± SEM | 25.05 ± 0.78 | 24.45 ± 0.88 | 0.613 |
| Height, cm, mean ± SEM | 182.8 ± 1.44 | 182.0 ± 1.49 | 0.701 |
| Weight, kg, mean ± SEM | 82.35 ± 1.58 | 80.75 ± 2.22 | 0.561 |
| BMI, kg/m$^2$, mean ± SEM | 24.40 ± 0.65 | 24.65 ± 0.39 | 0.748 |
| Marital status, % | | | 0.147 |
| Married | 10 | 5 | |
| Single | 90 | 95 | |
| Relationship, % | | | 0.739 |
| Short-term single | 15 | 10 | |
| Long-term single | 20 | 20 | |
| Alternating partners | 0 | 5 | |
| Long-term relationship (married) | 10 | 5 | |
| Long-term relationship (unmarried) | 55 | 60 | |
| Children, % | | | 0.147 |
| Yes | 0 | 10 | |
| No | 100 | 90 | |
| Education, % | | | 0.055 |
| General school graduation | 10 | 0 | |
| Secondary school without university entrance diploma | 25 | 5 | |
| Secondary school with university entrance diploma | 65 | 95 | |
| High education (ISCED), % | 20 | 15 | 0.677 |
| Professional qualification, % | | | 0.251 |
| Still in education | 60 | 75 | |
| Apprenticeship | 20 | 10 | |
| Apprenticeship with master craftsman's diploma | 10 | 0 | |
| University | 10 | 15 | |
| Professional group, % | | | 0.585 |
| Unskilled worker | 0 | 5 | |
| Skilled worker | 30 | 5 | |
| Lower professional group | 0 | 10 | |
| Middle professional group | 5 | 0 | |
| Higher professional group | 5 | 10 | |
| Self-employed | 5 | 0 | |
| Never worked before | 10 | 20 | |
| Unclear | 45 | 50 | |
| Professional situation, % | | | 0.543 |
| Full-time employment | 30 | 15 | |
| Part-time employment | 0 | 5 | |
| Casual employment | 5 | 5 | |
| In training | 65 | 75 | |
| Net income per month, % | | | 0.756 |
| <400 € | 25 | 15 | |
| 400–1,000 € | 35 | 30 | |
| 1,000–1,500 € | 5 | 0 | |
| 1,500–2,000 € | 15 | 15 | |
| 2,000–2,500 € | 15 | 25 | |
| 3,000–3,500 € | 0 | 5 | |
| 3,500–4,000 € | 5 | 10 | |
| High income (=more than 1,500 € net income per month), % | 35 | 55 | 0.204 |
| Daily contact with pets and/or farm animals, % | 35 | 0 | 0.002 |
| Nutrition, % | | | 0.147 |
| Meat-eating | 90 | 100 | |
| Vegetarian | 10 | 0 | |
| Alcohol consumption, % | | | 0.376 |
| Nondrinking | 10 | 5 | |
| Less than once a month | 15 | 25 | |
| Once a month | 0 | 10 | |
| More than once a month | 20 | 35 | |
| Once a week | 25 | 15 | |
| Two or 3 d a week | 25 | 10 | |
| Nearly daily | 5 | 0 | |

Shown is the mean ± SEM or the percentage of rural and urban participants raised in the presence or absence of animals, respectively, per group, and the *P* value provided by statistical analysis using either *t* test or $\chi^2$ test. ISCED, International Standard Classification of Education.

**Table S2. Summary of the results generated by the various questionnaires employed in the present study during recruiting, as well as before and after TSST exposure**

| Parameter | Rural | Urban | *P* value (*t* test; $\chi^2$) |
|---|---|---|---|
| STAI-S, mean ± SEM | | | |
| Before TSST | 33.7 ± 1.18 | 29.85 ± 0.92 | 0.014 |
| After TSST | 33.05 ± 1.34 | 27.75 ± 1.19 | 0.005 |
| BL, mean ± SEM | | | |
| Complaints | 5.95 ± 1.32 | 4.3 ± 0.99 | 0.324 |
| PASA, mean ± SEM | | | |
| Threat | 3.56 ± 0.26 | 2.68 ± 0.16 | 0.005 |
| Challenge | 4.59 ± 0.14 | 4.03 ± 0.21 | 0.032 |
| Self-concept of own abilities | 3.93 ± 0.27 | 4.28 ± 0.19 | 0.299 |
| Locus of control | 4.61 ± 0.19 | 4.64 ± 0.15 | 0.919 |
| Primary appraisal | 4.08 ± 0.16 | 3.36 ± 0.16 | 0.004 |
| Secondary appraisal | 4.27 ± 0.18 | 4.46 ± 0.11 | 0.388 |
| Stress index | −0.19 ± 0.29 | −1.08 ± 0.24 | 0.025 |
| HADS-D, mean ± SEM | | | |
| Anxiety | 4.25 ± 0.6 | 2.65 ± 0.35 | 0.027 |
| Depression | 2.15 ± 0.36 | 2.45 ± 0.49 | 0.626 |
| PSS-4, mean ± SEM | | | |
| Stress scale | 5.1 ± 0.58 | 4 ± 0.52 | 0.165 |
| CTQ, mean ± SEM | | | |
| Emotional abuse | 6.45 ± 0.64 | 5.58 ± 0.18 | 0.209 |
| Physical abuse | 6.45 ± 0.46 | 5.6 ± 0.28 | 0.125 |
| Sexual abuse | 5 ± 0 | 5 ± 0 | 1.000 |
| Emotional neglect | 9.6 ± 0.72 | 7.9 ± 0.66 | 0.089 |
| Physical neglect | 6.1 ± 0.29 | 5.5 ± 0.22 | 0.109 |
| CECA-Q, mean ± SEM | | | |
| Maternal aversion | 14.1 ± 1.54 | 11.45 ± 0.84 | 0.140 |
| Maternal neglect | 12.4 ± 1.26 | 12.2 ± 1.27 | 0.912 |
| Paternal aversion | 15.9 ± 1.34 | 14.35 ± 1.12 | 0.380 |
| Paternal neglect | 16.65 ± 1.49 | 16.15 ± 1.46 | 0.812 |
| SCID-I (telephone screening), No/Unclear/Yes in % | | | |
| Alcohol (times with more than five drinks at one occasion?) | 20/0/80 | 15/0/85 | 0.678 |
| Drugs (ever taken?) | 55/5/40 | 45/10/45 | 0.744 |
| Pharmaceuticals (felt dependent on or took more than prescribed?) | 100/0/0 | 100/0/0 | N.A. |
| Panic attacks (ever experienced?) | 100/0/0 | 100/0/0 | N.A. |
| Agoraphobia (ever experienced?) | 100/0/0 | 100/0/0 | N.A. |
| Social anxiety (ever experienced?) | 100/0/0 | 100/0/0 | N.A. |
| General anxiety (ever experienced?) | 95/0/5 | 90/0/10 | 0.548 |
| Compulsive thoughts (ever experienced?) | 100/0/0 | 100/0/0 | N.A. |
| Compulsive acts (ever experienced?) | 100/0/0 | 100/0/0 | N.A. |
| Particularly nervous or anxious (during last 6 mo?) | 85/5/10 | 60/25/15 | 0.155 |
| Extraordinarily lean (ever mentioned by others?) | 90/0/10 | 100/0/0 | 0.147 |
| Binge eating (ever occurred?) | 100/0/0 | 100/0/0 | N.A. |
| SCID-I (affective part), No in % | | | |
| Current major depression episode (questions A1, A2) | 100 | 100 | N.A. |
| Previous major depression episode (questions A38, A39) | 100 | 100 | N.A. |
| Current manic episode (question A55) | 100 | 100 | N.A. |
| Previous manic episode (question A90) | 100 | 100 | N.A. |
| Current dysthymia (question A121) | 100 | 100 | N.A. |

Shown is the mean ± SEM or percentage of rural and urban participants raised in the presence or absence of animals, respectively, per group, and the *P* value provided by statistical analysis using either *t* test or $\chi^2$ test. BL, list of complaints for quantitative analysis of current bodily and general complaints; CECA-Q, Childhood Experience of Care and Abuse Questionnaire; CTQ, Childhood Trauma Questionnaire; HADS-D, Hospital Anxiety and Depression Scale–German Version; IL, interleukin; PASA, Primary Appraisal Secondary Appraisal Scale; PBMC, peripheral blood mononuclear cell; PSS-4, Perceived Stress Scale-4; SCID-I, Structured Clinical Interview for DSM-IV Disorders; STAI-S, State–Trait Anxiety Inventory; TP, time point; TSST, Trier social stress test.