

Pupillometric Markers of Locus Coeruleus–Norepinephrine Activity Are Associated with Internalizing Psychopathology in Adolescents

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Abstract

Background

Stress influences psychopathology across diagnostic categories. Although most stress research has focused on the endocrine stress axis, the locus coeruleus–norepinephrine (LC-NE) system may represent a neurophysiological mechanism of the autonomic stress axis through its regulation of arousal. We therefore examined associations among LC-NE functioning, cortisol, and transdiagnostic psychopathology.

Methods

Self-reported internalizing and externalizing symptoms were assessed in a clinical sample of 106 adolescents (56 male, 50 female). During auditory and visual oddball paradigms, pupillometry indexed baseline pupil size (BPS) as LC-NE tonic activity and stimulus-evoked pupillary response (SEPR) as LC-NE phasic activity. Endocrine stress was assessed using hair cortisol concentration (HCC) as a retrospective marker and salivary cortisol concentration (SCC) as a current marker. Adjusted linear models tested associations among pupillometry, cortisol measures, and transdiagnostic psychopathology.

Results

Pupillometry and cortisol measures were uncorrelated but interacted in their associations with psychopathology. Higher BPS, lower HCC, and higher SCC were associated with more internalizing symptoms. The association between BPS and internalizing was stronger at lower SEPR or HCC levels. Only higher SCC was associated with more externalizing symptoms. Combined pupillometry and cortisol measures showed strong performance in classifying clinically relevant internalizing symptoms ($n = 42$ with $T\text{-score} > 65$; area under the curve [AUC] = .861 [.819, .903]).

Conclusion

Larger baseline pupil size may reflect hyperarousal indicating increased autonomic activity and serving as a quantitative marker of self-reported internalizing psychopathology. Elevated LC-NE tonic activity may index immediate stress processing distinct from endocrine stress measures and improve differentiation of clinical psychopathology

Introduction

Stress is a subjective appraisal [1] and correlates with psychopathology [2]. Human stress levels are based on an evaluation of the corresponding situation [3]. This does not only occur for challenging situations [4], rather any stimulus is contextually evaluated in an ebb and flow of individual stress levels throughout the day [5]. First, primary appraisal evaluates the stimulus as a stressor (“threatening, challenging, or harmful”) [6]. An autonomic stress response follows that acts via the sympatho-adrenomedullary (SAM) axis and prepares the body for immediate action [7]. Concurrently, an endocrine stress response is initiated [8] that acts delayed but sustained via the hypothalamic-pituitary-adrenal (HPA) axis by releasing cortisol for prolonged energy proliferation [9]. The effect of stress levels on psychopathology might be further decoded by identifying neurophysiological mechanisms that modulate this iterative stressor evaluation.

Occasional stress is considered adaptive for learning and growth [10], whereas excessive stress levels without sufficient recovery wears the body [11]. This negative effect of stress has been associated with a dysregulated HPA-axis function that exhibits elevated baseline cortisol and an attenuated endocrine response [12]. In youth mental health, such a dysregulated endocrine stress response has been reported in adolescents with conduct disorder [13] and depression [14] during the Trier Social Stress Test. In contrast, childhood adversity has been associated with lower baseline cortisol [15], and these lower baseline levels longitudinally predicted increased depressive symptoms [16]. Such contrasting findings may originate from a focus on the endocrine axis, whereas the autonomic stress axis is more proximate to immediate stressor evaluation [17] and might provide additional information in the characterization of interindividual stress levels.

A key mechanism of the autonomic stress axis is the locus-coeruleus – norepinephrine (LC-NE) system [18]. The locus coeruleus synthesizes cortical norepinephrine that is distributed during LC-NE activity [19]. LC-NE tonic activity is a low frequency (1–5 Hz) that corresponds to arousal levels [20]; whereas LC-NE phasic activity is a transient burst [21]. The associated NE release increases a neuronal signal-to-noise ratio [22], which favours unselective sensory processing for increased LC-NE tonic activity [23] and sensory selectivity to salience for increased LC-NE phasic activity [24]. Thus, LC-NE activity likely modulates stressor evaluation [25], which perpetuates to cognition [26] and could differentiate phenotypes of psychopathology [27].

In rodents, pharmacological blocking of LC-NE tonic and phasic activity reduced amygdala activity during fear conditioning [28], whereas disrupting LC-NE activity increased a susceptibility to social stress [29]. LC-NE tonic activity is upregulated during increased autonomic stress representing increased arousal [30, 31]. The SAM-axis has been proposed to modulate arousal via connectivity of the LC-NE with the vagal nerve [32]. Accordingly, experimentally increased NE levels predicted an autonomic stress responses that has been conceptualized as a mechanism of stress vulnerability [33]. Early within the endocrine stress response, the HPA-axis expresses the corticotropin-releasing hormone (CRH) that binds to the LC and increased LC-NE tonic activity with anxiety-like behaviour in rodents [34]. Animal studies emphasize the LC-NE as a modulator of stress levels, in which SAM- and HPA-axes activity is translated by increased LC-NE tonic activity to stress-related brain states.

In adults, anxiety and stress-related disorders such as post-traumatic stress disorder (PTSD) have been associated with increased LC-NE tonic activity [27] indicating hyper-arousal [35]. Excessive LC-NE tonic activity under stress might thus exacerbate anxiety symptoms [36]. In healthy adults, LC-NE phasic activity and functional coupling with the amygdala during a neurocognitive task predicted internalizing psychopathology after stressful life events [37]. Further, PTSD has been associated with elevated LC-NE phasic activity in response to loud noises [38]. In children, blunted LC-NE phasic activity could indicate a subtype of elevated externalizing psychopathology [39, 40]. LC-NE activity patterns may translate omnidirectional stress to different phenotypes of transdiagnostic psychopathology [41, 42]. However, LC-NE activity patterns have not been systematically investigated in youths for internalizing and externalizing psychopathology.

Pupillometry is an established marker of LC-NE activity [43, 44], where baseline pupil size (BPS) covaries with LC-NE tonic activity [45, 46] and stimulus-evoked pupillary responses (SEPR) to salient stimuli covary with LC-NE phasic activity [47, 48]. Here, we apply pupillometric markers of LC-NE activity to differentiate patterns of transdiagnostic psychopathology in adolescents. During an oddball task battery, derived LC-NE activity is related to endocrine cortisol measures and both explored in their interaction with self-reported psychopathology. We expect lower hair cortisol levels to be associated with increased self-reported psychopathology. We furthermore hypothesize that higher LC-NE tonic activity is associated with higher internalizing psychopathology (henceforth named as Internalizing), whereas lower LC-NE phasic activity might be associated with higher externalizing psychopathology (accordingly: Externalizing). This would support LC-NE activity as a neuro-modulatory mechanism of stress that differentiates psychopathology across diagnostic boundaries.

Methods

Sample

The sample comprised $n = 106$ adolescents ($n = 56$ male, $n = 50$ female) between 11 and 18 years of age with varying levels of self- and parent-reported psychopathology (see Table 1). Ethnicity was not assessed. Participants were recruited from our opt-in recruitment database and local advertisement. Most participants ($n = 76$) were associated with our clinical departments, where they received mental health diagnosis in clinical procedures according to ICD-10 criteria from trained clinical staff. Primary diagnoses were autism spectrum disorder (ASD, $n = 42$), depression ($n = 14$), attention deficit hyperactivity disorder (ADHD, $n = 11$), or anxiety disorder ($n = 9$). These also had high comorbidity rates ($n = 49$) including ADHD ($n = 21$), anxiety disorder ($n = 19$), conduct disorder ($n = 7$), eating disorders ($n = 5$), disorder of scholastic skills ($n = 5$), mood disorder ($n = 4$), tic disorder ($n = 4$), and others ($n = 5$). The sample was enriched with autistic adolescents (German Register for Clinical Trials: DRKS00028803). Here, we investigated a subsample with pupillometry and cortisol assessments concerning self-reported transdiagnostic psychopathology, which was controlled for a diagnosis of autism spectrum disorder. Exclusion criteria were conditions preventing pupillometry such as a nystagmus.

Table 1
Sample description.

age	n	mean	sd	min	max
	106	15.35	1.95	11.93	18.84
IQ	97	100.44	12.16	61.25	125.00
YSR Total (t)	106	61.31	10.31	40.00	91.00
YSR Internalizing (t)	106	62.74	12.61	34.00	94.00
YSR Externalizing (t)	106	54.51	7.53	31.00	70.00
CBCL Total (t)	105	61.00	11.90	31.00	84.00
CBCL Internalizing (t)	105	62.83	12.41	36.00	87.00
CBCL Externalizing (t)	105	54.65	10.41	36.00	81.00
SDQ-s Total (sum)	94	14.13	6.17	1.00	30.00
SDQ-s Internalizing (sum)	93	7.92	4.13	1.00	19.00
SDQ-s Externalizing (sum)	94	6.26	3.43	0.00	15.00
SDQ-p Total (sum)	93	12.30	7.17	0.00	28.00
SDQ-p Internalizing (sum)	93	7.16	4.68	0.00	16.00
SDQ-p Externalizing (sum)	93	5.14	3.84	0.00	15.00
hair cortisol concentration (HCC; pg/mg)	89	4.07	5.12	0.04	25.39
saliva cortisol concentration (before assessment; nmol/l)	106	5.17	3.41	1.19	22.34
saliva cortisol concentration (after assessment; nmol/l)	102	4.35	2.63	0.99	17.84
YSR = Youth Self Report 11–18, CBCL = Child Behavior Checklist 4–18, SDQ-s = Strength and Difficulties Questionnaire – self-report version, SDQ-p = Strength and Difficulties Questionnaire – caregiver-report version					

[Table 1]

Procedure

Written informed consent and assent have been obtained from participants and caregivers. The study was approved by the ethical committee of the Medical Faculty of the Goethe University Frankfurt am Main (reference: 20-1043; date: 04th of May 2022). Participants rated their transdiagnostic psychopathology with screening questionnaires before on-site assessments. On site, we estimated IQ with four subtests (block design, matrix reasoning, similarities, and vocabulary) of the Wechsler Intelligence Scale for Children (WISC-V) or Wechsler Adult Intelligence Scale (WAIS-IV) based on participants' age. Next, two oddball tasks were carried out with controlled artificial lighting at 580 lux with concurrent electroencephalography (EEG) and pupillometry. We assessed hair cortisol concentration (HCC) as a retrospective marker of endocrine stress and salivary cortisol concentration (SCC) as a current marker of endocrine stress. Participant compensation was 20 €.

Transdiagnostic psychopathology

Participants rated their transdiagnostic psychopathology with the Youth Self Report (YSR 11–18) [49] and the Strength and Difficulties Questionnaire self-report version (SDQ-s) [50]. Caregiver ratings were not considered here as self-reports likely are more proximate to target mechanisms in adolescents. We calculated scale sum scores as estimates of the bi-factor transdiagnostic psychopathology factors (Total, Internalizing, Externalizing), as previous research showed little benefit in estimating latent factor scores [51]. YSR and SDQ-s differ in their conceptualization of transdiagnostic psychopathology. For SDQ-s, Internalizing is estimated by combining the subscales Emotional Symptoms and Peer Problems, whereas Externalizing is estimated by combining the subscales Conduct Problems and Hyperactivity/Inattention. For YSR, Internalizing is estimated by combining the subscales Anxious/Depressed, Withdrawn/Depressed, and Somatic complaints, whereas Externalizing is estimated by combining the subscales Aggressive Behavior and Rule-Breaking Behavior. Thus, YSR versus SDQ-s scales are more comprehensive concerning symptom range, whereas SDQ-s includes ADHD symptoms in the

Externalizing scale [52]. Transdiagnostic psychopathology substantially varied within the sample (see Fig. 1) and suggested elevated psychopathology ($\sim +1$ SD) compared to the general population (see t-scores Table 1). Correlations showed conceptual overlap between questionnaires (self-rated Internalizing - YSR and SDQ-s: $r(91) = 0.79$, self-rated Externalizing - YSR and SDQ-s: $r(92) = 0.59$).

[Figure 1]

Oddball Tasks

A passive auditory and an active visual oddball task were applied in PsychoPy that are available online and were presented on a 24-inch monitor (EIZO EV 2451, 1980 x 1080 pixels resolution) in consecutive order. A gaze-contingency component paused both tasks and nudged participant to recentre gazes on the screen when overt attention waned.

The auditory oddball task duration was 25 minutes (Fig. 2 top). Participants were instructed to gaze at a centred, black fixation cross on a grey background and listen to the pure tone sequence. Pure tones were presented binaurally via over-ear headphones (Beyerdynamic DT 770 PRO) at 85 dB loudness. The task consisted of four blocks. Each block included 100 tones (80% standards, 20% oddballs) preceded by three additional standard tones to establish standards. Each tone was presented for 100 ms and followed by a jittered interstimulus-interval between 1800 and 2000 ms. Oddball and standard tones differed in pitch (500 Hz/750 Hz). The initial oddball pitch (500 Hz or 750 Hz) was pseudo-randomized between participants. Oddball pitch was counterbalanced within subjects between blocks before and after a manipulation to control for frequency-specific effects. The experimental manipulation applied an isometric handgrip exercise after the second block that aimed to manipulate LC-NE activity via a grip strength exercise (Mather et al., 2020). The manipulation effect was characterised in a previous publication [53].

The visual oddball task duration was 45 minutes (Fig. 2 bottom). Visual stimuli were pink circles (RGB: 255, 0, 255; HSL: 300%, 100%, 50%) presented centrally on a grey background (RGB: 128, 128, 128; HSL: 0%, 0%, 50%). Oddball and standard circles differed in size. Each circle was presented for 5 frames at 60Hz monitor refresh rate (83,35 ms) and followed by a jittered interstimulus-interval between 2250 and 2500 ms. Participants were instructed to respond as quickly as possible to smaller circles (oddballs, 20%) compared to larger circles (standards, 80%) by pressing the spacebar on the keyboard. The task consisted of four blocks presented in pseudo-random order. Each block included 150 circles preceded by three additional standards to establish standards. Stimulus salience and task utility were manipulated between blocks. For stimulus salience, the smaller oddball circle was either 75% (low salience) or 50% (high salience) of the fixed standard size. For task utility, a monetary reward of 10 cents was provided with respective feedback ("Well done! You won 10 cents!") for fast and correct responses (high utility) or no reward (low utility). Initially, a practice block including 13 trials of each condition defined median reaction time that was used as an individual threshold for reward delivery. At the end of the task, the total monetary reward was displayed and paid out.

[Figure 2]

Pupillometry

Pupillometry was assessed during both oddball tasks to estimate LC-NE tonic and phasic activity. Pupillometry was recorded at 300 Hz sampling rate with a Tobii Pro Spectrum (Tobii AB, Danderyd, Sweden). The eye tracker provides binocular pupil diameters using a 3D eye model that considers distance to screen. Before the tasks, a 6-point calibration with Tobii Pro Eye-Tracker manager (version 2.7) was performed. The subject's head could move freely, while visual feedback indicated when heads were off tracking space (distance: 55–75 cm, width: 42 cm, height: 26 cm). Data were pre-processed with R (version R 4.5.1) following established guidelines [54]. Preprocessing code is available here. Raw data were segmented into trials. Implausible diameter values (< 2 mm, > 8 mm) and speed outliers ($> 3 \times$ median absolute deviation) were excluded in a two-fold approach. Blinks were identified (missing range of 75–250 ms) and removed. Missing data up to 300 ms were linearly interpolated. Pre-processed data of both eyes were then averaged.

A baseline pupil size (BPS) was calculated as a mean of the pupil size during resting state sequences (5 seconds duration) that were recorded after each auditory task block and required participants to look at a central fixation cross. This BPS measure was highly correlated ($r = 0.8$, $p < 0.001$) with an alternative BPS measure that average the mean pupil size during the first 250 ms of each task trial, which we applied in prior studies [53, 55]. Here, we utilized the BPS during the resting-state sequence after the second auditory oddball block (sequence 3) to assess a baseline arousal during which participants were already accustomed to the experimental situation but not influenced by any task manipulation (see Figure S1, Fig. 3).

SEPR was defined as pupil size change with pupil diameter at 500–1500 ms after stimulus onset minus per-trial baseline pupil size for each trial across both tasks. This SEPR was averaged per participant across task conditions as we had no specific hypothesis on differential

effects of auditory-oddball versus visual-oddball SEPR on psychopathology. the correlation analysis below showed that BPS measures were highly correlated, whereas average SEPR was more closely associated with visual oddball SEPR.

[Figure 3]

Cortisol concentration

Saliva samples were collected at the beginning and end of the on-site assessment to capture saliva cortisol concentration (SCC). Prior to collection, participants were instructed to rinse their mouths with water a few minutes before sampling. They were also asked to refrain from consuming large meals within one hour before saliva collection. For collection, Sarstedt Salivettes® were used that were swapped in the mouth by the participants for 45–60 seconds. Samples were stored at – 20°C until shipment in dry-ice to the external contractor (DAACRO Contract Research, Trier). Analysis was performed using ELISA with single measurement sensitivity of 0.19 nmol/L. Each sample was divided before analysis into two probes that returned an intra-assay coefficient of variation (SCC before: 5.35/4.58, SCC after: 5.50/4.16 [m/SD in %]).

Hair samples were collected two centimetres below the occipital protuberance. This scalp region is particularly suitable for hair sampling given the low variation in cortisol levels compared to other scalp areas [56]. A thin strand of hair was isolated and secured with a thread. To obtain the most recent cortisol values, the strand was cut as close to the scalp as possible without pulling hair follicles that could result in contamination. We assessed three centimetres of hair to approximate cortisol levels for the last three months. Samples were stored dry and dark at – 20°C before shipping to the external contractor (DRESDEN LABservice, Dresden). Analysis was performed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) [57].

Hair cortisol concentration (HCC) estimated the retrospective endocrine stress level of the last three months, and SCC before the experiment estimated the acute endocrine stress level. A negative change of SCC from before to after the experiment suggested a reduction of the endocrine stress across participants. HCC and SCC were not normally distributed and thus log-transformed (see Fig. 3).

Statistical Analysis

Analyses were conducted in R 4.5.1 with additional packages (emmeans, effectsize, car, boot, pROC, caret). The analysis code is available online. Initially, we explored the effect of potential covariates (sex, age, IQ, and autism diagnosis) on questionnaire scores (YSR, SDQ-s), pupillometry (BPS, SEPR), and cortisol concentration (HCC, SCC change). Significant covariates were considered in all analyses to ensure parallel modelling.

As a main analysis, we applied the dimensions of transdiagnostic psychopathology (Externalizing or Internalizing) as outcome and explored the concurrent effect of pupillometry and cortisol concentration as well as their interaction as predictors. In addition, we applied a logistic regression as classification (80/20 training-test split) to investigate whether pupillometry or cortisol alone or combined as predictors including interactions can identify clinically significant cases (YSR t-score > = 65), while controlling for age, sex, and autism diagnosis. An area-under-the-curve (AUC) estimated classification performance with bootstrapping (k = 1000) for confidence intervals.

Full models are reported in the supplements. Here, we report significant fixed effects or interactions as standardized effect sizes (β) with 95% confidence intervals. Adjusted coefficient of determination (R^2) is provided as model fit. Significant interactions are post-hoc explored with contrasts based on estimated marginalized means. We estimated achieved power in linear models for a target effect size of $\beta = 0.3$ and assuming a standard error scaling of $1/\sqrt{n}$. With the given sample size (n) considering missing values in core variables, this resulted in a power = .85 to detect bivariate associations between predictor and outcome, and a power = .80 to detect a two-way interaction between predictors on the outcome.

Results

Correlative Patterns

We utilized SCC before the experiment as marker of current endocrine stress level. This was only moderately correlated (see Table 2) with SCC after the experiment ($r = .39$, $p < .001$) and negatively correlated with SCC change ($r = -.67$, $p < .001$) suggesting that higher current endocrine stress levels are associated with a stronger cortisol dropoff during the experiment. HCC as retrospective endocrine stress level showed small negative correlations with self-reported psychopathology. Further, results showed a strong correlation between BPS measures ($r > .73$, $p < .001$) indicating that underlying LC-NE tonic activity is a slow changing measure of arousal that is consistent throughout the task. SEPR was less consistent and indicates that SEPR rather reflects pupillary responses during the visual oddball task

irrespective of reward or stimulus salience ($r = .83-.94$, $p < .001$). Lastly, self-reported psychopathology measures showed moderate to strong intercorrelations.

Table 2
Correlation matrix of main measures.

Variable	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
SCC (1)																			
SCC after (2)	.39																		
SCC change (3)	-.67	.31																	
HCC (4)	.	.	.																
BPS (5)	.	.	.	.															
BPS avg (6)	.	.	.	.	.80														
BPS before (7)	.	.	.	.	.73	.83													
BPS after (8)	.	.	.	.	.74	.77	.73												
SEPR (9)	.	.	.	.	.	.	.29	.											
SEPR AO pre (10)	.	.	.	.	.	.	.	.	.										
SEPR AO post (11)	.	-.21	.	.	.34	.30	.29	.24	.30	.25									
SEPR VO ++ (12)	.	-.20	.	.	.	.22	.30	.23	.84	.	.								
SEPR VO -- (13)	.	.	.	.	.	.	.20	.	.83	.	.	.65							
YSR Total (14)	.19	.	.	-.27	.	.	.	.	.	.	.	.	.						
YSR Int (15)	.	.	.	-.22	.	.	.	.	.	.	.	.	.	.90					
YSR Ext (16)	.21	.	.	.	.	.	.	.	.	.	.	.	-.21	.70	.46				
SDQ Total (17)	.	.	.	-.28	.	.	.	.	.	.	.	.	.	.78	.72	.50			
SDQ Int (18)	.	.	.	.	.	.	.	.	.	.	.	.	.	.69	.78	.30	.85		

Note: Pearson correlation shown only for $p < .05$. Indent rows show auxiliary measures for pupillometry and cortisol. SCC = saliva cortisol concentration, HCC = hair cortisol concentration, BPS = baseline pupil size, BPS avg = average of all BPS measures, SEPR = stimulus-evoked pupillary response, AO pre = auditory oddball before experimental manipulation, AO post = auditory oddball after experimental manipulation, VO ++ = visual oddball, salience high + utility high condition, VO -- = visual oddball, salience low + utility low condition, YSR = Youth Self Report, SDQ = Strengths and Difficulties Questionnaire.

YSR = Youth Self Report 11–18 as sum scores, SDQ-s = Strength and Difficulties Questionnaire – self-report version.

BPS = baseline pupil size, SEPR = stimulus-evoked pupillary response.

Scatterplots were split based on global median splits of SEPR and HCC and show the association in these subgroups of BPS and self-reported Internalizing (YSR, SDQ-s). YSR = Youth Self Report 11–18, SDQ-s = Strength and Difficulties Questionnaire – self-report version. BPS = baseline pupil size, SEPR = stimulus-evoked response, HCC = hair cortisol concentration.

Variable	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
SDQ Ext (19)	.	.	.	-.28	.	.	.	.	.	.	.	.	.	.57	.36	.55	.77	.33	
Note: Pearson correlation shown only for $p < .05$. Indent rows show auxiliary measures for pupillometry and cortisol. SCC = saliva cortisol concentration, HCC = hair cortisol concentration, BPS = baseline pupil size, BPS avg = average of all BPS measures, SEPR = stimulus-evoked pupillary response, AO pre = auditory oddball before experimental manipulation, AO post = auditory oddball after experimental manipulation, VO ++ = visual oddball, salience high + utility high condition, VO -- = visual oddball, salience low + utility low condition, YSR = Youth Self Report, SDQ = Strengths and Difficulties Questionnaire. YSR = Youth Self Report 11–18 as sum scores, SDQ-s = Strength and Difficulties Questionnaire – self-report version. BPS = baseline pupil size, SEPR = stimulus-evoked pupillary response. Scatterplots were split based on global median splits of SEPR and HCC and show the association in these subgroups of BPS and self-reported Internalizing (YSR, SDQ-s). YSR = Youth Self Report 11–18, SDQ-s = Strength and Difficulties Questionnaire – self-report version. BPS = baseline pupil size, SEPR = stimulus-evoked response, HCC = hair cortisol concentration.																			

[Table 2]

Covariate Effects

A female versus male biological sex was associated with higher self-reported psychopathology for YSR total ($F(1,92) = 4.03$, $p = .047$, $\beta = 0.46$ [0.00, 0.92], table S1) but not SDQ-s total ($F(1,81) = 1.79$, $p = .185$, $\beta = 0.34$ [-0.16, 0.84], Table S2)). For baseline pupil size (BPS, Table S3), an older age was associated with a lower BPS ($F(1,82) = 10.17$, $p = .002$, $\beta = -0.35$ [-0.57, -0.13]), in line with our previous findings in independent samples [55]. For SCC (Table S4), we also explored the effect of coefficient of variation, time of day, and body mass index (BMI), but found no significant effects. For HCC (Table S6), we also explored the effect of hair mass and BMI. Higher age ($F(1,69) = 3.84$, $p = .023$, $\beta = 0.25$ [0.04, 0.47]) was associated with higher HCC. Given the significant covariates, we controlled for age and sex in all subsequent models. We also controlled for the “autism diagnosis” in all models, as the sample overrepresented autistic individuals compared to the general population.

Combined Effects of Pupillometry and Cortisol Concentration on Transdiagnostic Dimensions

For YSR Internalizing ($R^2 = .37$, Table S8), increased Internalizing was associated with a higher BPS ($F(1,60) = 10.52$, $p = .002$, $\beta = 0.37$ [0.14, 0.60]), lower HCC ($F(1,60) = 6.65$, $p = .012$, $\beta = -0.34$ [-0.60, -0.08]), and higher SCC ($F(1,60) = 4.73$, $p = .034$, $\beta = 0.23$ [0.02, 0.45]). The effect of BPS was specified by an interaction of BPS x SEPR ($F(1,60) = 11.22$, $p = .001$) and BPS x HCC ($F(1,60) = 5.38$, $p = .024$). Post-hoc contrasts showed that the positive effect of BPS on YSR Internalizing was only evident for SEPR levels on average ($z = 0$: $\beta = 0.36$ [0.14, 0.59]) or below-average ($z = -1$: $\beta = 0.89$ [0.48, 1.31]), as well as HCC levels on average ($z = 0$: $\beta = 0.35$ [0.12, 0.57]) or below-average ($z = -1$: $\beta = 0.60$ [0.29, 0.91]).

For SDQ Internalizing ($R^2 = .35$, Table S9), the main effect of BPS ($F(1,57) = 6.93$, $p = .012$, $\beta = 0.30$ [0.07, 0.53]) and interaction of BPS x SEPR ($F(1,57) = 16.64$, $p < .001$) and BPS x HCC ($F(1,57) = 15.85$, $p < .001$) were replicated. Post-hoc contrasts showed that higher BPS was associated with increased Internalizing, when SEPR or HCC levels were on average ($\beta = 0.26$ [0.05, 0.51], $\beta = 0.26$ [0.04, 0.49]) or below-average ($\beta = 0.98$ [0.53, 1.43], $\beta = 0.72$ [0.39, 1.05]). Overall, higher BPS was strongly associated with higher self-reported internalizing, when SEPR or HCC were below-average levels (see Fig. 4).

[Figure 4]

For YSR Externalizing ($R^2 = .01$, Table S10), the overall model fit was poor and thus not further interpreted. For SDQ-s Externalizing ($R^2 = .06$, Table S11), increased externalizing was associated with higher SCC ($F(1,58) = 6.96$, $p = .011$, $\beta = 0.31$ [0.08, 0.55]).

Classification

The clinical cutoffs ($T > 65$) in the sample were reached for YSR Internalizing in $n = 42$ (40%) and for YSR Externalizing for $n = 7$ (7%). No consensual clinical cutoffs are available for SDQ scores. Thus, classification analysis was limited to YSR Internalizing. For cortisol measures alone, the logistic regression achieved a classification performance of $AUC = .659$ [.601, .729]. For pupillometry alone, the logistic regression achieved a classification performance of $AUC = .666$ [.603, .734]. For combined classification with pupillometry and cortisol measures, the logistic regression achieved a significantly higher classification performance of $AUC = .861$ [.819, .903].

Discussion

The current study demonstrated LC-NE tonic activity as estimated by pupillometry as a transdiagnostic marker of self-reported internalizing psychopathology. We conceptualized LC-NE activity as a neurophysiological mechanism of autonomic stress or SAM-axis that was compared to established measures of cortisol concentration as endocrine stress.

LC-NE activity was not directly associated with endocrine stress levels as markers of the HPA-axis, which is in line with previous research [58] that established an effect of stress induction on LC-NE activity but did not report an association of LC-NE activity and cortisol concentration. It might be argued that LC-NE activity as part of the autonomic SAM-axis is faster and more reactive compared to the endocrine HPA-axis, which would reveal correlations only after experimental manipulations of stress responses [13] that are absent in the current study.

However, increased YSR Internalizing across measures was associated with lower HCC, which has been established in adults specific to anxiety disorders [59] and PTSD [60]. One seminal study reported lower HCC in children after adversity exposure that predicted later depressive symptoms [16]. It suggests a dysregulated HPA-axis in Internalizing that expresses as a net reduction in cortisol levels. In contrast, increased YSR Internalizing as well as SDQ Externalizing was associated with increased SCC before the experiment. This might suggest that a combination of hair and salivary cortisol might differentiate between internalizing and externalizing psychopathology, but the explained variance in the externalizing model was rather low.

Further, the findings replicate that higher self-reported psychopathology in youth can be associated with higher current cortisol levels [61], but might not differentiate between Internalizing and Externalizing like a cortisol stress response did in previous research [62]. Interestingly, lower retrospective, cumulative cortisol levels as well as higher current cortisol levels were associated with higher Internalizing. We speculate that retrospective cortisol level indicates the dysregulated HPA-axis in psychopathology that results in an overproportional cortisol release in the anticipation of stress as measured with the current cortisol level right before the experiment.

As a main finding, increased LC-NE tonic activity was associated with increased Internalizing. This supports previous findings of increased LC-NE tonic activity [27] indicating a general hyperarousal in Internalizing disorders [35]. Recent optogenetic findings in mice showed that increased LC-NE tonic activity engages functional networks of associative thinking [63], which could facilitate a cognitive rumination tendency in Internalizing. Importantly, in our transdiagnostic sample, the quantitative association of increased Internalizing and increased LC-NE tonic activity is emphasized in a subgroup with concurrent markers of elevated psychopathology (lower HCC) or attenuated sensory selectivity (lower SEPR) [53]. Increased LC-NE tonic activity might serve as a quantitative marker of Internalizing in those individuals with elevated risk factors of psychopathology [36]. Together with cortisol levels, it underlines the utility of LC-NE activity as additional clinical marker of stress. This is supported by our classification analysis that emphasized pupillometry combined with cortisol levels in its sufficient ability to differentiate between individuals below and above clinical cutoffs for Internalizing.

This is the first study to explore LC-NE functioning as a neurophysiological mechanism of stress in transdiagnostic psychopathology. The assessment did not induce a substantial stressor with a stress response (Table 1), which limits the interpretation of SCC as a marker of current cortisol levels. This does not limit the interpretation of HCC that provided the most coherent findings in the current analysis. Finally, pupillometry as a measure of LC-NE activity is established but indirect (like the BOLD signal in fMRI) [43, 44] and other neuro-modulatory pathways may converge on pupil size [64, 65].

Conclusions

We conclude that elevated LC-NE tonic activity indicates an upregulated autonomic stress reactivity and hyperarousal in Internalizing, which is corroborated by lower baseline cortisol levels. Based on stress-research in animal models [18] and one seminal longitudinal study [37], our data in youths emphasizes LC-NE activity as a neuromodulatory mechanism of psychopathology across diagnostic boundaries. LC-NE functioning is expected to translate an objective stressor to a subjective evaluation of stress, which shapes and quantifies transdiagnostic dimensions in adolescents with elevated psychopathology. This stress evaluation informed by the autonomic response might act on a faster timescale than measures of the endocrine stress response can reliably detect. Pupillometry combined with hair and saliva cortisol levels provided a good classification of clinically relevant cases of self-reported Internalizing. Future studies should test this objective and easily accessible biomarker for its predictive clinical validity.

Declarations

Competing Interest

CMF receives royalties for books on autism and ADHD. Since 2025 she serves as scientific advisor for Infectopharm on possible future studies related to ASD. Currently, her research is funded by the German Research Foundation (DFG) and the European Commission. NB receives royalties for lecturing at institutes for training in psychotherapy and receives research funding by the German Research Foundation (DFG), the Daimler and Benz Foundation, and the Johanna Quandt Young Academy. The remaining authors have declared that they have no competing or potential conflicts of interest.

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Author Contribution

NB, CL, and CMF contributed to the study conceptualization. NB and CL acquired the funding. NB designed the study concept, provided the preprocessing of pupillometric data and supervised the findings of this study. AKM and SH performed the data acquisition. NB carried out the data analysis. The original draft of the manuscript was written by NB. NB and AKM, and AB reviewed and edited the manuscript. All authors discussed the results and interpretation and agreed to the final draft.

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Data Availability

The code and preprocessing scripts used in this study are publicly available at https://github.com/nicobast/SEGA_transdiagnostic_stress. The raw data cannot be shared due to legal and ethical restrictions under the General Data Protection Regulation (GDPR), as the dataset contains sensitive/personal information. Researchers interested in accessing the data may contact the corresponding author to discuss whether access can be granted under appropriate legal, ethical, and institutional approvals.

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Figures

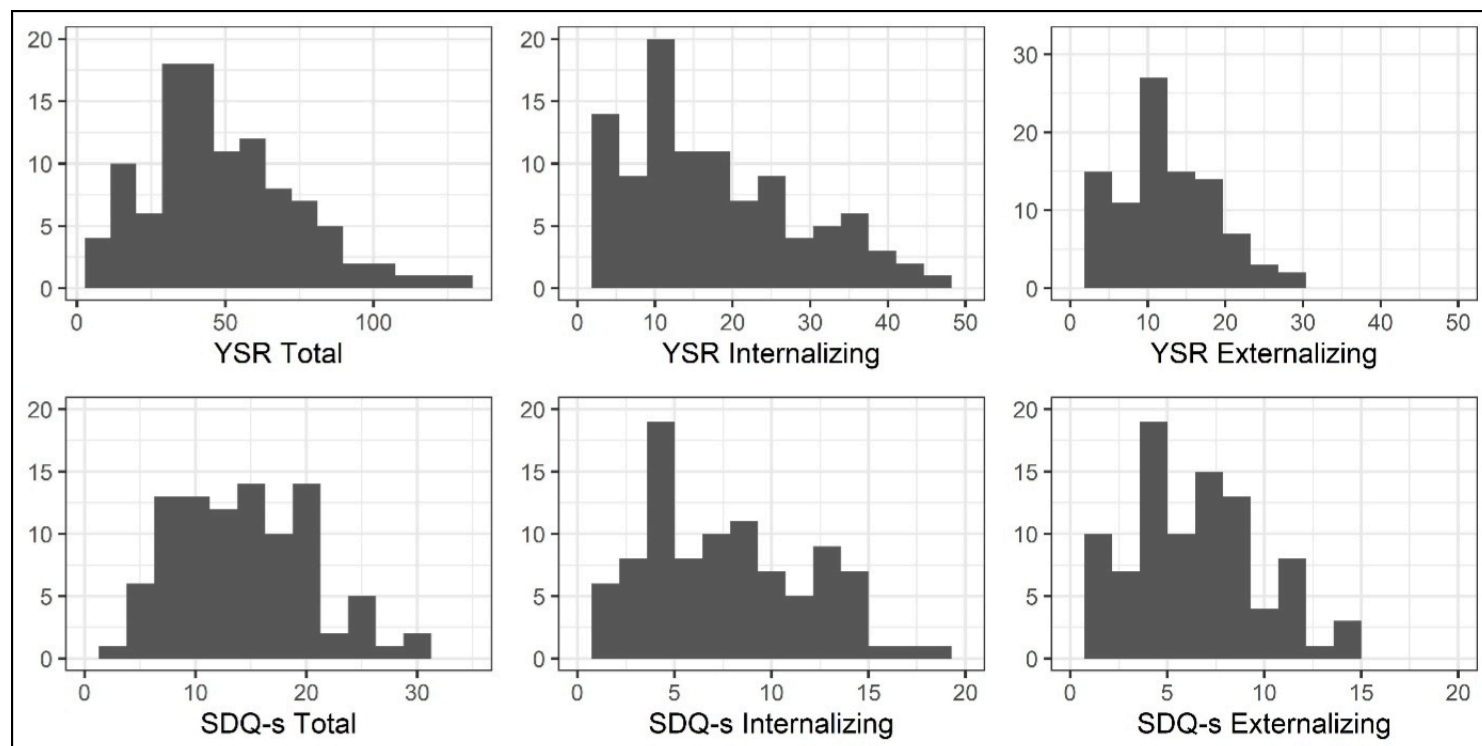


Figure 1

Distribution of self-reported transdiagnostic psychopathology.

YSR = Youth Self Report 11-18 as sum scores, SDQ-s = Strength and Difficulties Questionnaire – self-report version.

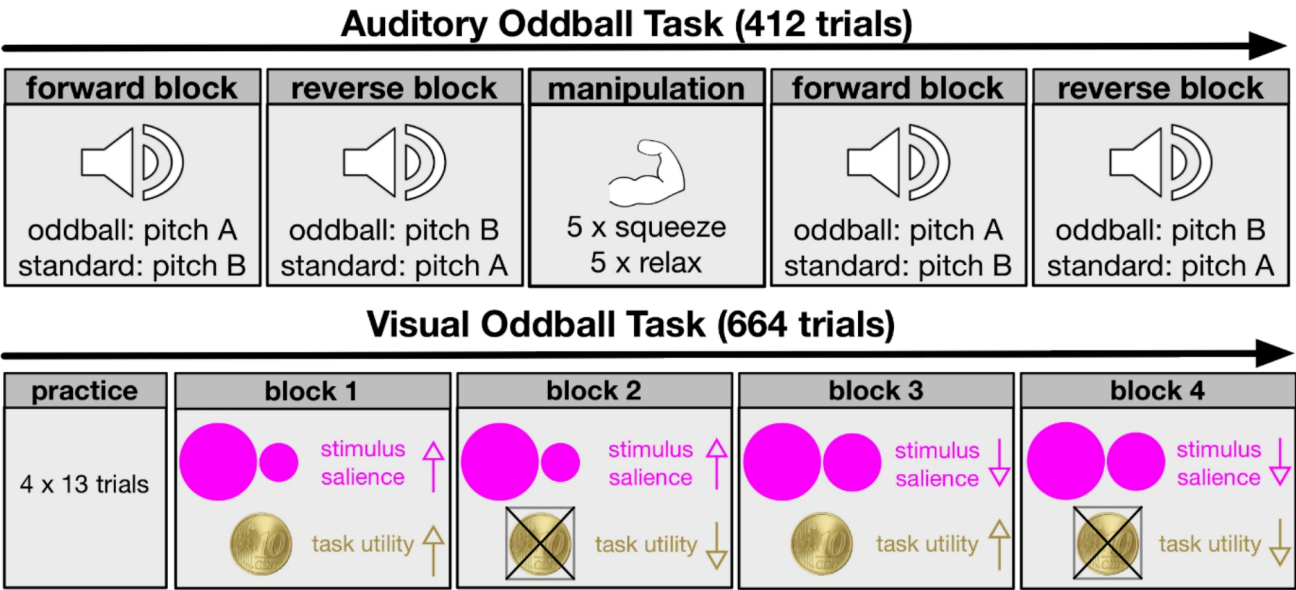


Figure 2

Oddball Tasks.

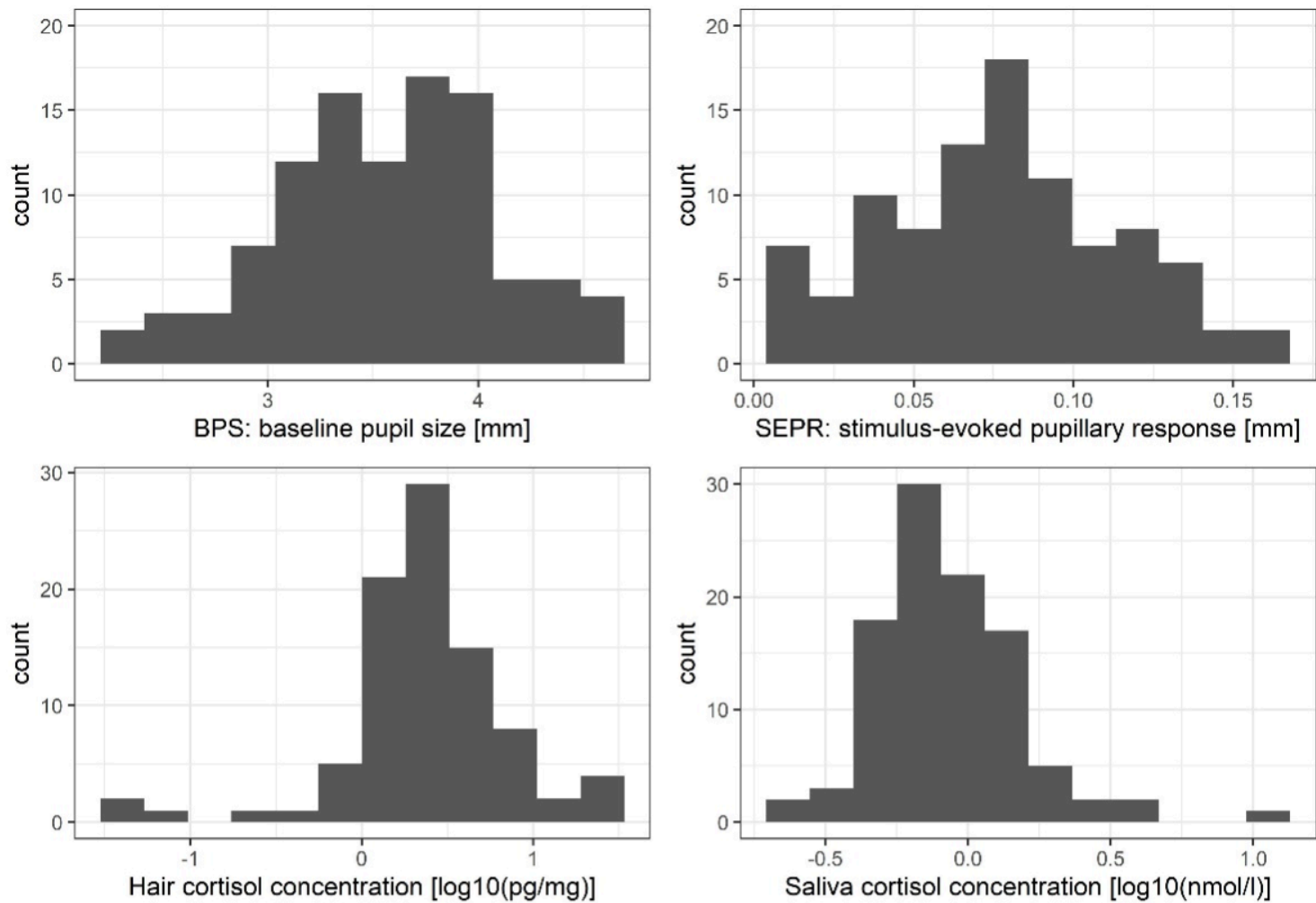


Figure 3

Distribution of pupillometry and cortisol concentration estimates.

BPS = baseline pupil size, SEPR = stimulus-evoked pupillary response.

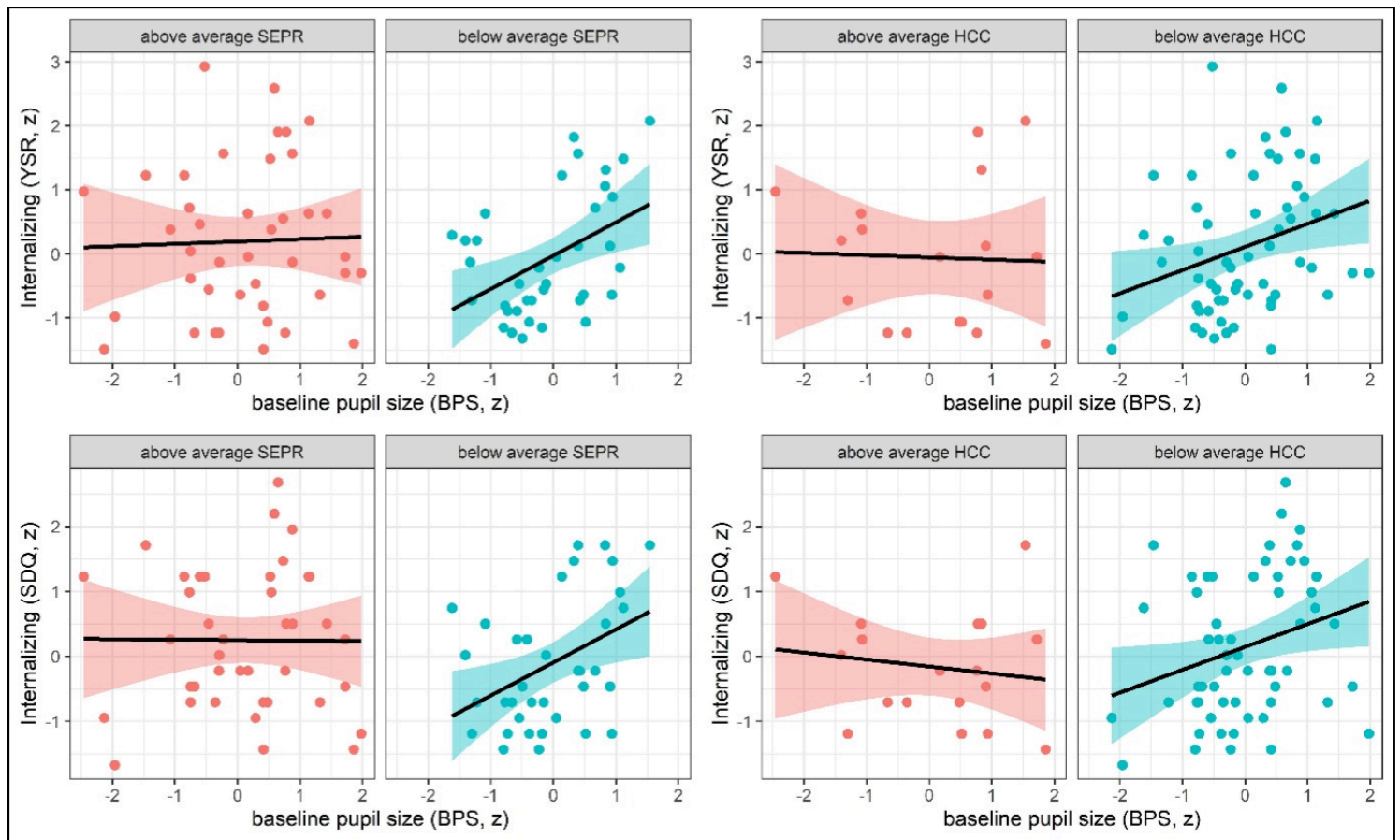


Figure 4

Association of baseline pupil size (BPS) and self-reported Internalizing (YSR, SDQ) in subgroups of different hair cortisol level (HCC) and pupillary response (SEPR).

Scatterplots were split based on global median splits of SEPR and HCC and show the association in these subgroups of BPS and self-reported Internalizing (YSR, SDQ-s). YSR = Youth Self Report 11-18, SDQ-s = Strength and Difficulties Questionnaire – self-report version. BPS= baseline pupil size, SEPR = stimulus-evoked response, HCC = hair cortisol concentration.

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