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Hair Cortisone Predicts Lower Stress-induced Salivary Cortisol Response: Resting-state Functional Connectivity Between Salience and Limbic Networks

Yipeng Ren,[a,b1] Yadong Liu,[a,b1] Xiaolin Zhao,[a,b] Weiyu Hu,[a,b] Zhenni Wei,[a,b] Zihan Tang,[a,b] Jiahao Luo,[a,b] Mengyi Li,[a,b] Kaige Guo,[a,b] Yan Wu[c] and Juan Yang[a,b]*

[a] *Faculty of Psychology, Southwest University, Chongqing 400715, China*

[b] *Key Laboratory of Cognition and Personality (Ministry of Education), Southwest University, Chongqing, China*

[c] *Key Laboratory of Child Development and Learning Science (Ministry of Education), Southeast University, Nanjing, China*

Abstract—Previous studies revealed that high long-term hypothalamic–pituitaryadrenal (HPA) axis activity measured by the hair cortisol concentrations predicts lower acute stress cortisol response and reported the influences of hair cortisol on brain activity during acute stress exposure. However, considering that long-term HPA axis activity has a close relationship with the brain's resting-state functional connectivity (RSFC), the current study aimed to explore the role of RSFC between limbic and salience network in this relationship. Seventy-seven healthy participants underwent resting-state imaging scans before performing the acute ScanSTRESS task. Saliva samples were collected to assess the levels of acute stress salivary cortisol. Hair samples were also collected, and the corticosteroid concentration extracted from these samples were used as a biomarker of long-term HPA axis activity. High hair cortisone (HairE) levels predicted lower acute stress cortisol response. Moreover, high HairE levels were significantly correlated with enhanced RSFC between limbic and salience networks, while RSFC was negatively associated with acute stress cortisol response. Importantly, the RSFC between left insula and left parahippocampus mediated the association between HairE and acute cortisol stress response. Taken together, this study uncovers the important role of RSFC between salience and limbic networks in the long-term relationship between HairE and acute cortisol response and contributes to a deeper understanding of the individual differences in acute stress response.

Key words: hair cortisone, salivary cortisol, resting-state functional connectivity, limbic network, salience network.

INTRODUCTION

Stress is a normal part of life; although discomforting, it is commonly thought of as an adaptive, allostatic response to perceived threats in our environment. When an individual is confronted with a stressor, the hypothalamic–pituitaryadrenal (HPA) axis, which is a major stress regulatory system, will be rallied, mobilizing or storing energy through the release of cortisol to facilitate threat resolution and environmental adaptation (de Kloet et al., 2005). Salivary cortisol, the terminal product of the HPA axis, has been utilized in a substantial number of acute stress-induction studies, and elevated cortisol is used as a gold standard for determining the "stress response" in a laboratory setting (Hellhammer et al., 2009).

A tremendous inter-individual variability was observed in the response of the HPA axis to acute stress (Zänkert, 2019). Several biological, environmental, social, and psychological factors may be responsible for the pronounced heterogeneity in stress response (Rab and Admon, 2021). For example, individuals who experienced long-term stress including adverse life events in childhood, maltreatment or bullying, and past stressful life events had lower cortisol responses to acute stressors (Loft et al., 2007; Tomiyama et al., 2011; Armbruster et al., 2012; Raffington et al., 2018; Lam et al., 2019). In particular, a recent study found that high long-term HPA axis activity, measured based on the hair cortisol concentration, predicted a lower acute stress cortisol response (Sandner

*Correspondence to: Juan Yang, Faculty of Psychology, Southwest University, No. 2 Tiansheng Road, Beibei, Chongqing 400715, China. E-mail address: valleyqq@swu.edu.cn (J. Yang).

[1] They equally contributed to this work.

Abbreviations: ANOVA, analysis of variance; AUCg, area under the curve with respect to the ground; CI, confidence interval; CON, control network; DAN, dorsal attention network; DMN, default mode network; FD, frame-wise displacement; fMRI, functional magnetic resonance imaging; HairE, hair cortisone; HairF, hair cortisol; HPA, hypothalamic–pituitaryadrenal; LBN, limbic network; MRI, magnetic resonance imaging; PHG, parahippocampus; ROI, region-of-interest; RSFC, resting-state functional connectivity; SD, standard deviation; SMN, somatomotor network; SN, salience network; TPN, temporal parietal network; VN, visual network.

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et al., 2020). Hair cortisol concentrations is a systematic marker of long-term HPA axis activity and is an indicator of recent stress experiences (Russell et al., 2012; Mewes et al., 2017; Iob and Steptoe, 2019). Therefore, exposure to greater recent cumulative stress was a prominent predictor of decreased salivary cortisol responses. Although a previous study has suggested the importance of alterations in the long-term HPA axis as a significant factor accounting for individual differences in acute stress cortisol response, the possible neural pathways remained unknown. The identification of neural pathways contributing to this relationship is of particular interest as the brain regulates response to stressful events and is further linked to the development of psychiatric disorders (de Rooij, 2013; Phillips et al., 2013).

The brain's functional connectivity at rest provides a task-free way to eliminate any confounding factors associated with performance (Gusnard and Raichle, 2001). Both long-term and basal HPA axis activities showed a close relationship with the brain's resting state. For example, cortisol awakening response could predict the intrinsic functional connectivity of the medial prefrontal cortex in the afternoon of the same day (Wu et al., 2015). Moreover, a higher cortisol awakening response in two consecutive weekdays is correlated with more left-sided cortical activity in the frontocentral regions in a resting state (Duan et al., 2019). Higher hair glucocorticoid levels were associated with lower frontal lobe oxygenation, measured by resting state near-infrared spectroscopy (Feeney et al., 2021). Furthermore, resting-state functional connectivity (RSFC) has been primarily used to elucidate the neural basis of some physiological or psychological phenomena that deviate from the norm within a long-term period. For example, individuals with high perceived stress in the previous month had stronger RSFC between the anterior cingulate cortex and amygdala (Taren et al., 2015). Considering the close relationship between the long-term HPA axis activity and brain's functional connectivity during a resting state, the current study aimed to explore the role of the brain's functional connectivity during a resting state in the relationship between the long-term HPA axis activity and acute stress-induced salivary cortisol response.

The salience network (SN) is particularly associated with stress responsivity and regulation (Grinband et al., 2006; Thomason et al., 2011). Insula, which is a pivotal node in the SN, located in the boundary between the cognitive, homeostatic, and affective systems of the human brain, provides a liaison between stimulus-driven processing and brain areas engaged in the surveillance of the interior milieu (Menon and Uddin, 2010). The insula can compare the normal situation underlying the maintenance of homeostasis and the incoming stimulus to determine whether a certain event is recognized as consistent with the current homeostatic processes (Panerai, 2011). Moreover, the hippocampus/parahippocampus (PHG), which is located in the limbic network (LBN), can provide a negative feedback loop necessary for the long-term maintenance of appropriate glucocorticoid levels. The hippocampus is well enriched with glucocorticoid receptors, making it vulnerable to the "stress hormone cascade," psychosocial stress leading to hippocampal atrophy, and reduced neurogenesis and synaptogenesis (McEwen et al., 2016). The activation of PHG correlates negatively with the cortisol response level (Davies et al., 2022). Hence, the SN and LBN as well as the insula and hippocampus/PHG may play a collaborative role of long-term HPA axis activity in predicting a decreased acute salivary cortisol response.

In the presented study, we aimed to explore the association between long-term HPA axis activity (hair glucocorticoids: cortisol and its inactive metabolite cortisone) and acute salivary cortisol reactivity and to determine the role of RSFC between the limbic network (LBN) and salience network (SN) as well as the RSFC between the insula and hippocampus/PHG in this relationship.

EXPERIMENTAL PROCEDURES

Participants

Seventy-seven healthy participants (35 women) aged 18–26 years (M = 20.18, *standard deviation (SD)* = 1.97) were recruited for the study. All women were in the luteal phase. The participants were instructed to avoid performing vigorous exercise, smoking, eating, consuming alcohol or caffeine, or brushing teeth 1 h prior to the magnetic resonance imaging (MRI) experiment. Two participants who demonstrated excessive head motion during the resting state functional MRI (fMRI) (mean frame-wise displacement (FD) Jenkinson > 0.2), three participants with missing hair samples, and another participant with missing saliva samples were excluded. Finally, 71 participants (31 women) aged 18–26 years (M = 20.21, SD = 1.96) were included in the data analysis. All participants provided informed consent and received a small amount of monetary compensation. The study was approved by the ethics board of Southwest University (H22008).

Measurements

Hair samples and analysis. Considering an average monthly hair growth speed of 1 cm (Wennig, 2000), the most proximal 1-cm segment of hair samples contains the cumulative cortisone and cortisol secretion in the previous month. Moreover, since the proximal 1–3-mm hair strands were deeply embedded in the scalp and were not perfectly cut with scissors (LeBeau et al., 2011), hair collection was performed 2 weeks after performing the acute stress task to allow the previously uncut hair to grow; at this point, the time of the most proximal 1-cm segment of hair samples was matched with the time span (1 month) before the acute stress task was performed. A hair sample of approximately 150 strands was cut from the scalp at the posterior vertex position, which has the steadiest growth rates (Wennig, 2000). The hair samples were wrapped in an aluminum foil and stored in a dry and dark place at room temperature until analysis. The most proximal 1-cm segment of hair strands was used for analysis.

The hair strands were subjected to a series of treatments including washing, grinding, extraction, and evaporation (Greff et al., 2019). The cortisone and cortisol concentrations were determined using a high-performance liquid chromatography-tandem mass spectrometer (ABI 3200 QTRAP, USA) equipped with atmospheric pressure chemical ionization sources (liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry/mass spectrometry) (Gao et al., 2013; Zhang et al., 2018). The method showed the limits of detection and quantitation of 0.3 and 1.1 pg/mg for hair cortisone (HairE) and 0.3 and 0.9 pg/mg for hair cortisol (HairF), respectively. The intra-day and inter-day coefficients of variation were both < 10%, and the recovery rate ranged between 85% and 120% at standard concentrations. The hair corticosteroid data were logarithmized to base 10 to minimize the typical data skewness.

Acute stress task and saliva samples. The participants underwent the ScanSTRESS paradigm (Fig. 1(**A**)) for the induction of acute social stress through the performance of a high cognitive load task as well as social appraisal threat (Streit et al., 2014). Under a stress condition, the participants were required to perform continuous subtraction and mental rotation tasks while being monitored and received feedback from an investigator panel. The participants were asked to select the answer within a limited time and press the button that corresponds to their answer. The speed and difficulty of the task were continuously adjusted according to the individual participant's performance to ensure that the number of correct answers remains at 50%. Under controlled conditions, the participants performed non-demanding tasks including number matching and figure matching without time pressure and monitoring.

Saliva samples were collected five times for each participant (Fig. 1(**B**)). Specifically, the first saliva sample was collected 30 min after the participant arrived at the laboratory (S1), while the second and third samples were collected upon completion of the first (S2) and second (S3) ScanSTRESS sessions. The last two samples were collected after a 17-min relax (S4) and a 10-min relax (S5), respectively. The saliva samples were collected using the Salivette sampling device (Sarstedt AG & Co, Nümbrecht, Germany) and were stored at −20 °C until analysis. The cortisol concentrations were determined using the enzyme-linked immunosorbent assays (IBL, Hamburg, Germany) with a sensitivity of 0.005 μg/dL. The inter-assay coefficient of variation was 8.55%. The salivary cortisol data were logarithmized to base 10 to minimize the typical data skewness. A repeated measures analysis of variance (ANOVA) with time (five levels) as the within-subject variable was performed to examine whether variations in cortisol concentrations were influenced by the paradigm. Then, we separately calculated the area under the curve with respect to the ground (AUCg; Pruessner et al., 2003) of saliva cortisol for each participant to denote the total cortisol output.

Brain image data acquisition. Images were acquired using a 3T Siemens Prisma scanner (Erlangen, Germany). Three-dimensional magnetization-prepared rapid gradient echo high-resolution T1-weighted neuroanatomical images were obtained for each participant (192 slices; TR = 2,530 ms; TE = 2.98 ms; slice thickness = 1 mm; FOV = 256 × 256 mm^2; voxel size = 0.5 × 0.5 × 1.0 mm^3, flip angle = 7°).

Moreover, each participant underwent a resting-state fMRI examination, which included the acquisition of 240 volume-functional images. Field map images were acquired to correct for distortions of echo-planar images (TR = 620.0 ms; TE1 = 4.92 ms; TE2 = 7.38 ms; slice thickness = 2.0 mm; FOV = 224 × 224 mm^2; voxel size = 2.0 × 2.0 × 2.0 mm^3; flip angle = 60°).

Procedure. Details of the experiment are illustrated in Fig. 1(**B**). All experiments were conducted between 12:00 and 18:00 to control for the effect of cortisol secretion rhythms on acute salivary cortisol changes. Upon arrival at the laboratory, the participants were informed of the study procedure and completed a brief training session of the ScanSTRESS to familiarize the task requirements. Afterward, they were asked to rest for 30 min. Thereafter, the participants were positioned in the MR scanner, underwent a T1 weighted image scan, a resting state fMRI scan with their eyes open, and performed the ScanSTRESS task. Before the participants left the laboratory, they were informed of the real purpose of the experiment and relieved of their negative emotions. Throughout the experiment, five saliva samples were collected to observe for changes in stress induction on the objective level. Two weeks following the fMRI experiment, hair samples were collected from each participant.

Data analyses

Brain imaging data preprocessing. Preprocessing was conducted using the Data Processing & Analysis for Brain Imaging toolbox (Yan et al., 2016). The images were corrected for scanning time differences between slices and realigned for head motion. The anatomical images were co-registered to the functional image and segmented into gray matter, white matter, and cerebral spinal fluid. The functional images were normalized using DARTEL and smoothed with a 4-mm full-width at half-maximum Gaussian kernel to optimize the signal-to-noise ratio.

Resting state fMRI analysis. The RSFC analysis was conducted using the CONN toolbox (Whitfield-Gabrieli and Nieto-Castanon, 2012). The predefined regions-of-interest (ROIs) were defined using the templates of 17 networks developed by Schaefer et al. (2018) including two SN subnetworks (SN_A including supplementary motor area, midcingulate cortex, supramarginal gyrus, Rolandic operculum, and insula; SN_B including middle frontal gyrus, anterior cingulate cortex, and insula), three default mode network (DMN) subnetworks (DMN_A including superior frontal gyrus, precuneus, and angular gyrus; DMN_B including medial superior frontal gyrus, left

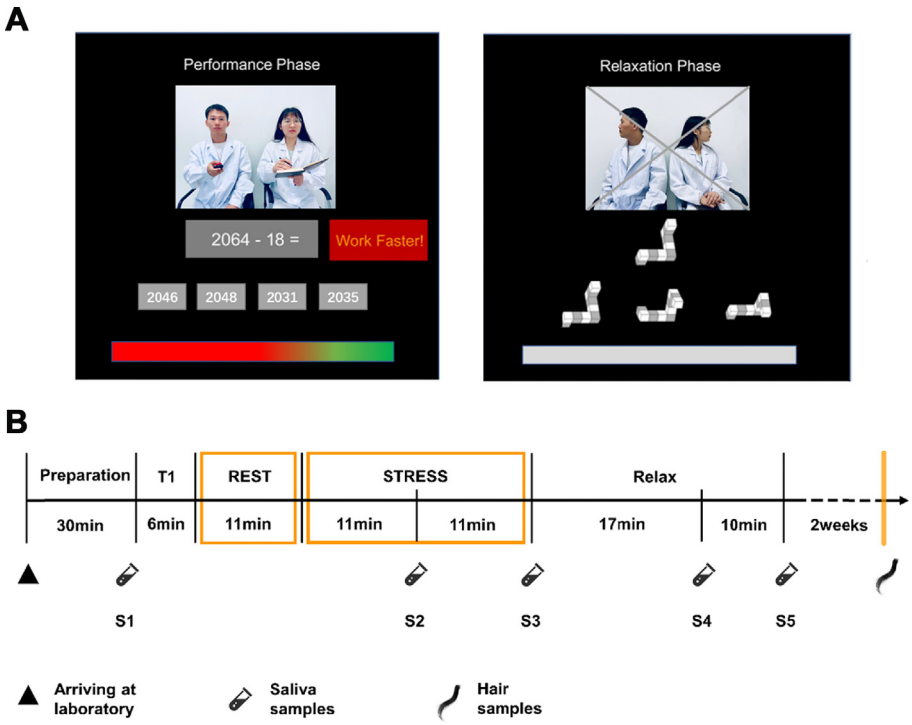

Fig. 1. ScanSTRESS paradigm and experimental procedure. (A) The design of the ScanSTRESS paradigm. The left and rnight images represent the interface viewed by the participants under a stress condition (left: taking the continuous subtraction task as an example) and a control condition (right: taking the figure matching task as an example), respectively. **(B)** The overall experimental procedure.

middle frontal gyrus, and inferior frontal gyrus; DMN_C including calcarine sulcus, and middle occipital gyrus), two dorsal attention network (DAN) subnetworks (DAN_A including superior parietal gyrus, inferior temporal gyrus, and middle temporal gyrus; DAN_B including supramarginal gyrus, superior frontal gyrus, precuneus and superior parietal gyrus), three control network (CON) subnetworks (CON_A including inferior parietal gyrus, middle frontal gyrus, and angular gyrus; CON_B including medial superior frontal gyrus, middle frontal gyrus, and inferior frontal gyrus; CON_C including middle cingulate cortex and precuneus), temporal parietal network (TPN, including superior temporal gyrus, middle temporal gyrus, supplementary motor area, and precentral gyrus), two visual network (VN) subnetworks (VN_A including fusiform gyrus, lingual gyrus, and middle occipital gyrus; VN_B including calcarine sulcus, cuneus, and lingual gyrus), two LBN subnetworks (LBN_A including inferior temporal gyrus, fusiform gyrus, and parahippocampus; LBN_B including orbitofrontal cortex and rectus gyrus), and two somatomotor network (SMN) subnetworks (SMN_A including postcentral gyrus and precentral gyrus; SMN_B including superior temporal gyrus, heschl gyrus, Rolandic operculum, and postcentral gyrus).

The ROI wise functional connectivity matrices for each participant were created, and their correlation with HairE concentrations or acute stress endocrine response (cortisol AUCg) was examined. Results that remained unchanged after correcting for multiple comparisons were considered significant ($p_{\_FDR} \leq 0.05$). Finally, the Pearson's correlation coefficients of RSFC between networks for each participant were extracted and included in a mediation analysis. Furthermore, after finding the brain network connections associated with both variables, we computed all connections of every component of one network to the other network's components and included them in the mediation models.

Correlational and mediation analysis. To test our hypothesis of the negative relationship between hair corticosteroid concentration and acute stress reactivity, we determined the correlation of HairE or HairF with acute stress endocrine response (cortisol AUCg). A partial correlation analysis of the hair corticosteroid concentration and cortisol AUCg was further performed, and age, sex, childhood trauma, daily stress, perceived stress, and hair-washing frequency were included as covariates of no interest (Staufenbiel et al., 2015; Ren et al., 2022).

Furthermore, to verify if HairE influenced the cortisol AUCg through the resting state functional network connectivity, a mediation analysis was carried out using the bootstrapping approach (Hayes, 2013). We constructed two mediation models with HairE as the independent variable, stress reactivity as the dependent variable, and RSFC between salience and limbic networks as well as RSFC between insula and PHG as the mediator variables.

RESULTS

Endocrine stress response to ScanSTRESS

The overall change in stress reactivity is shown in Fig. 2 (**A**). On the endocrine level, the ANOVA manifested a remarkable main effect of time (F (4,71) = 5.14, $p < 0.01$), indicating that the cortisol concentration significantly changed over the course of the experiment, which proved the effect of stress induction.

Relationship between hair corticosteroid concentration and stress reactivity

Results of the correlation analysis revealed that HairE was negatively correlated with overall cortisol stress response ($r = -0.27$, $p = 0.03$, Fig. 2(**B**)), even after controlling the effect of sex, age, childhood trauma, daily stress, perceived stress, and hair-washing frequency ($r = -0.26$, $p = 0.04$). However, HairF was not significantly correlated with cortisol AUCg ($r = 0.03$, $p = 0.80$, Fig. 2(**C**)).

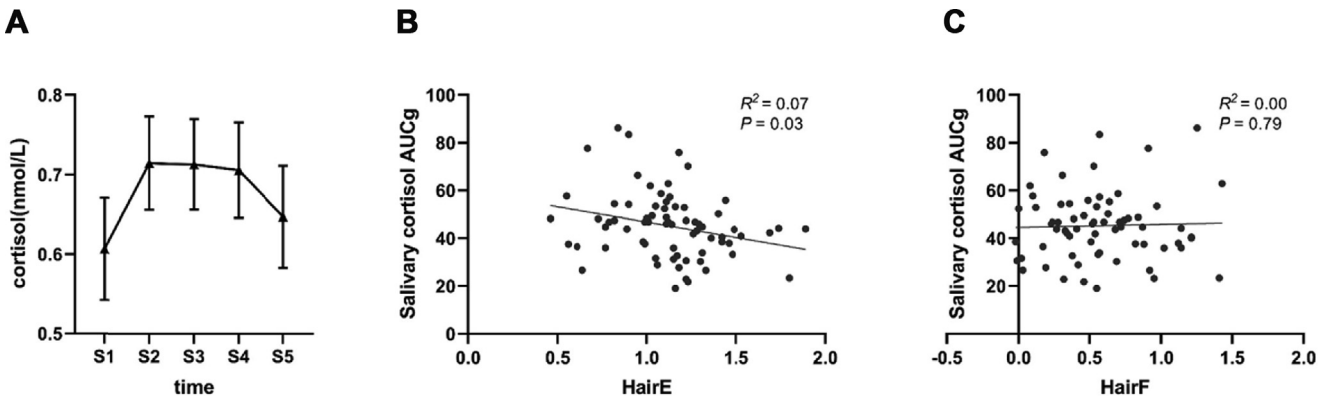

Fig. 2. Stress induction on the endocrine level and results of the correlation analysis. (A) Salivary cortisol concentration at all time points. **(B)** HairE is significantly associated with acute HPA cortisol AUCg. **(C)** HairF is not significantly associated with acute HPA cortisol AUCg. HPA, hypothalamic–pituitaryadrenal; AUCg, area under the curve with respect to the ground; HairE, hair cortisone; HairF, hair cortisol.

Resting-state functional connectivity and the associations with hair corticosteroid concentration and acute stress response

A higher HairE concentration, indicative of long-term HPA axis activity, was significantly associated with stronger functional coupling between SN_A – LBN_A (r = 0.37, p_{FDR} = 0.03, Fig. 3(**A**)). Moreover, a higher salivary cortisol AUCg, indicative of greater acute stress response, was significantly associated with weaker functional coupling between SN_A – LBN_A (r = −0.43, p_{FDR} = 0.00). Furthermore, the salivary cortisol AUCg was related to weaker SN_B – LBN_A (r = −0.34, p_{FDR} = 0.02) and LBN_A – TPN (r = −0.33, p_{FDR} = 0.02). The salivary cortisol AUCg was also related to enhanced RSFC between LBN_A – DMN_C (r = 0.35, p_{FDR} = 0.02), LBN_B – DMN_C (r = 0.33, p_{FDR} = 0.03), and within-DMN connectivity (r = 0.39, p_{FDR} = 0.01). All six connections related to cortisol AUCg are displayed in Fig. 3(**B**).

The mediation analysis showed that the RSFC between SN_A – LBN_A mediated the relationship between the HairE and cortisol AUCg (indirect effect = −6.92, 95% confidence interval (CI) = −14.94 to −1.71, p < 0.05, Fig. 4(**A**)). We also found that RSFC between left INS – left PHG mediated the relationship between the HairE and cortisol AUCg (indirect effect = −4.28, 95% CI = −10.94 to −0.51, p < 0.05, Fig. 4(**B**)).

DISCUSSION

The current study aimed to explore the role of brain's functional connectivity during resting state in the relationship between long-term HPA axis activity and acute salivary cortisol stress response and results showed the prominent role of RSFC between salience and limbic networks in this relationship.

With regard to the relationship between long-term HPA axis activity and acute salivary cortisol stress response, the current study found high hair cortisone levels predicted the lower acute salivary cortisol stress response, which is consistent with the concept of allostasis and allostatic load (McEwen and Gianaros, 2011). The process by which the body attempts to maintain homeostasis in the presence of environmental disturbances is referred to as allostasis, while the wear and tear generated by overexposure to stress and/or excessive or protracted reactions is referred to as allostatic load (McEwen, 2007). From this perspective, the reduction in acute stress response is attributable to allostatic load and initial excessive stress reactivity. The long-term HPA axis functions of the physiological stress system have been exhausted (Allison et al., 2019; Planche et al., 2019), and this leads to the downregulation of HPA axis reactivity (Alink et al., 2008). Similarly, the diminished responsiveness of stress hormones to acute psychosocial stressors is deemed as a consequence of stress habituation. Specifically, it occurs after low to moderate intensity stimuli are presented at a higher frequency (Grissom and Bhatnagar, 2009).

Interestingly, inconsistent with a previous study (Sandner et al., 2020), HairE rather than HairF was associated with acute cortisol stress response. This result can be explained in several aspects. First, cortisone is less polar than cortisol and can be incorporated into the hair from the bloodstream (Raul et al., 2004); hence, the HairE concentration is three to four times higher than the HairF concentration (Stalder et al., 2013). This may contribute to the more successful quantification of HairE concentration than the HairF concentration. Furthermore, a previous study suggested that the cortisone content in the saliva is the preferred biomarker for precise evaluation of serum free cortisol levels under basal and stimulated conditions (Perogamvros et al., 2010). The cortisone concentration in the hair can be used to more accurately assess the serum free cortisol levels over a long-term period. Hence, HairE may serve as a more stable measurement of long-term HPA activity compared with HairF.

The current study also found high HairE levels enhanced the RSFC between SN – LBN. A recent study showed that the perceived stress in the previous month could be primarily predicted by the interaction between SN (e.g., the thalamus) and LBN (e.g., the PHG and orbital gyrus), which were associated with emotion regulation and salience attribution. The critical nodes in the high precision predictive model included regions

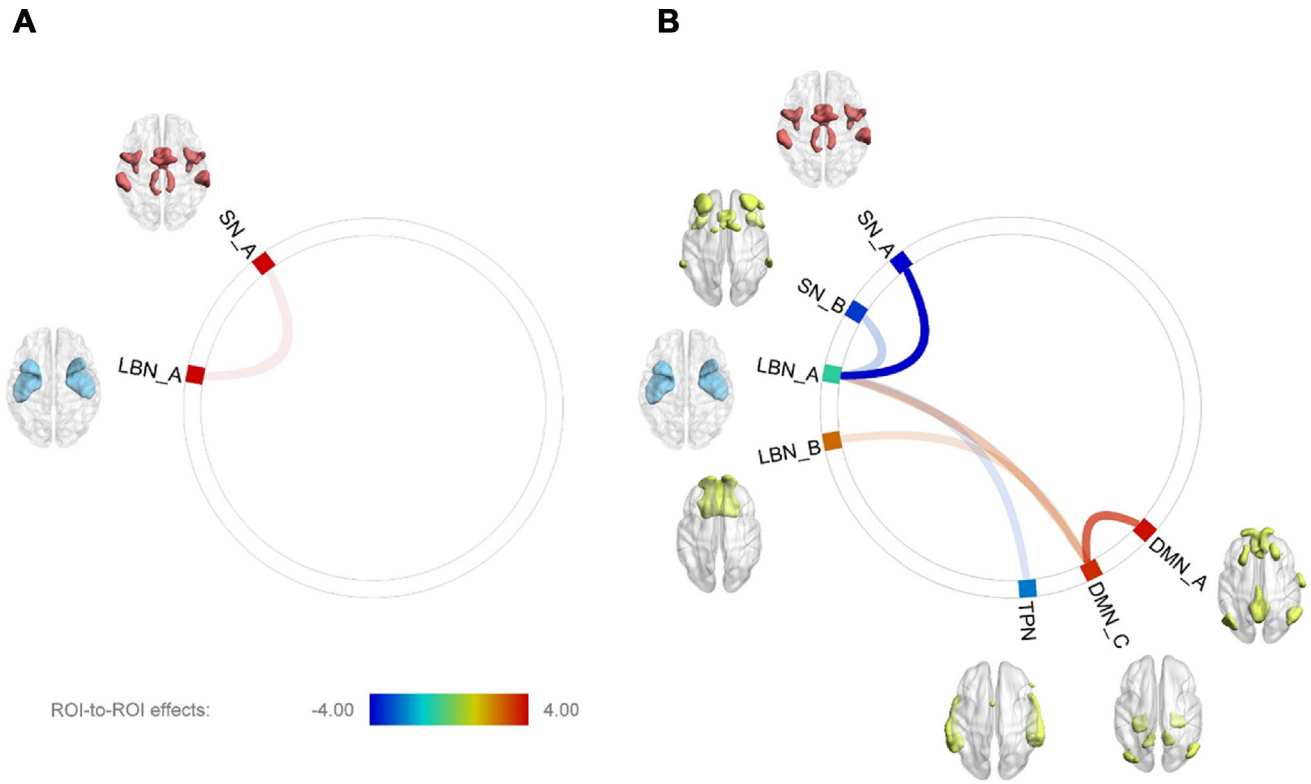

Fig. 3. Associations between RSFC and HairE or AUCg. RSFC correlates significantly with **(A)** HairE and **(B)** salivary cortisol AUCg. Depicted lines indicate the pairs of ROIs that demonstrate positive (red lines) or negative (blue lines) relationship between RSFC and HairE or AUCg. RSFC, resting-state functional connectivity; HairE, hair cortisone; AUCg, area under the curve with respect to the ground; SN_A and SN_B, two salience subnetworks; LBN_A and LBN_B, two limbic subnetworks; DMN_A and DMN_C, two of three subnetworks of default mode network (DMN_A, DMN_B, DMN_C); TPN, temporal parietal network.

predominately situated in the limbic systems and temporal lobe (Liu et al., 2021). Although perceived stress and hair corticosteroid levels are not related, they may still have a great intersection. Furthermore, enhanced FC between LBN – DMN as well as within-DMN connectivity was positively associated with acute salivary cortisol stress response. Previous studies have found that acute stress response is surprisingly associated with increased activity in the DMN during stress induction (van Oort et al., 2017). DMN reflects self-referential processing; this finding may indicate that individuals with a greater tendency for self-referential processing have a stronger response to an upcoming stress. Furthermore, LBN is an extremely important node related to the secretion of cortisol.

Importantly, the RSFC between SN – LBN and RSFC between left insula – left PHG act as meditators in the relationship between hair cortisone and acute salivary cortisol stress response. The insula can compare the normal situation underlying the maintenance of homeostasis and the incoming stimulus in order to determine whether this event is recognized as consistent with the current homeostasis (Panerai, 2011). The hippocampus and PHG, as essential components of the amygdala-MTL subsystems, are involved in the allostatic-interoceptive system (Ruiz-Rizzo et al., 2020). If the long-term HPA axis activity is excessive, it may mobilize to connect with the hippocampus and PHG in order to re-establish homeostasis. Due to the smooth operation during preprocessing and other reasons, overlapping portions may exist in various regions along the left PHG and left hippocampus. Hence, this result may be related to the negative feedback loop of the hippocampus. The threshold for the activation of the negative feedback mechanism is reduced, leading the low baseline and peak response to the approaching stressor. Furthermore, the PHG has an important role in the regulation of stress and emotion and the encoding and retrieval of episodic memory (Grinband et al., 2006; Aminoff, 2013). This finding suggests that long-term HPA axis activity may be related to those stressful experience.

Notably, a previous study found that a self-reported high daily stress level can predict a low acute stress response (Ren et al., 2022). This may suggest that both the long-term stress exposure and the daily stress exposure might decrease an individual's response to acute psychosocial stressors. Furthermore, individuals who experienced a greater number of stressful events in the previous day showed less hippocampal activation in response to the acute stress task. This finding is consistent with our results, suggesting that this blunting phenomenon may have protective implications. In addition, the limbic system is presumably activated prior to the performance of a stress task.

Our study has several limitations that should be acknowledged. First, the relatively small sample size subjected to resting-state fMRI examination limited the statistical power of this study. Therefore, these discoveries should be evaluated in a larger sample size.

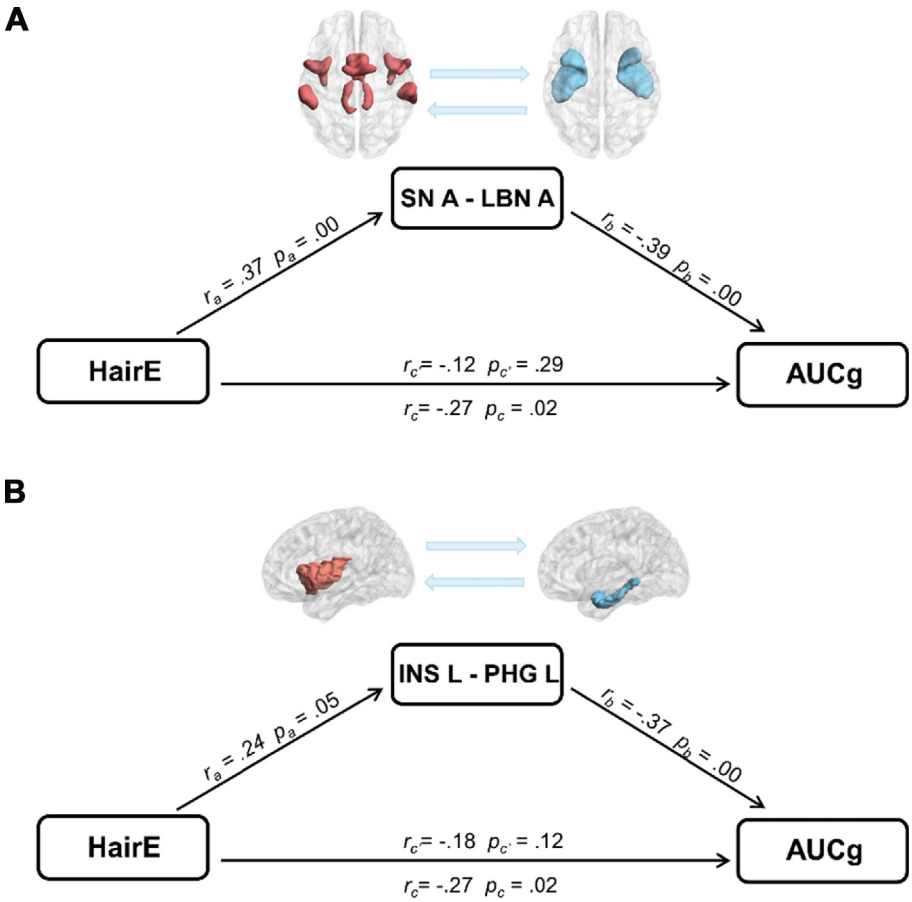

Fig. 4. Result of the mediation analysis. (A) RSFC between SN_A – LBN_A had an indirect effect on the association between HairE and acute stress cortisol response AUCg. **(B)** RSFC between left insula and left PHG had an indirect effect on the association between HairE and acute stress cortisol response AUCg. RSFC, resting-state functional connectivity; HairE, hair cortisone; AUCg, area under the curve with respect to the ground; SN_A, one salience subnetwork; LBN_A, one limbic subnetwork; INS_L, left insula; PHG_L, left parahippocampus.

Second, our resting state data are likely to be affected by anticipatory stress, because the participants may feel nervous about the next task and most of them have been exposed to the scanner environment for the first time; hence, future researchers need to strictly exclude this disturbance. Third, socioeconomic status and sexuality are highly correlated to stress; however, we did not consider these covariates.

In summary, the current study suggested that enhanced RSFC between LBN – SN, more specifically enhanced RSFC between left INS – left PHG, can mediate the negative relationship between long-term HPA axis activity indicated by HairE level and acute cortisol reactivity. In general, the present study provides empirical evidence that the brain activity mediates the cortisol response attenuated by the hair corticosteroid levels, which may help deepen our understanding of the individual differences in HPA stress responses.

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CONFLICT OF INTEREST

All authors disclosed no relevant relationships.

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Xiaolin Zhao and Prof. Juan Yang contributed to the design of the study. Yipeng Ren, Xiaolin Zhao, Weiyu Hu, Yadong Liu, Zhenni Wei and Zihan Tang contributed to the collection of the data. Yipeng Ren, Yadong Liu, Mengyi Li, Jiahao Luo and Kaige Guo contributed to the analysis of the data and chart making. Yipeng Ren and Yadong Liu contributed to the interpretation of the results and the drafting of the manuscript. We are grateful to Prof. Huihua Deng and Yan Wu for their support in the qualification of hormones from hair samples.

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