Idle Behaviors of the Hippocampus Reflect Endogenous Cortisol Levels in Youth

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Objective: Compelling evidence indicates that disruption in functional connectivity (FC) in brain networks underlies many psychiatric and developmental disorders. Current theory posits that biological (i.e., cortisol) and environmental (i.e., stress) experiences in early life are strong determinants in the development of functional brain systems and formative in the genesis of such disorders. The objective of this study was to examine the extent to which individual differences in cortisol concentrations during FC magnetic resonance imaging (MRI) would map onto variability in hippocampal to default mode network (DMN) connectivity in typically developing youth. **Method:** Salivary cortisol and FC MRI data were collected concurrently in 33 scan-naive 7- to 15-year-old participants. Twenty-nine of these participants previously completed the Trier Social Stress Test. Hippocampal to DMN FC and endogenous cortisol variability during MRI were examined. A possible association between MRI cortisol and cortisol response to the Trier Social Stress Test during the preceding visit or a participant's ratings of anxiety during MRI was tested. **Results:** There were significant positive relations between MRI cortisol levels and measurements in the following 3 areas: hippocampal to DMN FC during the resting state, cortisol levels during the Trier Social Stress Test, and fear/anxiety ratings during MRI. Fear/anxiety ratings during MRI also related to self-reported anxiety on standardized measurements. **Conclusions:** This study shows for the first time that FC of the hippocampus is altered with changing cortisol responsivity in youth. Altered FC during the resting state may represent altered alertness or monitoring resulting from variation in glucocorticoid function in youth, which carries implications for the effect of stress on response monitoring and decision making. J. Am. Acad. Child Adolesc. Psychiatry, 2013;52(6):642–652. **Key Words:** cortisol, default mode, functional connectivity, hippocampus, resting state

In the face of real or imagined adversity and/or uncertainty, the human body readily engages in a rapid and adaptive physical and chemical stress response. The hypothalamic-pituitary-adrenal (HPA) axis resides at the center of this regulatory stress response, in which adrenal glands are stimulated to increase the production of glucocorticoids, including cortisol. Cortisol helps the body to mobilize an adequate or appropriate response to stress or uncertainty by rapidly altering function in the metabolic, cardiovascular, and immune systems.[1]

Given that cortisol has such a widespread impact on brain and bodily functions, it is not surprising that the cumulative response of the body to stress has a profound effect on overall health. In fact, the past several decades have produced substantial research showing that an altered response in the HPA system influences growth and development in humans and predisposes select individuals to endocrine, psychiatric, cardiovascular, or immunologic disorders.[2,3] Psychiatric disorders linked to dysregulated HPA axis function include depression, panic disorder, posttraumatic stress disorder, and obsessive-compulsive disorder, to name a few.

An area of the brain that has been identified as regulatory over HPA function[4] and vulnerable to prolonged HPA activation is the hippocampus, situated bilaterally in the medial temporal lobes. Indeed, studies in children and adults have demonstrated that chronic or repeat HPA activation decreases hippocampal volume,[5] modifies

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surface morphology,[6] and changes hippocampal function.[7,8] Animal studies have indicated that excessive exposure to glucocorticoids results in widespread hippocampal changes, including altered synaptic plasticity, decreased neurogenesis,[9] decreased dendritic branching,[10] altered activity and protein expression,[11] and increased neuronal atrophy.[12] There are major gaps in understanding of the interplay between HPA function and neural functional connectivity (FC), and these are understood to an even lesser extent in youth, a period when the brain is putatively more vulnerable to the harmful physiologic effects of stress.[13] An establishment of the associations between brain FC and naturally varying HPA axis function in early life may facilitate a better understanding of the relation between dysregulated HPA axis function and mental health.

Resting-state FC magnetic resonance imaging (MRI) methodology enables the extraction of brain intrinsic connectivity networks. The best described and most widely studied of the human brain intrinsic connectivity networks is the "default mode network" (DMN). Identified 15 years ago by Shulman *et al.*,[14] the DMN is comprised of the medial prefrontal cortex (mPFC), posterior cingulate cortex/precuneus, bilateral parietal cortex, and bilateral medial temporal lobes, including the hippocampus. The DMN reflects the intrinsic processing demands of the brain and is regarded as essential to states of alertness, vigilance, and monitoring.[15] Moreover, abnormal DMN function has been associated with several neurologic and psychiatric diseases (e.g., schizophrenia,[16] depression,[17-19] Parkinson's disease,[20] autism,[21] posttraumatic stress,[22] and attention deficit disorders[23]) and has been associated with decrements in task performance[24] that mirror the performance deficits observed under conditions of chronic stress.[25] DMN function may contribute to psychological health in part by enabling a readiness to initiate rapid, adaptive responses to environmental demands, and the hippocampus is likely to play a central role in this form of behavioral vigilance.[26,27] Because individuals vary to a great extent in their HPA responses to stressful/uncertain events, it is possible to evaluate associations between HPA stress reactivity and DMN neural connectivity even in healthy volunteers and learn about associations between neuroendocrine function and individual differences in psychological health.

In recent years, novel observations about human brain development have arisen from FC MRI studies in infants and children. These studies have shown that fundamental networks such as the DMN are apparent in primitive form in infancy[28] and appear relatively mature by early childhood.[29] They have demonstrated maturation proceeds in a shift from diffuse local FC to increased long-range connectivity.[30] They have also shown that children's genes,[31] anxiety levels during scanning,[32] and biological sensitivity to stress[33] alter FC, highlighting a degree of individual variability in the composition of these functional networks in typically developing youth. FC studies in infants and children have been the subject of recent reviews,[34,35] and the reliability in FC networks in youth has been empirically demonstrated.[36]

Given the central role of the DMN in healthy psychological function,[25] and given that early developmental mechanisms influence the tendency of individuals to express maladaptive responses to threatening stimuli (i.e., heightened anxiety; see Gross and Hen[37]), the authors tested DMN FC and individual differences in HPA function in youth. In late childhood and early adolescence, brain limbic circuitry is still developing,[38] and protracted maturational brain changes are likely to affect the effectiveness of emotion regulation in children.[39] Seed-based connectivity analysis was applied to resting-state data to define the brain DMN in 33 children and young adolescents; then, whole-brain linear regression was performed on DMN FC maps to determine regions in which FC was altered with heightened HPA response. The primary objective of this study was to test the hypothesis that within the DMN hippocampal connectivity would vary with cortisol response during MRI. Additional stress reactivity measurements and self-ratings of stress were obtained to test the following secondary predictions: an increased cortisol response would be observed in youth reporting higher levels of anxiety during the MRI scan session and individuals with a high cortisol response during the Trier Social Stress Test for Children (TSST-C)[40] would show high cortisol response activity during their MRI study.

METHOD

Participants

Thirty-three children and adolescents 7 to 15 years old (mean = 11.1, SD = 2.4) were recruited through advertisements on the Wayne State University website, Craigslist (metro Detroit), or printed flyers. None of the participants reported a history of brain injury or

learning disorder and all were fluent in English. Parents and participants gave informed consent and assent, respectively, as approved by the Wayne State University institutional review board.

Visit 1 Procedure

Laboratory Visit. Before being scheduled for a functional MRI (fMRI) experimental session, all participants visited the behavioral laboratory for cognitive and affective testing. Introductory laboratory visits began at 9:00 or 9:30 A.M. to mitigate time-of-day cortisol effects. The visits included administration of the Kaufman Brief Intelligence Test, 2nd edition,[41] and the TSST-C,[40] during which salivary cortisol was collected 4 times at 15-minute intervals. In brief, the TSST-C began quickly and at the beginning of the visit (after consent/assent) and included 5-minute speech preparation, 5-minute speech performance at a microphone in front of 2 interviewers seated behind a desk, and 3-minute serial subtraction while still at the microphone. Participants were given positive encouragement to speak for the entire duration. Children were asked to start over at the beginning of the subtraction task at points when mathematical errors were made. Overall, administration of the TSST-C closely followed prior work.[40] During this visit, participants and parents also viewed a video to prepare them for the MRI scan session and completed several self-report measurements.

Visit 2 Procedure

Cortisol Collection and Analysis. Participants refrained from eating and drinking (except water) for 1 hour before arrival at the MRI testing facility. The experimental sessions began at 9:30 or 11:00 A.M. to mitigate time-of-day cortisol effects. Saliva samples were collected at 30-minute intervals throughout the visit using Salivettes (Sarstedt, Nümbrecht, Germany). Each time a cortisol sample was collected, the participants were asked to rate their comfort/anxiety level. These collection times were based on meta-analytic findings indicating that peak cortisol response occurs 21 to 40 minutes after the onset of an acute stressor and that complete recovery to baseline values occurs within 41 to 60 minutes.[42] Saliva samples were stored at −20°C until assayed using commercially available enzyme-linked immunoassay kits (DRG International, Springfield, NJ). Before assay, saliva samples were allowed to thaw at room temperature and then centrifuged at 3,000 rpm for 10 minutes. All samples were assayed in duplicate and the average of the duplicates was used in all analyses. Optical densities were read at 450 nm using an Epoch plate reader (BioTek, Winooski, VT). Concentrations (nanograms per milliliter) were interpolated from the calibration curve using a 4-parameter logistics curve fit. The mean intra-assay coefficient of variation was 6.53% and the mean interassay coefficient of variation was 14.8%. Outlying cortisol and visual analog scale (VAS) measurements (Figure 1) were winsorized to 2 SD from the mean, as in previous work.[33] Correlations between participants' MRI cortisol area under the curve (AUC) and cortisol AUC to the laboratory TSST (~2 weeks previously) and mean VAS ratings during MRI were tested using the Pearson bivariate correlation, implemented in IBM SPSS Statistics 20 (SPSS, Inc. Chicago, IL). Participants' mean cortisol response during MRI was used in resting-state regression models.

Ascertainment of Fear/Anxiety Ratings. As described in previous studies,[43] VAS ratings were obtained by showing participants an array of cartoon faces varying in expression (Figure 1) and then asking each participant to indicate the face that best indicated that participant's feelings at that time. Participants performed the VAS rating twice before entering the MRI scanner. Once in the scanner, children were not shown the faces, but an experimenter went into the room and queried the child as follows: "Remember those faces you saw where 1 indicated no fear or anxiety, 5 indicated high fear and anxiety, and 3 was neither good nor bad? Which one of those faces best represents how you are feeling now?" Participants responded verbally and expressed no difficulty with these instructions. Four VAS measurements were used to calculate mean MRI VAS scores (Figure 1 shows measurement timeline).

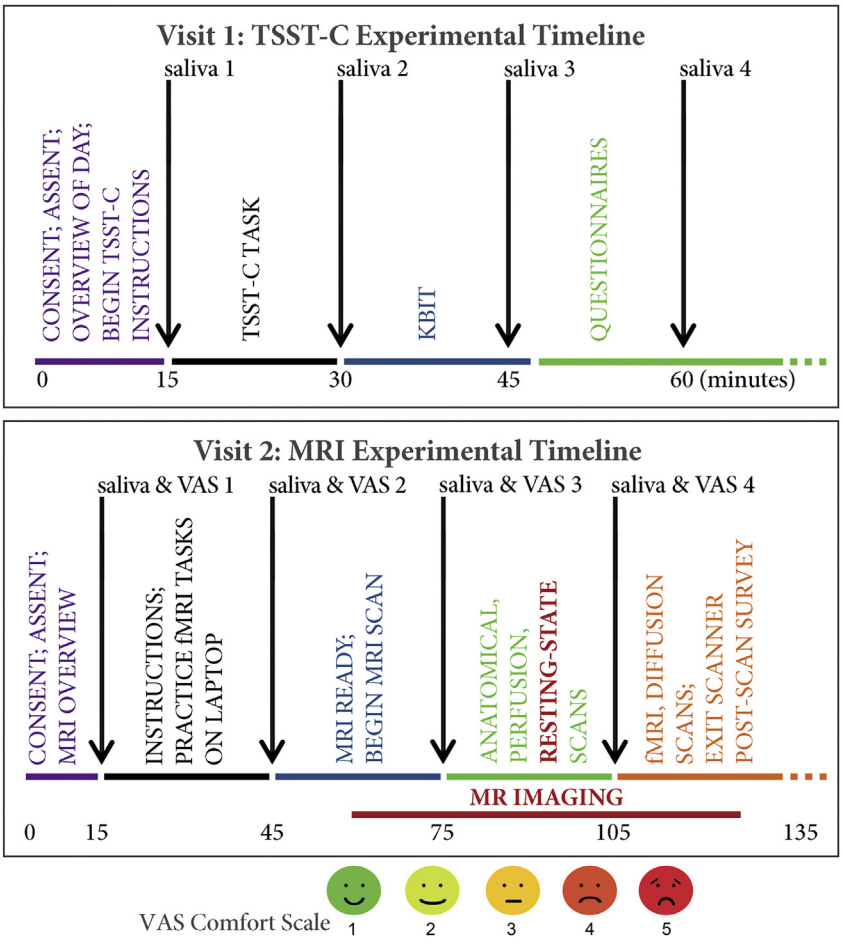
Associations between average MRI VAS scores and total and subscale scores of the 41-item revised Screen for Child Anxiety Related Emotional Disorders (SCR-C; an empirically derived self-report instrument that screens for anxiety disorders)[44] were tested. The SCR-C includes 5 subscales corresponding to panic/somatic concerns, general anxiety, separation anxiety, social anxiety, and avoidance. Family health history was assessed using a self-report questionnaire. Statistical analyses were performed in IBM SPSS Statistics 20.

MRI Data Acquisition. MRI was performed on a 3.0-T Verio scanner (Siemens, Munich, Germany) on average 2 weeks after the laboratory visit. Participants were positioned in an 8-channel transmit-receive head coil and stabilized by clamps to decrease motion-related artifacts during scanning. During the resting-state experiment, participants completed a 6-minute scan during which they were instructed to lay still with their eyes closed. During the resting-state scan, a single run of 180 29-slice (4-mm-thick) functional echoplanar images were acquired (interleaved ascending acquisition) using a repetition time of 2,000 ms, echo time of 25 ms, field of view of 220 × 220 (full brain coverage), flip angle of 90°, and a voxel size of 3.44 × 3.44 × 4 mm.

Data Analysis

fMRI Preprocessing. Data preprocessing was completed using Statistical Parametric Mapping software (SPM8; www.fil.ion.ucl.ac.uk/spm/software/spm8/). Preprocessing included slice timing correction, image realignment, and coregistration of functional and anatomic images. Functional images were normalized to the

FIGURE 1 Experimental timeline for each of the study visits. Note: Visits were separated on average by 2 weeks. During visit 1, saliva measurements were taken every 15 minutes, with measurements 1 and 2 flanking the Trier Social Stress Test for Children (TSST-C). The TSST-C occurred at the beginning of the visit. During visit 2, saliva and visual analog scale (VAS) measurements were repeated 4 times at 30-minute intervals, with the first 2 measurements taking place during preparations before scanning and measurements 3 and 4 while in the magnetic resonance imaging (MRI) system. The VAS was presented to participants with each cortisol assessment throughout the MRI visit. KBIT = Kaufman Brief Intelligence Test, 2nd edition.



Montreal Neurological Institute template using participant-specific transformation parameters created by fitting mean functional images to the single-reference echo planar imaging standard SPM template. Data were not resampled during normalization; thus, data retained the native resolution (3.44 × 3.44 × 4 mm) for subsequent analyses. After normalization, images were smoothed with an 8-mm gaussian kernel.

Movement. Siemens MRI motion correction software was used to retrospectively measure 6 parameters of rigid-body translation and rotation for each time frame and produce a corrected time series using an affine transformation. After this correction, motion statistics were extracted and are reported in Table S1 (available online). In addition, correlations between movement and cortisol AUC were tested. The results demonstrated that residual movement was well within accepted standards (<0.5 mm) and that movement was not significantly related to cortisol AUC.

Seed-Based Connectivity Analysis. FC analysis was performed using the CONN fMRI FC toolbox (version 12.p; www.nitrc.org/projects/conn[45]). The DMN region-of-interest mask was generated using WFU PickAtlas 3.0 (http://fmri.wfubmc.edu/software/PickAtlas). The DMN mask was comprised of 3 6-mm radius binarized spheres around Talairach coordinates 5, −51, and 12 in right Brodmann area 29; −2, 55, and −8 in left Brodmann area 10; and 49, −61, and 23 in right Brodmann area 39, drawn from a previously published independent components FC MRI analysis of 65 children and adolescents 9 to 15 years old.[36]

To account for noise confounds, which can introduce spurious correlations across the brain, especially in FC analysis, and to avoid issues inherent in regressing out

the global signal,[46] the CONN fMRI toolbox uses the anatomic component correction method of removing physiologic and other spurious sources of noise on a voxel-by-voxel basis.[47] The anatomic component correction noise correction method basically applies a principal components approach to remove signals from white matter and cerebral spinal fluid regions of interest with regression. Residual head motion parameters (3 rotation and 3 translation parameters plus another 6 parameters representing their first-order temporal derivatives) also were regressed out. Functional scans were bandpass filtered between 0.01 and 0.08 Hz to investigate low-frequency correlations, which are most consistently produced within this range.[48]

Definition of DMN. Random effects analyses were performed in SPM8 on *t* statistic DMN volume images generated by the CONN fMRI toolbox for every subject. One-sample *t* tests were used to identify significant DMN whole-brain connectivity for the sample. Participant age and gender were included as covariates in the model to account for interindividual differences within the study sample. A statistical height threshold of $p < .01$ using family-wise error correction[49] ($T_{threshold} = 6.291$) was used to identify significant DMN whole-brain connectivity for the sample.

Cortisol-DMN Regression Analysis. Regression analysis was applied to evaluate the relation between cortisol during MRI and DMN FC. Regression analysis was performed for the entire brain (for descriptive purposes) and within an anatomically defined bilateral hippocampal mask (effectively restricting the number of comparisons to the a priori region of interest). A statistical height threshold of $p < .005$ ($k = 5$) was used to identify significant FC differences related to cortisol levels during the MRI study. Multiple testing correction was performed using the AFNI (Bloomington, IL) subroutine, AlphaSim, which used 10,000 Monte Carlo simulations to estimate the number of contiguous voxels one would expect to observe in a significant cluster given the *p* threshold used and number of comparisons made. The correction is based on the principle that true regions of activation will tend to occur over contiguous voxels, whereas noise has much less tendency to form clusters of activated voxels.[50] For the present study data, AlphaSim was applied using the hippocampal mask, comprised of 6,306 voxels (resolution = 2 mm^3), a *p* threshold of .005, and an 8-mm full width at half maximum smoothing kernel.

Hippocampal Region of Interest Analyses. Possible associations between FC in the left hippocampus and participant self-reported VAS ratings during MRI were evaluated. Using WFU PickAtlas, a sphere was generated in the left hippocampus centered at −22, −32, and −4 (Montreal Neurological Institute) and the FC for each participant was extracted. FC strength in the left hippocampus was compared with a participant's mean VAS score. These correlations were performed in IBM SPSS Statistics 20.

RESULTS

Participant Characteristics

Five participant families (15.2%) reported the presence of parental psychopathology (Axis 1 mood/anxiety disorders) in 1 biological parent; 1 family (3.0%) reported parental psychopathology in both parents. Thus, the prevalence of parental psychopathology in the study sample appeared representative of national averages.[51] The distribution of female (n = 21) and male (n = 12) participants in the sample did not differ ($\chi^2_1 = 2.5$, $p = .12$). With the exception of 1 male participant who was left-handed, all participants were right-handed. Participants were 17 African Americans (51.5%), 13 Caucasians (39.4%), 2 Latinos/Hispanic Americans (6.1%), and 1 participant of multiracial descent (3.0%). The mean Kaufman Brief Intelligence Test intelligence score for the sample was 106 (SD = 12).

Association Between Cortisol AUC and FC Within the DMN

Whole-brain omnibus analysis revealed that individual variation in FC was associated with endogenous cortisol levels in some brain regions. Specifically, the bilateral hippocampus, bilateral visual cortex, and regions of the temporal cortex exhibited greater FC to core DMN regions (e.g., mPFC, lateral parietal, posterior cingulate cortex) in individuals with higher cortisol during MRI. Conversely, those individuals with higher cortisol levels during MRI exhibited decreased connectivity to the mean DMN trace in 2 areas in the left posterior cerebellar lobe, thalamus, and anterior cingulate. Whole-brain regression results are presented in Table 1 ($p < .005$ threshold). A hippocampal mask was used for small volume correction and to address the authors' hypothesis that altered FC to the hippocampus would be observed in individuals with high cortisol levels. Both hippocampal clusters survived multiple testing correction performed using AlphaSim. DMN regression results are shown in Figure 2.

Self-Reported Anxiety and VAS Response During MRI

The group average for the psychometric measurement of anxiety was within the normal range (SCR-C total, mean = 15, SD = 12). Similarly, no abnormal values were obtained for subscale SCR-C scores (panic, mean = 3.7, SD = 4.7; generalized anxiety, mean = 3.4, SD = 3.7; separation

TABLE 1 Significantly Altered Functional Connectivity in Youth With Higher Cortisol Levels During Magnetic Resonance Imaging

	Brodmann Area	X	Y	Z	*t* Statistic	Voxel No.
Increased FC in youth with higher cortisol levels						
Hippocampus	L-27	−22	−36	−4	4.12	152
Cuneus	R-18	14	−102	8	3.60	207
Superior temporal gyrus	R-38	46	24	−22	3.13	8
Middle occipital cortex	L-18	−20	−102	8	3.11	214
Superior frontal gyrus (supplementary motor)	R-6	5	16	68	3.10	78
Postcentral gyrus	R-40	26	−32	44	3.09	42
Middle temporal gyrus	L-39	−50	−80	24	3.03	34
Posterior insula (rolandic operculum)	L-40	−50	−22	16	2.93	12
Transverse temporal gyrus	R-42	64	−18	10	2.92	32
Inferior frontal gyrus	L-11	−26	34	−22	2.88	6
Hippocampus	R	30	−38	−4	2.83	8
Decreased FC in youth with higher cortisol levels						
Cerebellum posterior lobe (tonsil)	L	−20	−42	−40	3.40	124
Thalamus	R	6	−16	16	3.27	65
Middle temporal gyrus	R-21	70	−42	−6	2.99	14
Cerebellum posterior lobe (tonsil)	L	−46	−48	−42	2.91	77
Inferior parietal lobe	L-40	−46	−48	38	2.82	8
Anterior cingulate	R-32	10	46	2	2.79	28

Note: Regression summarized at $p < .005$, *cluster minimum = 5. Coordinates are presented according to the Montreal Neurological Institute. FC = functional connectivity; L = left; R = right.*

anxiety, mean = 2.8, SD = 2.7; social anxiety, mean = 4.1, SD = 3.6; avoidance, mean = 1.4, SD = 1.5). Pearson correlations were used to test associations between the average VAS reported by participants and SCR-C total and subscale scores. Individuals' VAS ratings were significantly correlated with their overall SCR-C score ($r_{31} = .39$, $p = .024$). That is, compared with children who reported lower levels of anxiety, children with high levels of self-reported anxiety on the SCR-C rated the MRI as evoking higher levels of fear and anxiety (VAS). SCR-C subscales that showed a significant positive correlation with MRI VAS included panic ($r_{31} = .35$, $p = .043$) and avoidance ($r_{31} = .35$, $p = .044$).

Stress Reactivity

Using Pearson correlation, the authors found that the AUC for MRI was significantly and positively related to the mean VAS score obtained during the MRI session ($r_{31} = .411$, $p = .017$). Thus, the VAS scores obtained by youth in this study corresponded well to the measured cortisol response. In addition, the AUC for the MRI visit was significantly positively correlated with the cortisol AUC to TSST-C during the prior laboratory visit ($r_{27} = .57$, $p = .001$), suggesting that, within subjects, cortisol response is stable across time. TSST-C and MRI cortisol measurements, VAS during MRI, and strength of DMN to left hippocampus FC values are provided for all subjects in Table S2 (available online).

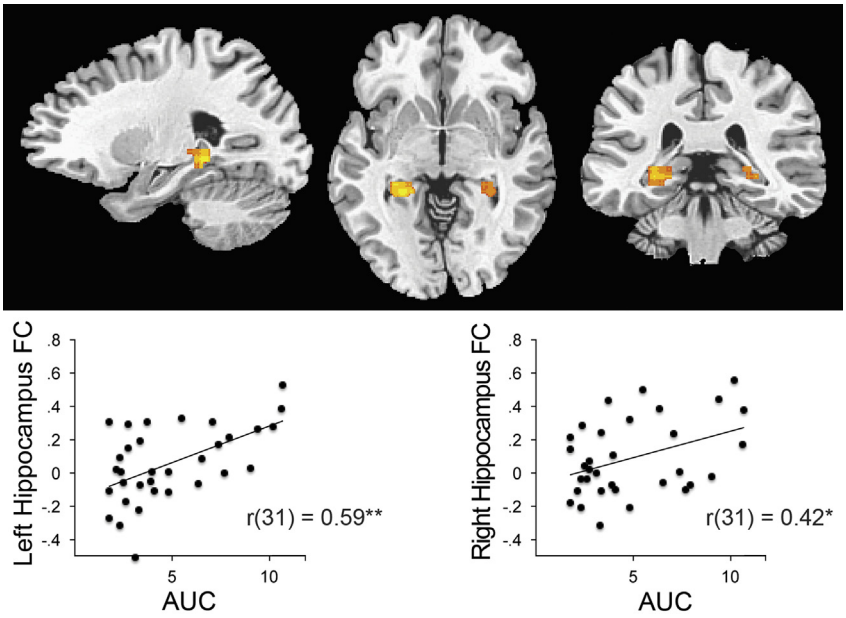
DMN Connectivity in Youth

Across the 33 subjects, DMN connectivity was significant across many regions, including the mPFC, posterior cingulate/precuneus, lateral parietal, and bilateral medial temporal regions (including the hippocampus; Figure 3). These DMN results are in good agreement with prior reports in similar age groups.[29,36]

DISCUSSION

The primary result of this study is the observed positive correlation between the cortisol AUC during MRI and hippocampal to DMN connectivity during the resting state. This indicates that participants experiencing greater biological stress during the visit (i.e., higher cortisol AUC) had different patterns of resting neural connectivity that signify increased involvement of the hippocampus in DMN-related processing. The unconstrained processes of the DMN are regarded as essential to states of alertness, vigilance, and monitoring.[15] The hippocampus plays a critical role in arousal/autonomic components

FIGURE 2 Cortisol regression results at $p < .005$, corrected for multiple comparisons. Note: Significant bilateral hippocampal clusters indicate areas in which default mode network connectivity was higher in scan-naive individuals with higher cortisol levels during magnetic resonance imaging. Whole-brain results for this regression are presented in Table 1. Scatterplots illustrate the correlations between magnetic resonance imaging cortisol area under the curve (AUC) strength of hippocampal functional connectivity (FC) and the default mode network. *$p = .014$, **$p = .001$.

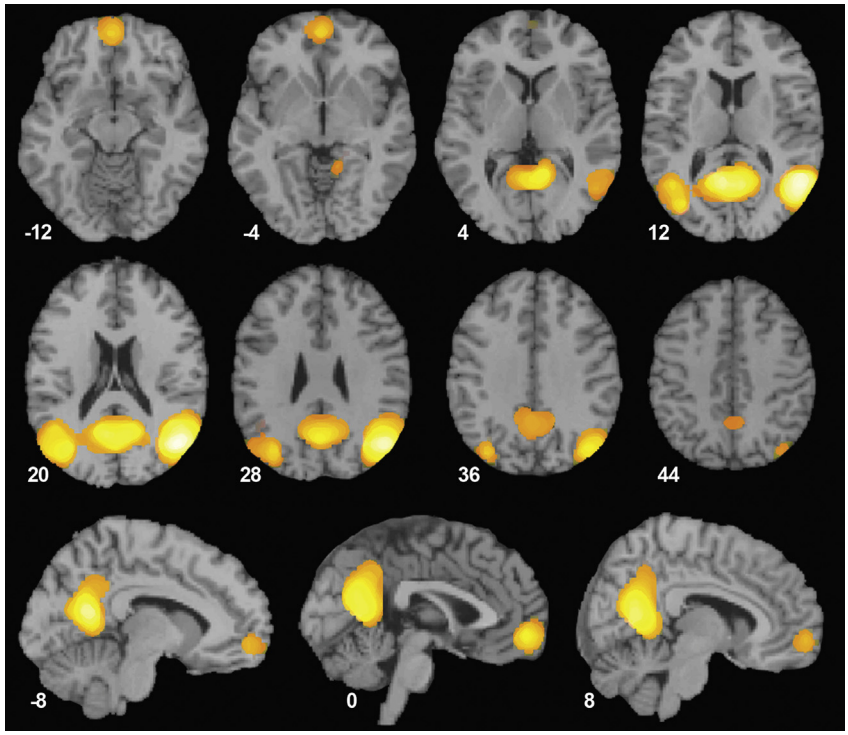


of anxiety[52] and in generating appropriate responses to ambiguous aversive stimuli.[53] The hippocampus is also the flagship structure of learning and memory[54] and more recently has been attributed to prospective thought[55] and decision making.[56] Thus, this observed association between stress response and hippocampal FC offers insight into functional shifts that may disrupt proper resource allocation during idle behavioral states.

The authors suggest that connectivity between the hippocampus and DMN during rest may affect the ability of the system to appropriately tune into relevant information and may hinder readiness to respond appropriately to environmental input. The push and pull of the DMN and executive networks of the brain have been demonstrated well in adults[57] and children.[29] In addition, an inability to effectively switch dominance between DMN and executive systems has been linked to deficits in cognitive control[58] and to depression[19] and posttraumatic stress.[22] Further, stress negatively affects cognition (see review by Lupien *et al.*[59]). An important direction for future work will be to examine whether the impact of stress on cognitive performance is be due in part to altered DMN connectivity. Overall, the ability to engage DMN and executive systems appropriately and reciprocally has been increasingly acknowledged as important for mental and cognitive health, and FC in the DMN was related to cortisol variability in typically developing youth in the present study.

At first glance, it may seem contradictory that previous task-based fMRI studies have shown decreases in hippocampal activity in individuals with increased cortisol (e.g., during presentation of negatively valenced emotional images[60,61] and during declarative memory retrieval[62]). However, decreased hippocampal activity during a task can arise from less engagement during the task or from higher basal activity. The present connectivity data suggest the latter explanation may account for decreased hippocampal activation during an emotionally evocative task. That is, in task-based activation studies, the blood oxygen level-dependent (BOLD) signal amplitude of fMRI may be decreased by baseline differences in resting-state activity. If the default mode (baseline) fMRI signal is higher in the medial temporal lobes, then the comparison of an activated state with baseline would produce a decreased BOLD signal change.

FIGURE 3 Default mode network functional connectivity across all study participants. Note: Results of 1-sample *t* test are presented for values $p < .01$ with family-wise error correction. Transverse and sagittal slices of the default mode network are projected over a template brain to provide anatomic reference. Corresponding network peak coordinates are presented in Table 1.



In contrast to task-based fMRI, FC MRI is concerned not with activation levels, but with connectivity between regions. In fact, under normal physiologic conditions, connectivity is largely impervious to absolute activation levels. Studies that have examined brain connectivity and HPA status in adults have observed interactions primarily in the amygdala/mPFC[63,64] and hippocampal[65] regions. Further, studies using positron-emission tomography have described increased cerebral blood flow in the hippocampus in anticipation of public speaking in individuals with social phobia[66] and decreased cerebral blood flow in the hippocampus in patients with social phobia who respond well to pharmaceutical treatment.[67] The peak cortisol effects observed in the present youth sample were in hippocampal regions where altered connectivity has previously been associated with trait anxiety in adults.[68] Overall, available positron-emission tomographic and fMRI data[69] support the conclusion that hippocampal activity is heightened in individuals with high anxiety. The present study adds to this the observation that hippocampus to DMN connectivity is increased in youth with higher cortisol levels during resting-state MRI.

The authors also observed rather high stability in children's measurements of stress and anxiety in self-report and biological domains. Self-reported anxiety on panic and generalized anxiety SCR-C subscales was correlated with self-reported VAS anxiety during scanning. That is, children who reported being generally more anxious also reported higher fear/anxiety during an MRI study. In addition, cortisol reactivity to the well-established TSST-C in the laboratory was significantly correlated with cortisol reactivity during MRI, which occurred on average 2 weeks later. With the increase in MRI research in children, there has been growing discussion about MRI as a potential stressor and the concomitant neural and psychological effects of stress during MRI. Suggestions have been made about mitigating the psychological impacts of MRI-related stress in youth,[70-72] but less has been done to evaluate and respond to the potential neural effects of biological stress variation. The present data suggest that cortisol levels should be taken into account, particularly when between-group

contrasts include groups that may differ significantly in stress response (i.e., children versus adults, individuals with emotional psychopathology versus healthy controls). However, the authors found that MRI-associated cortisol was significantly related to VAS, which suggests that, in the absence of cortisol measurements, repeat VAS assessment of fear/anxiety at 30-minute intervals across the visit may suffice.

Because the systems that support emotional development in children develop beyond adolescence,[38] one imagines that the behavioral tendencies of early life shape the mature form of these large-scale neural systems. Because all the present participants were scan-naive, the MRI examination was a real-world, prolonged exposure to an unfamiliar environment that mimics adaptive demands that would be elicited in any new environment, demands that are present with greater frequency in the early developmental years. In a theory discussed in *The Neuropsychology of Anxiety* by Gray and McNaughton,[52] the hippocampus acts to resolve ambiguity or conflict by increasing weight to affectively negative information. Thus, the human hippocampus is a key structure in initiating appropriate responses in the face of uncertainty. The hippocampus is likely to develop response tendencies through the experiences of early life, when amplification of negative affective information to achieve a felt response is learned.

The limitations of this study warrant mention. First, seed-based analyses require a priori selection of regions of interest. Although DMN seed regions were selected based on coordinates determined using independent components analysis in an independent sample of 65 children, the authors cannot declare that the seed points used were optimal for testing variation in DMN connectivity in this study group. Second, the authors took a narrow approach in studying only DMN connectivity. Prior FC MRI research in youth[33] and in adult males[63-65] has indicated that correlates of HPA function also may be observable in the mPFC, insula, and amygdala. Thus, broader brain network connectivity differences may underlie variability in HPA axis function. However, maintaining a narrow focus assured mitigation of the multiple-comparisons problem that arises from testing tens of thousands of voxels simultaneously, a problem potentially inflated by temporal autocorrelation in FC MRI.[73] Third, as in most BOLD fMRI studies, the observed associations were intrinsically correlative; it was impossible to conclude whether decreased hippocampal connectivity reflected or caused decreased inhibition of HPA function. Causal distinctions are important for the development of mechanistic models, and thus this presents a limitation of the present study and an important avenue for future research. Fourth, although participants in this study spanned a wide age-range (7–15 years), the study was not sufficiently powered to assess the effect of age on study outcomes. Therefore, the authors attempted to control for age by including age as a nuisance covariate in the regression model applied to DMN neuroimaging data.

In the present study, an FC neuroimaging strategy was used to identify neural-systems level mechanisms corresponding to heightened HPA axis activity during MRI. Whole-brain analysis implicated significant FC differences in bilateral hippocampal regions. Further, the authors found that anxiety ratings in children were stable across time, and that children's anxiety ratings during MRI were correlated with cortisol AUC during MRI. The latter suggests that in the absence of cortisol measurements, children's self-reports may be a useful indicator of HPA function during MRI. These data highlight the tight relation between unconstrained brain FC and individual variation in biological and psychological responses to stress. The authors propose that interactions between brain regions influence the individuals' ability to rapidly initiate appropriate responses, and they highlight that altered resting FC represents a quantifiable means for querying neurodevelopmental processes that may lead to maladaptive behaviors or represent vulnerability to developing psychiatric disorders. &

CG Clinical Guidance

- Stress responses in youth (e.g., children, adolescents) are reflected in different patterns of connectivity in the brain.
- Youth who report high fear/anxiety during magnetic resonance imaging (MRI) exam also show higher cortisol reactivity during MRI.
- Youth who report high fear/anxiety during MRI also report higher trait anxiety.
- Differences in brain connectivity may underlie cognitive deficits associated with stress.

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TABLE S1 Descriptive Statistics for Movement Parameters and Results of Bivariate Correlation With Measured Cortisol Response and Age

Motion Parameter	Mean	SD	MRI AUC (*r*)	MRI AUC (sig.)
X mean (mm)	0.02	0.02	−0.18	0.34
Y mean (mm)	0.04	0.03	0.00	0.98
Z mean (mm)	0.04	0.04	0.03	0.87
Pitch mean (°)	0.06	0.05	0.04	0.82
Roll mean (°)	0.02	0.02	−0.25	0.18
Yaw mean (°)	0.03	0.03	0.08	0.67
X max excursion (mm)	0.14	0.10	−0.11	0.57
Y max excursion (mm)	0.28	0.17	−0.01	0.95
Z max excursion (mm)	0.28	0.12	−0.02	0.93
Pitch max excursion (°)	0.38	0.25	0.01	0.95
Roll max excursion (°)	0.16	0.10	−0.17	0.36
Yaw max excursion (°)	0.17	0.13	−0.17	0.34

Note: AUC = area under the curve; excursion = frame-to-frame displacement; max = maximum; mean = average movement across the scan; MRI = magnetic resonance imaging; r = Pearson correlation; sig. = 2-tailed.

TABLE S2 Cortisol, Visual Analog Ratings of Fear/Anxiety Obtained During Magnetic Resonance Imaging, and Functional Connectivity Measurements

ID	TSST-C AUC	MRI AUC	MRI VAS	Hippoc-DMN FC
1	2.68	5.47	1.63	0.32
2	2.77	4.82	1.75	−0.11
3	2.32	3.38	1.75	0.19
4		9.02	3.00	0.03
5		3.38	1.00	−0.07
6	1.21	2.16	1.00	0.02
7	11.15	7.40	2.00	0.17
8	2.97	3.70	1.00	0.30
9	4.90	7.96	1.50	0.21
10	4.63	2.62	1.00	−0.18
11	8.11	3.96	1.50	0.01
12	2.78	1.78	1.50	−0.11
13	3.05	3.27	2.99	−0.22
14	9.50	4.82	1.25	0.00
15	6.00	2.49	1.00	−0.06
16	1.83	1.78	1.00	−0.28
17	10.40	10.69	1.75	0.53
18	5.18	2.30	1.00	−0.32
19	11.52	3.09	1.00	−0.51
20	5.82	2.77	2.00	0.15
21	4.50	10.24	2.00	0.28
22	4.93	3.93	2.00	−0.05
23	19.88	7.72	2.00	−0.01
24	22.29	10.63	3.00	0.38
25	14.22	7.09	1.25	0.31
26	6.14	6.38	1.00	−0.07
27		6.55	1.00	0.08
28		9.41	2.00	0.26
29	3.66	1.78	1.25	0.30
30	1.96	2.31	2.50	0.09
31	12.37	2.38	1.00	0.01
32	2.14	2.76	2.50	0.29
33	1.35	4.07	1.00	−0.11

Note: AUC = area under the curve; FC = functional connectivity; Hippoc-DMN FC = correlation between time course in left hippocampal peak at −22, −32, and −4 (Montreal Neurological Institute; 10-mm sphere) and default mode network (DMN) whole-network time course; MRI = magnetic resonance imaging; TSST-C = Trier Social Stress Test for Children; VAS = visual analog scale.