Psychoneuroendocrinology 102 (2019) 68–78

Contents lists available at ScienceDirect

Psychoneuroendocrinology

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

Resting amygdala connectivity and basal sympathetic tone as markers of chronic hypervigilance

Olena Kleshchova[a,b], Jenna K. Rieder[a,b], Jack Grinband[c], Mariann R. Weierich[a,b,*]

[a] Hunter College, The City University of New York, 695 Park Avenue, New York, NY 10065, USA
[b] The Graduate Center, The City University of New York, 365 Fifth Avenue, New York, NY 10016, USA
[c] Department of Radiology and Neurology, Columbia University, 622 W 168th St, New York, NY 10032, USA

ARTICLE INFO

Keywords:
Trauma
Hypervigilance
Resting-state fMRI
Amygdala connectivity
Ventral anterior cingulate cortex
Salivary alpha-amylase

ABSTRACT

Background: Chronic hypervigilance, a state of sustained alertness and hyperarousal in the absence of threat, has been shown to predict poorer clinical outcomes post-trauma. An exaggerated and persistent amygdala alerting response to affective information has been proposed as a reactivity-based, and thus indirect, marker of hypervigilance. However, because chronic hypervigilance is a persistent rather than reactive state, it should be directly observable under resting-state conditions without the need for exposure to affectively charged stimuli.
Objective: We tested resting amygdala connectivity and basal sympathetic and hypothalamic-pituitary-adrenal axis activity as direct neural and neuroendocrine markers of chronic hypervigilance.
Participants: 24 trauma-exposed women (age $M = 22.9$, $SD = 5.5$) and 20 no-trauma controls (age $M = 21.1$, $SD = 3.2$).
Measures: Amygdala connectivity was measured using functional magnetic resonance imaging at rest and during viewing of novel and familiar affective scenes. Elevated amygdala connectivity during the viewing of novel scenes (exaggerated alerting response) and familiar scenes (persistent alerting response) was used as a reactivity-based index of hypervigilance. Resting amygdala connectivity and basal salivary alpha-amylase (sAA) and cortisol were tested as neural and neuroendocrine markers of hypervigilance, respectively.
Results: Compared to no-trauma controls, trauma-exposed women showed greater connectivity between the left amygdala and the ventral anterior cingulate cortex (vACC) both during affective processing and at rest. Exaggerated neural novelty response was associated with greater resting left amygdala-vACC connectivity and higher basal sAA, but not cortisol.
Conclusions: Greater synchronization of threat-detection circuitry in the absence of threat and basal sympathetic tone might serve as complementary resting-state markers of the cognitive and physiological components of chronic hypervigilance, respectively.

1. Introduction

Hypervigilance is a behavioral, cognitive, and physiological state of sustained hyperarousal and alertness for potential threat. The cognitive component of hypervigilance (i.e., increased alertness) is mediated by activation of threat-detection neural circuitry centered around the amygdala (e.g., Yoon and Weierich, 2016), whereas its physiological component (i.e., elevated arousal) is mediated by activation of the neuroendocrine stress systems (e.g., Pole, 2007). In potentially dangerous situations, hypervigilance is adaptive, as it facilitates threat detection when an actual threat appears and enables a prompt response by mobilizing necessary resources. However, when hypervigilance becomes a chronic state, characterized by unnecessary activation and failed downregulation of the alerting response in the ongoing absence of threat, it is psychologically and physiologically taxing and can impair quality of life. Exposure to traumatic events can lead to chronic hypervigilance even in safe environments (e.g., Kimble et al., 2013), which in turn contributes to the episodic onset and maintenance of other trauma-related symptoms and predicts poorer clinical outcomes in trauma survivors (Schell et al., 2004).

Despite the potential prognostic utility of chronic hypervigilance, it is typically assessed using self-report, which can be unreliable, and there are still no widely accepted objective and direct biological markers of this phenomenon. One previously proposed objective neural marker of chronic hypervigilance is exaggerated and persistent reactivity of the threat-detection circuitry (e.g., Yoon and Weierich, 2016,

* Corresponding author at: Department of Psychology, Hunter College CUNY, 695 Park Avenue, New York, NY, 10065, USA.
E-mail address: mariann.weierich@hunter.cuny.edu (M.R. Weierich).

https://doi.org/10.1016/j.psyneuen.2018.11.036
Received 17 August 2018; Received in revised form 16 November 2018; Accepted 24 November 2018

2017). Consistent with chronic post-trauma hypervigilance, trauma-exposed (TE) people show heightened amygdala reactivity not only to aversive and trauma-related information (e.g., Patel et al., 2012), but also to any novel – and thus potentially threat-related – information (e.g., van den Bulk et al., 2016). In addition, whereas the normative alerting amygdala response to novel information tapers rapidly with repeated exposure (e.g., Weierich et al., 2010), TE people show impaired amygdala habituation to repeated affective stimuli (e.g., van den Bulk et al., 2016).

Although exaggerated and persistent amygdala novelty response might be a clinically useful marker, it is a reactivity-based – and thus an indirect – measure, because an acute amygdala response to affective stimuli (reactivity) is taken as an index of sustained amygdala hyperactivity at baseline (chronic hypervigilance). In addition, measurement of amygdala novelty response relies upon presentation of affectively charged and potentially trauma-related information, and thus imposes additional burden on the already strained stress systems of trauma survivors. Therefore, objective and direct measurement of chronic hypervigilance requires an alternative to reactivity-based measures.

Given that chronic hypervigilance is a persistent rather than reactive state, it should be observable under resting-state conditions (i.e., in the absence of affective stimuli), for example, as specific patterns of resting amygdala connectivity. Although there is evidence of abnormal resting amygdala connectivity in TE people, the results are inconsistent, with reports of both weaker (e.g., Jin et al., 2014) and stronger (e.g., Thomason et al., 2015) resting connectivity of the amygdala and prefrontal cortex, as well as greater coupling of the amygdala and insula (e.g., Rabinak et al., 2011) in TE people compared to controls. In addition, it is not known whether resting amygdala connectivity differences in TE people underlie hypervigilance. As the amygdala is a key node of the threat-detection circuitry, abnormal resting amygdala connectivity in TE people might represent sustained activation of the neural alerting response that persists in the absence of threat, consistent with chronic hypervigilance. Therefore, the first objective of this study was to test trauma-related alterations in resting amygdala connectivity as a resting-state neural marker of chronic hypervigilance in TE people.

Given that a hypervigilant state includes both a cognitive component (increased alertness) and a physiological component (hyperarousal), a comprehensive model of chronic hypervigilance must account for both components. Consistent with chronic hyperarousal, TE people show abnormalities in basal function of the two neuroendocrine stress systems: the sympathetic nervous system (SNS) and the hypothalamic-pituitary-adrenal (HPA) axis. For example, people with post-traumatic stress disorder (PTSD) consistently show heightened basal sympathetic tone, indexed by elevated heart rate, blood pressure, startle reflex, and galvanic skin response (e.g., Pole, 2007). Basal SNS hyperactivity post-trauma also has been indexed by salivary alpha-amylase (sAA; e.g., Keeshin et al., 2015), an enzyme secreted by the salivary glands in response to increased central noradrenergic drive (e.g., Warren et al., 2017). SAA is a biomarker of sympathetic activity, which increases in response to physical and psychological stressors and is associated with stress-related increases in circulating norepinephrine (e.g., Ditzen et al., 2014) and physiological markers of SNS activity (e.g., Nater and Rohleder, 2009).

In contrast to reliable SNS hyperactivity, most evidence suggests HPA blunting in TE people, and especially those with a history of early-life trauma (e.g., Galatzer-Levy et al., 2017). Blunted HPA activity, indexed by lower basal and reactive cortisol, is common in stress-related disorders and might represent an allostatic protection against the deleterious effects of a prolonged stress response (e.g., Fries et al., 2005). Although basal measures of SNS and HPA dysregulation have been associated with trauma-related symptoms (e.g., Vigil et al., 2010), their utility as biomarkers of chronic hypervigilance has not been tested. Therefore, a second objective of this study was to test basal SNS and HPA function as resting-state neuroendocrine markers of chronic hypervigilance.

We tested resting amygdala connectivity and basal SNS and HPA activity as resting-state markers of the cognitive and physiological components of chronic hypervigilance, respectively. We hypothesized that, compared to no-trauma controls, TE people would show greater resting connectivity between the amygdala and other regions involved in threat detection, and weaker resting connectivity between the amygdala and regions involved in affect regulation. We also hypothesized that trauma-related resting-state abnormalities in amygdala connectivity would persist during affective processing and would be associated with an exaggerated and persistent neural novelty response to affective information (i.e., reactivity-based neural measure of hypervigilance). Finally, we hypothesized that TE people would show previously reported differences in basal SNS and HPA activity, and that these differences would be associated with an exaggerated and persistent neural novelty response to affective information.

2. Method

2.1. Participants

We recruited 24 trauma-exposed (TE) women (age $M = 22.9$, $SD = 5.5$) and 20 no-trauma control women (age $M = 21.1$, $SD = 3.2$) from a pool of undergraduate students. Trauma-exposed participants were recruited based on their responses to an online questionnaire which included items from the Life Events Checklist, a self-report of exposure to potentially traumatic events (Gray et al., 2004). Controls were specifically recruited from potential participants who did not endorse any trauma exposure on that measure. Trauma exposure was subsequently verified using the Criterion A of the PTSD module of the Diagnostic and Statistical Manual of Mental Disorders IV (event characterized by actual or perceived threat to life or physical integrity and accompanied by feelings of extreme fear, helplessness, or horror).

Five TE participants reported using prescribed medication (1 Prozac, 1 benzodiazepine, 1 unspecified birth control medication, 1 tramadol, 1 antihistamine and 1 unspecified prescription medication), as did one control participant (Wellbutrin and Lexapro). We included medication use as a nuisance covariate in subsequent analyses. All participants were right-handed and eligible for MRI. Demographic characteristics of our sample are presented in Table 1. We recruited an all-female sample to avoid confounding effects of sex on stress system activity (e.g., Kajantie and Phillips, 2006) and cortico-limbic connectivity (e.g., Kilpatrick et al., 2006).

2.2. Procedure

The study consisted of two sessions. Session 1 included a clinical interview, saliva collection, and completion of questionnaires. Session 2 was scheduled either several days later or on the day of Session 1 and included an MRI scan. We obtained informed consent at the beginning of Session 1 and fully debriefed the participants at the end of the study. The study protocol was approved by the Institutional Review Board and carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

2.2.1. Clinical interview

During Session 1, we conducted all modules of the Structured Clinical Interview for DSM-IV (SCID) to assess trauma exposure and symptoms of PTSD and other Axis I disorders. Clinical interviews were conducted by rigorously trained post-bacc and doctoral level laboratory personnel, each of whom had no fewer than 40 h of training. Reliability checks showed high rater consistency for individual SCID items (i.e., whether a symptom meets threshold or not).

Total number of PTSD symptoms in our sample ranged from 0 ($n = 4$) to 15 ($n = 1$), with a mean of 6.9 and a standard deviation of 4.8. Five of 24 TE women (20.8%) met the criteria for a provisional current DSM-IV PTSD diagnosis. We tested the effects of current PTSD

Table 1
Participants.

Variable	Trauma (n = 24)	Controls (n = 20)
Age in years, *M* (*SD*), range	22.9 (5.5), 18-37	21.1 (3.2), 18-29
Race/ethnicity, *n* (%)		
Asian/Pacific Islander	8.0 (33.3)	4.0 (20.0)
Black, non-Hispanic	4.0 (16.7)	3.0 (15.0)
Hispanic	1.0 (4.2)	2.0 (10.0)
White, non-Hispanic	5.0 (20.8)	10.0 (50.0)
Other	4.0 (16.7)	1.0 (5.0)
Multiple	2.0 (8.3)	0.0 (0.0)
Perceived Stress Scale, *M* (*SD*)	22.7 (6.6)**	16.5 (6.1)
STAI-S, *M* (*SD*)	46.2 (12.5)**	36.2 (10.7)
BDI II, *M* (*SD*)	16.0 (6.9)**	8.5 (6.0)
PANAS (state negative affect), *M* (*SD*)	9.2 (2.8)**	6.3 (1.9)
PANAS (state positive affect), *M* (*SD*)	11.9 (3.7)	13.2 (4.4)
Basal alpha-amylase (T1) in U/ml, *M* (*SD*)	55.1 (55.0)	53.8 (57.4)
Basal cortisol (T2) in µg/dl, *M* (*SD*)	0.27 (0.23)	0.35 (0.22)
Trauma type, *n* (%)		
Physical assault	10.0 (45.5)	N/A
Sexual assault	8.0 (36.4)	N/A
Motor vehicle accident	6.0 (27.3)	N/A
Sudden, unexpected death of loved one	6.0 (27.3)	N/A
Other serious accident	5.0 (22.7)	N/A
Fire/explosion	3.0 (13.6)	N/A
Life-threatening injury/illness	3.0 (13.6)	N/A
Witness violent death	2.0 (9.1)	N/A
Natural disaster	1.0 (4.5)	N/A
Other unwanted sexual experience	1.0 (4.5)	N/A
Caused serious injury/death of another	1.0 (4.5)	N/A
Other very stressful event	6.0 (27.3)	N/A
Years since the most recent trauma	3.1 (4.0)	N/A
Years since the earliest trauma	8.4 (5.4)	N/A
Total PTSD symptoms	6.9 (4.8)	N/A
Re-experiencing (Cluster B)	2.5 (1.6)	N/A
Avoidance & numbing (Cluster C)	2.6 (1.8)	N/A
Hyperarousal (Cluster D)	1.8 (1.9)	N/A
Current PTSD diagnosis, *n* (%)	5.0 (20.8)	N/A

** $p < 0.01$.

diagnosis in subsequent analyses.

2.2.2. Saliva collection

Participants were asked to refrain from eating, smoking, and drinking for one hour prior to the study. All participants had been awake for a minimum of one hour prior to the study session, ensuring that our time points did not overlap with the cortisol awakening response. We collected two saliva samples. All sessions were scheduled at 10am to control for diurnal variation in cortisol and salivary alpha-amylase. We collected the first sample immediately after informed consent at approximately 1005 h (time point T1), which provided a resting basal measure of sAA. We collected the second sample approximately 20 min later (time point T2), which provided a resting basal measure of cortisol, which takes ~20 min to reach the concentration that reflects the state of the system 20 min prior. In other words, sAA at 1005 h and cortisol 20 min later both index the state of the system at 1005 h. For each sample, the participant placed an oral swab (Salimetrics, LLC) under her tongue for two minutes and then placed the swab in a sealed plastic tube. Samples were stored in a −20C freezer until the time of analysis.

2.2.3. Questionnaires

After the interview, each participant completed questionnaires, measuring past and current affect, including the Perceived Stress Scale (PSS, Cohen et al.,1983), the Beck Depression Inventory II (BDI-II, Beck et al., 1996), the State-Trait Anxiety Inventory—State Version (STAI-S, Spielberger et al., 1983), and the Positive and Negative Affect Schedule (PANAS, Watson et al., 1988). The PSS is a 10-item measure of the degree to which life events over the past month are perceived as stressful, uncontrollable, and unpredictable. PSS scores above 20 are consistent with high perceived stress. The BDI-II is a 21-item measure of depressed mood over the past two weeks. BDI-II scores of 30 and above are consistent with severe depressed mood. The STAI-S is a 20-item measure of state anxiety. STAI-S scores of 40 and above are consistent with high state anxiety. Finally, the PANAS is a 20-item measure of state negative and positive affect.

2.2.4. MRI scan

The MRI scan included a 7-min sequence of structural scans and field maps, during which the participants acclimated to the scanner, followed by a 6.5-min resting-state scan. During the resting-state scan, we asked the participants to keep their eyes open and not to think about anything in particular. After the resting-state scan, the participants completed a 20-min image-viewing task. We selected the task images from a stimulus set that is currently being normed in our laboratory. The selected images depicted complex social and non-social scenes (rather than isolated people/animals/objects). The images were selected to reflect a range of affective responses to visual stimuli typically encountered in daily life. The selected scenes were thus characterized by a more restricted range of arousal and valence ratings than the International Affective Picture System. The valence and arousal of the selected scenes were rated on a 1–9 scale by an independent sample of 748 young adults (negative valence: $M = 2.61$, $SD = 1.02$, arousal: $M = 5.60$, $SD = 1.02$; neutral valence: $M = 5.59$, $SD = 0.84$, arousal: $M = 3.88$, $SD = 0.65$; and positive valence: $M = 6.85$, $SD = 0.86$, arousal: $M = 4.58$, $SD = 0.69$).

The task consisted of four runs. During each run, the participants viewed 60 full-color images of complex negative, positive, and neutral scenes (20 scenes in each valence category). The first two runs consisted of novel scenes, and the last two runs consisted of the same scenes as the first two runs, presented for the second time (familiar scenes). For each scene, the participants indicated via button press whether it was indoors or outdoors[1]. We optimized stimulus presentation order for the fast event-related design using the Optseq2 sequence optimization tool (https://surfer.nmr.mgh.harvard.edu/optseq/). Each image was presented for 3500 ms, followed by a fixation cross presented for a jittered inter-trial interval ranging from 2000 ms to 6500 ms. Each task run was 332 s long. The task was designed and presented using E-Prime software (Psychology Software Tools, Pittsburgh, PA).

2.2.5. MRI image acquisition

MRI data were acquired on a Siemens Magnetom Trio Tim 3 T MRI scanner with a 32-channel gradient head coil. A high-resolution T1-weighted image was acquired using a whole-brain MPRAGE sequence (TR/TE/flip angle = 2.17 s/4.33 ms/7°, FOV = 256 × 256mm^2, matrix = 256 × 256, 160 slices, voxel size = 1 × 1 × 1.2 mm^3). Functional images were acquired using an interleaved echo-planar T2*-weighted sequence (rest: TR/TE/flip angle = 2.0 s/30 ms/90°, FOV = 240 × 240mm^2, matrix = 64 × 64, 33 slices, voxel size = 3.75 × 3.75 × 3.80mm^3; task: TR/TE/flip angle = 2.0 s/30 ms/90°, FOV = 220 × 220mm^2, matrix = 64 × 64, 30 slices, voxel size = 3.44 × 3.44 × 4.0mm^3).

[1] Due to experimenter error, 9 TE women and 3 controls rated their arousal, instead of indicating if a scene was indoors or outdoors. As rating affect has been shown to reduce amygdala activation (Lieberman et al., 2007), we tested potential effects of task instructions on amygdala connectivity. Amygdala-vACC connectivity during either novel or familiar runs did not differ by task instructions in either group (all p-values > 0.05), and group differences in connectivity remained even after including task instructions as a covariate (see Supplementary Figure S1).

2.3. Data preprocessing

2.3.1. MRI data preprocessing

One TE participant did not complete the resting-state scan, and one other TE participant did not complete the fourth fMRI task run. Neuroimaging data were preprocessed and analyzed using FSL (version 5.0.6) and MATLAB (2015b) software packages. Structural images were skull-stripped using FSL's Brain Extraction Tool (Smith, 2002) and bias-field corrected improve registration using FSL's FAST tool (Zhang et al., 2001). Resting-state and task fMRI data were preprocessed and cleaned in a similar way, described below. The first 5 functional volumes were discarded to allow for magnetic equilibration, such that a total of 195 volumes (resting-state fMRI) and 166 volumes (per task run) per participant were included in the analyses. The resting and task functional data were motion corrected to the middle volume using FSL's MCFLIRT tool (Jenkinson et al., 2002). Mean displacement due to motion did not exceed 1 mm in any of the participants. The data were slice-timing corrected using Fourier-space time series phase-shifting, smoothed using a Gaussian kernel of FWHM = 5 mm, and resampled to 4 mm. We applied a 0.02 Hz high-pass filter to task fMRI data. We removed linear trends from resting-state fMRI data using a high-pass temporal filter of 0.001 Hz. We chose such a minimal high-pass to avoid eliminating any resting-state neural signal, which has been observed not only in the traditionally bandpass filtered 0.01-0.08 Hz range, but also at frequencies higher than 0.1 Hz (e.g., Boubela et al., 2013).

To remove the structured temporal noise, including physiological noise, hardware artifacts, and participant motion from both resting-state and task fMRI data, we used FSL's MELODIC (Beckmann and Smith, 2004) which decomposes the fMRI time series into automatically estimated and variance normalized spatially independent components that represent neural signal and structured noise. Each independent component was visually inspected and classified as either artifact or neural signal, based on the criteria described by Kelly et al. (2010). Unique variance contributed by the artifactual components and the 24 motion parameters was regressed out of the preprocessed functional data using FMRIB's ICA-based Xnoiseifier (Griffanti et al., 2014). This approach has the advantage of selectively removing noise components while preserving the neural signal and is therefore preferable to traditional indiscriminate cleanup techniques, such as bandpass filtering or censoring of high-motion volumes (e.g., Smith et al., 2013). In addition, data-driven ICA-based cleanup has been shown to increase the reproducibility of resting connectivity results (Griffanti et al., 2016).

Functional images were registered to the structural image using boundary-based registration and to the standard-space MNI152 T1 2 mm template via 12-degree-of-freedom transformation using FSL's FLIRT (Jenkinson et al., 2002).

2.3.2. Immunoassays of salivary biomarkers

Samples were assayed for salivary alpha-amylase (sAA) using Salimetrics kinetic reaction assay kits. The assay utilizes a chromogenic substrate, 2-chloro-p-nitrophenol linked to maltotriose. The amount of sAA present in each sample is directly proportional to the increase in absorbance. The intra- and inter-assay coefficients of variation for these kits are less than 7.5% and 6%, respectively.

Samples were assayed for cortisol using Salimetrics enzyme immunoassay kits. The assay utilizes a microtiter plate coated with monoclonal anti-cortisol antibodies. Cortisol in samples and standards competes with cortisol conjugated with peroxidase for the antibody binding sites. The amount of cortisol enzyme conjugate detected is inversely proportional to the amount of cortisol present in the sample. The intra- and inter-assay coefficients of variation for these kits are less than 7% and 10%, respectively. All assays were conducted in-house by laboratory personnel.

2.4. Data analysis

2.4.1. Resting amygdala connectivity analysis

To test trauma-related differences in resting amygdala connectivity, we used whole-brain seed-based connectivity and region-of-interest (ROI) analyses. For whole-brain functional connectivity analyses, we chose the left and right amygdalae as seed regions. Anatomical amygdala masks were created using the Harvard-Oxford subcortical probability atlas. The masks were inverse-transformed from the standard MNI152 space to individual functional space using FLIRT. Mean amygdala time courses were then extracted from the cleaned functional data and used as regressors in two separate first-level mass univariate regressions – one for each hemisphere – implemented using FSL's FEAT (version 6.0). FSL's FILM was used to correct for temporal autocorrelation (Woolrich et al., 2001). To test group differences in resting amygdala connectivity in TE women and controls, we conducted separate robust group-level mixed-effects analyses for each amygdala seed using FSL's FLAME 1 with automatic outlier de-weighting (Beckmann et al., 2003; Woolrich, 2008). We used cluster-extent based inference and corrected for multiple comparisons using Gaussian random field theory implemented in FSL. Statistical maps were thresholded using the cluster-determining threshold (CDT) of $p < 0.005$ and an FWE-corrected cluster significance threshold of $p = 0.05$.

To test resting amygdala connectivity as a marker of chronic hypervigilance, we tested associations between resting amygdala connectivity (mean parameter estimates from the ROI; Fig. 1) and trauma-related symptoms, amygdala connectivity during novelty processing (reactivity-based measure of hypervigilance), and basal sAA and cortisol (candidate neuroendocrine markers of hypervigilance).

2.4.2. Analysis of amygdala connectivity during novel and familiar runs

To determine whether alterations in resting amygdala connectivity represented a stable characteristic in TE women, we tested group differences in amygdala connectivity during processing of novel and familiar affective information using whole-brain seed-based connectivity and ROI analyses. This analysis was confirmatory and served the purpose of confirming that the identified region was a marker of hypervigilance, by testing the relation between amygdala-vACC connectivity at rest and during novelty processing (a reactivity-based marker of hypervigilance).

We used FSL's FEAT to compute seed-based amygdala connectivity during novel and familiar runs separately, while regressing out the mean neural response to task stimuli and motor responses (i.e., button presses). For each task run, we constructed a separate model with five regressors: stimulus onsets for negative, positive, and neutral trials, onsets of motor responses, and mean amygdala time course across the run. As a result, each participant had four amygdala connectivity statistical maps, one for each run, that captured the unique variance accounted for by the amygdala time course. These first-level maps were then fed into two separate second-level fixed-effects analyses, testing for common connectivity patterns across the two novel and the two familiar runs separately. We then used the resulting second-level statistical maps to extract mean parameter estimates from the cluster that showed group differences in resting connectivity with the amygdala (Fig. 1). We tested heightened amygdala connectivity during novel runs (exaggerated alerting response to novelty) and impaired habituation of amygdala connectivity (persistent alerting response to familiar information) in TE women *vs.* controls using independent-samples *t*-tests.

Although our resting-state and task fMRI data were collected from the same participants and are not independent in that sense, our analyses do not suffer from circular assumptions. The use of the same sample is problematic only when the data used for ROI specification and for inferential statistics have non-independent noise (Kriegeskorte et al., 2009, Supplementary Information pp.18–19). However, we selected the ROI and conducted inferential analyses on two independent sets of fMRI scans (resting scan and task scans) that were collected at

different times. As such, the noise distributions of the resting and task scans are independent of one another, thus avoiding the circularity problem.

The use of a functional localizer scan for ROI selection is a widely used technique in neuroimaging and is recommended for ensuring ROI independence (e.g., Kriegeskorte et al., 2009; Poldrack and Mumford, 2009). Our resting data analysis was exploratory and served the purpose of identifying a candidate resting-state neural marker of tonic hypervigilance. It can therefore be considered analogous to an independent functional localizer scan recommended for identification of independent ROIs. Our task data analysis, on the other hand, was confirmatory, that is it served the purpose of confirming that the identified region was indeed a marker of hypervigilance, by testing the relation between resting connectivity between the amygdala and the vACC with their connectivity during novelty processing (a reactivity-based marker of hypervigilance). As such, our resting and task data analyses aimed to answer two distinct questions. First, which brain regions show differences in functional connectivity with the amygdala in TE people vs. controls at rest? Second, do these same regions also show differences in amygdala connectivity during novelty processing in the same participants?

2.4.3. Analysis of salivary biomarkers

As the main goal of this study was to test candidate basal salivary markers of tonic hypervigilance, we used sAA at the first collection time point (T1), which reflects SNS activity at the time of saliva collection, and in this case just after the participant signed the consent form and had a few minutes to acclimate to the laboratory environment. We used salivary cortisol at the second collection time point (T2) as a measure of basal cortisol, because salivary cortisol reflects HPA axis activity ~20 min prior to sample collection, such that this time point reflected basal levels just after consent.

We applied a log transformation to raw values of salivary biomarkers, because the data were positively skewed. All subsequent statistical tests were conducted on transformed values. To test basal sAA and cortisol as neuroendocrine biomarkers of hypervigilance, we tested associations between sAA and cortisol, trauma-related symptoms, and amygdala connectivity during novel and familiar runs (reactivity-based measure of hypervigilance).

3. Results

3.1. Self-report measures

TE women and controls did not differ in age, $t(42) = 1.27$, $p = 0.210$, $d = 0.39$. Because age was associated with higher perceived stress (PSS; $r = 0.35$, $p = 0.022$) and depressed mood (BDI; $r = 0.29$, $p = 0.056$), all subsequent associations with PSS and BDI scores were tested with age as a covariate. Compared to controls, TE women reported higher perceived stress (PSS), $F(1,40) = 7.63$, $p = 0.009$, partial $\eta^2 = 0.15$ (without age as a covariate: $t(41) = 3.18$, $p = 0.003$); depressed mood (BDI), $F(1,40) = 11.45$, $p = 0.002$, partial $\eta^2 = 0.21$ (without age as a covariate: $t(41) = 3.75$, $p < 0.001$); state anxiety (STAI-S), $t(41) = 2.78$, $p = 0.008$, $d = 0.86$; and state negative affect (PANAS-Negative), $t(41) = 3.97$, $p < 0.001$, $d = 1.24$. Group differences remained significant even when people with current PTSD were excluded: PSS, $F(1,35) = 5.16$, $p = 0.029$, partial $\eta^2 = 0.12$; BDI, $F(1,35) = 7.54$, $p = 0.009$, partial $\eta^2 = 0.17$; STAI-S, $t(36) = 2.79$, $p = 0.008$, $d = 0.91$; and PANAS-Negative, $t(36) = 4.42$, $p < 0.001$, $d = 1.44$. PTSD symptoms were not associated with PSS, BDI, STAI-S, or PANAS scores (all p-values > 0.05). As depression has been linked to altered amygdala-prefrontal connectivity (e.g., Pezawas et al., 2005), we tested the associations between depressed mood and amygdala connectivity. When there was a statistically significant association, we included depressed mood as a covariate in analyses of amygdala connectivity.

We did not include state anxiety or perceived stress as covariates in the analyses, as these two factors overlap with our construct of interest, trauma-related hypervigilance. Both state anxiety and hypervigilance involve a high-arousal, negatively valenced physiological state that has been associated with increased activation and connectivity of affective circuitry such as the salience network (e.g., Hermans et al., 2011; Seeley et al., 2007; Simpson et al., 2001). Thus, including state anxiety as a covariate in analyses of amygdala connectivity would result in removal of variance of interest.

3.2. Resting amygdala connectivity

TE women showed greater resting connectivity between the left amygdala and the perigenual and subgenual anterior cingulate cortex (henceforth referred to as the ventral ACC or vACC) relative to controls (Fig. 1; medication use as a covariate, minimal significant cluster size $k = 311$; cluster size $k = 366$; maximal Z-score = 3.98; MNI coordinates of peak voxel in mm: x = 14, y = 46, z = -4; Brodmann's Area 10). The group difference in left amygdala-vACC resting connectivity remained after exclusion of the 4 people who met criteria for PTSD: $t(37) = 3.47$, $p = 0.001$, $d = 1.11$; and after including depressed mood as a covariate: $F(1,39) = 14.12$, $p < 0.001$, partial $\eta^2 = 0.27$. Compared to no-trauma controls, TE women did not show weaker connectivity between the left amygdala and any other regions. Resting amygdala-vACC connectivity was not associated with the total number of PTSD symptoms or any cluster separately (all p-values > 0.05). The groups did not differ in resting connectivity of the right amygdala. Therefore, we conducted all subsequent task fMRI analyses using the left amygdala as the seed region.

3.3. Amygdala-vACC connectivity during processing of novel and familiar affective information

3.3.1. Group differences in amygdala-vACC connectivity during affective processing

To determine whether increased amygdala-vACC connectivity represented a stable characteristic in TE people, we tested group differences in amygdala-vACC connectivity during processing of novel and familiar affective scenes using separate independent-samples t-tests for novel and familiar runs (Bonferroni-corrected $\alpha = 0.05/2 = 0.025$). Compared to controls, TE women showed greater amygdala-vACC connectivity during both novel, $t(42) = 2.43$, $p = 0.020$, $d = 0.76$; no PTSD: $t(37) = 2.55$, $p = 0.015$, $d = 0.81$; medication use as a covariate: $F(1,41) = 5.48$, $p = 0.024$, partial $\eta^2 = 0.12$; and familiar runs, $t(41) = 4.40$, $p < 0.001$, $d = 1.35$; no PTSD: $t(36) = 3.94$, $p < 0.001$, $d = 1.28$; medication use as a covariate: $F(1,40) = 25.2$, $p < 0.001$, partial $\eta^2 = 0.36$ (Fig. 2). Depressed mood was associated with amygdala-vACC connectivity during familiar runs ($\rho = 0.42$, $p = 0.006$), but not during novel runs ($p > 0.05$). In addition, age was associated with amygdala-vACC connectivity during familiar runs ($\rho = 0.36$, $p = 0.019$), but not during rest or during novel runs (both p-values > 0.05). Therefore, we tested all subsequent associations with amygdala-vACC connectivity during familiar runs with and without depressed mood and age as covariates. Group differences in amygdala-vACC connectivity during familiar runs remained significant even after we included depressed mood as a covariate: $F(1,39) = 8.99$, $p = 0.005$, partial $\eta^2 = 0.19$, and after we included age as a covariate: $F(1,40) = 16.3$, $p < 0.001$, partial $\eta^2 = 0.29$. Amygdala-vACC connectivity during either novel or familiar runs was not associated with the total number of PTSD symptoms or any symptom cluster separately (all p-values > 0.05).

3.3.2. Reactivity to novelty and habituation of amygdala-vACC connectivity

To test the hypothesis that increased amygdala-vACC connectivity is part of the neural alerting response to novelty, we compared amygdala-vACC connectivity during novel *vs.* familiar runs in each group

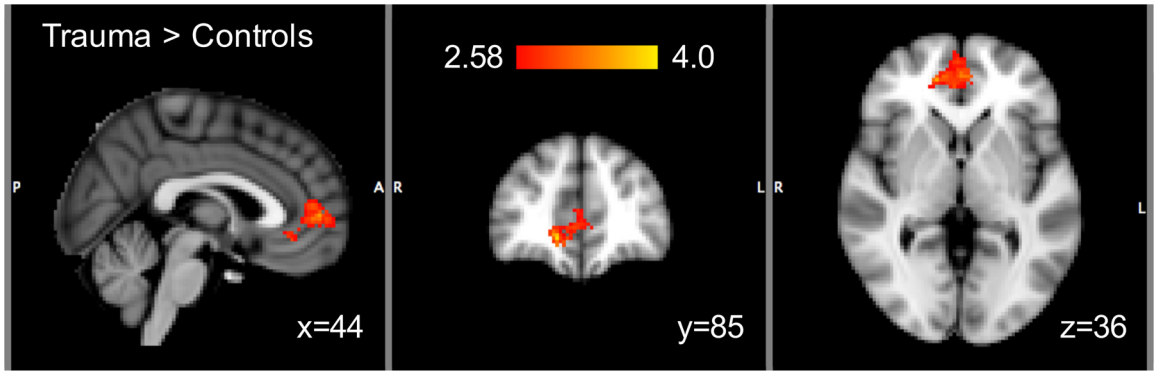

Fig. 1. Group difference in resting amygdala connectivity. Trauma-exposed women showed greater resting connectivity between the left amygdala and the ventral anterior cingulate cortex (vACC) than no-trauma controls (MNI standard space; coordinates in voxels; colorbar represents Z-scores; cluster-forming threshold: $p < 0.005$; FWE-corrected cluster significance: $p = 0.05$; minimal significant cluster size: $k = 311$).

separately using paired-sample *t*-tests and repeated-measures ANOVAs (Bonferroni-corrected $\alpha = 0.05/2 = 0.025$). Consistent with a normative novelty response, controls showed greater amygdala-vACC connectivity during novel runs compared to familiar runs (Fig. 2), $t(19) = 1.88$, $p = 0.075$, $d = 0.33$, main effect of novelty with age as a covariate: $F(1,17) = 8.36$, $p = 0.010$, partial $\eta^2 = 0.33$, main effect of novelty with depressed mood as a covariate: $F(1,15) = 7.89$, $p = 0.013$, partial $\eta^2 = 0.34$. In contrast, TE women showed no difference in amygdala-vACC connectivity during novel *vs.* familiar runs, consistent with impaired habituation, $t(22) = -0.63$, $p = 0.536$, $d = -0.24$; main effect of novelty with age as a covariate: $F(1,20) = 1.94$, $p = 0.179$, partial $\eta^2 = 0.09$; main effect of novelty with depressed mood as a covariate: $F(1,19) = 3.44$, $p = 0.079$, partial $\eta^2 = 0.15$.

3.3.3. Amygdala-vACC connectivity during novelty processing and at rest

To test resting amygdala-vACC connectivity as a neural marker of hypervigilance, we conducted bivariate Spearman's rank correlations between amygdala-vACC connectivity at rest and during novel and familiar runs (reactivity-based neural measures of hypervigilance) (Bonferroni-corrected $\alpha = 0.05/2 = 0.025$). Resting amygdala-vACC connectivity was associated with amygdala-vACC connectivity during novel runs ($\rho = 0.39$, $p = 0.010$; no PTSD: $\rho = 0.42$, $p = 0.008$; with medication use as a covariate: $F(1,40) = 8.43$, $p = 0.006$, partial $\eta^2 = 0.17$; Fig. 4) and familiar runs ($\rho = 0.36$, $p = 0.019$; no PTSD: $\rho = 0.46$, $p = 0.004$; with depressed mood as a covariate: $F(1,38) = 5.52$, $p = 0.024$, partial $\eta^2 = 0.13$; with medication use as a covariate: $F(1,39) = 5.30$, $p = 0.027$, partial $\eta^2 = 0.12$; with age as a covariate: $F(1,39) = 8.06$, $p = 0.007$, partial $\eta^2 = 0.17$). When we tested amygdala-vACC connectivity during novel and familiar runs as two separate predictors of resting amygdala-vACC connectivity using multiple regression to control for shared variance, only connectivity during novel runs predicted resting connectivity ($F(1,39) = 6.14$, $p = 0.018$, partial $\eta^2 = 0.14$). Amygdala-vACC connectivity during novel runs was associated with resting amygdala-vACC connectivity even when subject to robust regression ($F(1,41) = 7.25$, $p = 0.010$, Adj. $R^2 = 0.13$), suggesting that this relation was not driven by influential values.

3.4. Effects of head motion on connectivity estimates

As head motion has been shown to bias estimates of functional connectivity, we tested potential effects of head motion indexed by mean framewise displacement (FD) on amygdala-vACC connectivity. Our participants showed little in-scanner head motion, which did not exceed 0.016 mm on average (Rest: Total sample $M = 0.016$, $SD = 0.005$; TE $M = 0.016$, $SD = 0.006$; Controls $M = 0.015$, $SD = 0.004$; Novel runs: Total sample $M = 0.015$, $SD = 0.004$; TE $M = 0.015$, $SD = 0.003$; Controls $M = 0.015$, $SD = 0.004$; Familiar runs: Total sample $M = 0.016$, $SD = 0.005$; TE $M = 0.016$, $SD = 0.005$; Controls $M = 0.016$, $SD = 0.005$).

However, given that even micro-movements can bias functional connectivity, we tested group differences in mean FD using independent-samples *t*-tests. TE people did not differ from no-trauma controls in mean FD (Rest: $t(41) = 0.41$, $p = 0.682$, $d = 0.13$; Novel runs: $t(42) = -0.26$, $p = 0.794$, $d = -0.08$; Familiar runs: $t(42) = 0.03$,

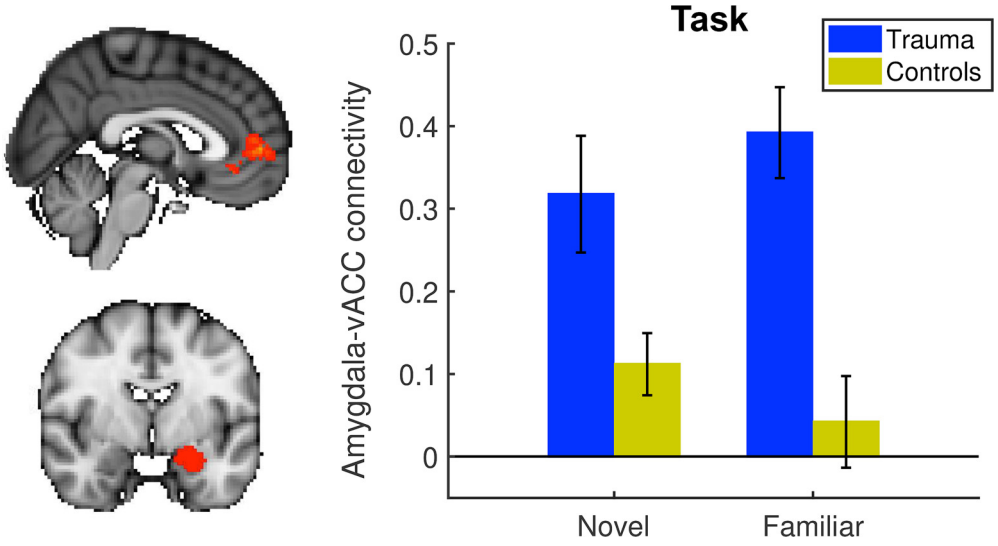

Fig. 2. Group differences in the neural alerting response, indexed by functional connectivity between the left amygdala and the ventral anterior cingulate cortex (vACC) during novelty processing. Trauma-exposed women showed greater amygdala-vACC connectivity during viewing of novel and familiar affective scenes (exaggerated and persistent neural novelty response, respectively) and impaired habituation of amygdala-vACC connectivity. Amygdala-vACC connectivity was greater during novel *vs.* familiar runs in no-trauma controls only. Bars represent mean parameter estimates (unstandardized betas; arbitrary units) extracted from the vACC region (top left panel). Error bars represent standard errors.

$p = 0.974$, $d = 0.01$). We also tested associations between mean FD and amygdala-vACC connectivity during rest and task scans using Spearman's correlations. FD was not correlated with amygdala-vACC connectivity (Rest: $\rho = 0.15$, $p = 0.335$; Novel runs: $\rho = -0.01$, $p = 0.942$; Familiar runs: $\rho = -0.12$, $p = 0.463$). These results suggest that our preprocessing pipeline was successful in removing motion artifacts, and that head motion is not likely to have had an effect on our connectivity estimates or to drive the observed group differences in amygdala connectivity.

3.5. Salivary biomarkers

To confirm previously reported alterations in basal neuroendocrine stress system activity post-trauma, we compared basal sAA and cortisol in TE women *vs.* controls using independent-samples *t*-tests. Twenty-two TE participants and 18 controls provided saliva samples with adequate volume for sAA assay. The groups did not differ in basal sAA, $t(38) = 0.08$, $p = 0.938$, $d = 0.02$ (Fig. 3). Twenty-two TE participants and 19 controls provided saliva samples with adequate volume for cortisol assay. Basal cortisol was lower in the TE group ($d = 0.44$), although the group difference did not reach statistical significance, $t(39) = 1.40$, $p = 0.170$ (Fig. 3). Neither analyte was correlated with the total number of PTSD symptoms or any symptom cluster separately (all *p*-values > 0.05).

To test basal sAA and cortisol as neuroendocrine biomarkers of hypervigilance, we tested the effect of trauma exposure on associations between the salivary biomarkers and (1) resting amygdala-vACC connectivity (candidate resting-state neural marker of hypervigilance) and (2) amygdala-vACC connectivity during novel and familiar runs (reactivity-based neural measures of hypervigilance) using robust regression to mitigate the effects of potentially influential values (Bonferroni-corrected $\alpha = 0.05/6 = 0.008$). There was no main effect of basal cortisol and no interaction between trauma exposure and basal cortisol on amygdala-vACC connectivity either at rest or during novel or familiar runs. There was a main effect of trauma exposure ($F(1,35) = 7.63$, $p = 0.009$, partial $\eta^2 = 0.18$) and an interaction between trauma exposure and basal sAA ($F(1,35) = 5.22$, $p = 0.028$, partial $\eta^2 = 0.13$) on resting amygdala-vACC connectivity (full model: $F(4,35) = 4.39$, $p = 0.01$, Adj. $R^2 = 0.21$; Fig. 4). The model was significant after we excluded participants with PTSD ($F(4,32) = 4.78$, $p = 0.007$, Adj. $R^2 = 0.25$). There was no main effect of medication use ($F(1,34) = 0.33$, $p = 0.567$, partial $\eta^2 < 0.01$) or depressed mood ($F(1,34) = 0.16$, $p = 0.691$, partial $\eta^2 < 0.01$).

There was a main effect of trauma exposure ($F(1,36) = 5.62$, $p = 0.023$, partial $\eta^2 = 0.14$), a main effect of basal sAA ($F(1,36) = 5.47$, $p = 0.025$, partial $\eta^2 = 0.13$), and an interaction between trauma exposure and basal sAA ($F(1,36) = 4.13$, $p = 0.049$, partial $\eta^2 = 0.10$) on amygdala-vACC connectivity during novel runs (full model: $F(4,36) = 5.12$, $p = 0.005$, Adj. $R^2 = 0.24$; Fig. 4). The model remained significant after we excluded participants with PTSD ($F(4,32) = 5.67$, $p = 0.003$, Adj. $R^2 = 0.29$). There was no main effect of medication use ($F(1,35) = 0.16$, $p = 0.692$, partial $\eta^2 < 0.01$) or depressed mood ($F(1,35) = 0.51$, $p = 0.478$, partial $\eta^2 = 0.01$).

There was no main effect of basal sAA ($F(1,35) = 1.78$, $p = 0.191$, partial $\eta^2 = 0.05$) and no interaction between trauma exposure and basal sAA ($F(1,35) = 0.02$, $p = 0.879$, partial $\eta^2 < 0.01$) on amygdala-vACC connectivity during familiar runs.

4. Discussion

4.1. Greater amygdala-vACC connectivity as a sustained neural alerting response

Trauma-exposed (TE) women showed greater resting connectivity between the left amygdala and the ventral anterior cingulate cortex (vACC), which are critically implicated in threat detection (e.g., Liddell et al., 2005) and regulation of the behavioral and neuroendocrine responses to threat (e.g., Herman et al., 2012; Vogt and Derbyshire, 2009). Moreover, in TE women, greater amygdala-vACC connectivity was present both during processing of affective information and at rest. These results are consistent with previous reports of greater amygdala-vACC connectivity in TE people at rest (e.g., Thomason et al., 2015) and during affective tasks, such as symptom provocation (e.g., Osuch et al., 2008) and recall of negative autobiographical memories (St. Jacques et al., 2011). Similarly, in normative samples, increased amygdala-vACC connectivity has been observed during various stress-related tasks, including threat anticipation (Gold et al., 2015), response to subliminal threat (e.g., Carlson et al., 2009), and after acute stress (e.g., Hermans et al., 2011), suggesting that greater amygdala-vACC connectivity might reflect a higher-arousal state that is associated with hyper-reactivity to actual threat-relevant information and hypervigilance for potential (but not present) threat.

Persistence of greater amygdala-vACC connectivity in TE women during affective processing and at rest suggests a fairly stable characteristic, which is consistent with a chronic state. Such stability is consistent with previous studies that demonstrated temporal stability and reproducibility of individual differences in functional connectivity identified through resting-state fMRI (e.g., Chen et al., 2015; Choe et al., 2015; Shah et al., 2016; Xu et al., 2016). Stable connectivity differences permit accurate identification of individuals (e.g., Finn

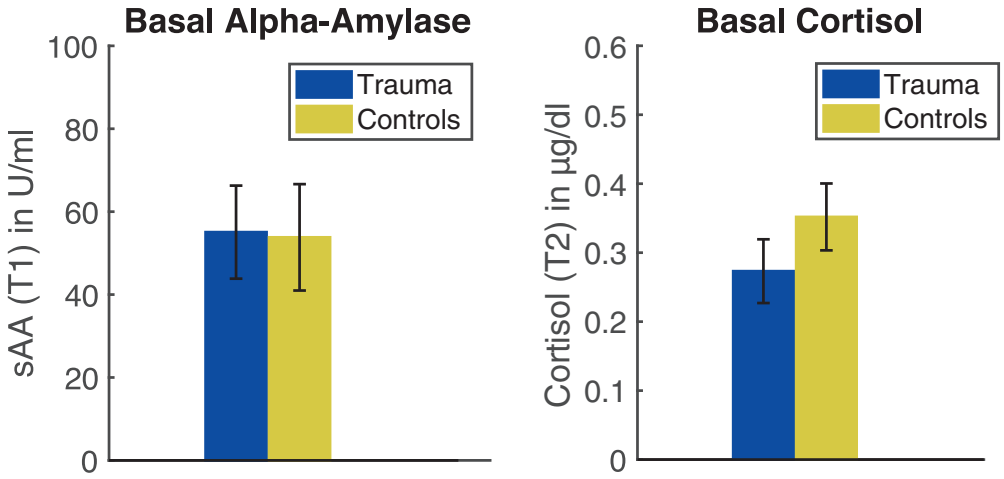

Fig. 3. Basal neuroendocrine biomarkers by group. Basal salivary alpha-amylase did not differ by group. Trauma-exposed women showed a non-significant trend ($p = 0.170$, $d = 0.44$) towards lower basal cortisol compared to no-trauma controls. Error bars represent standard errors.

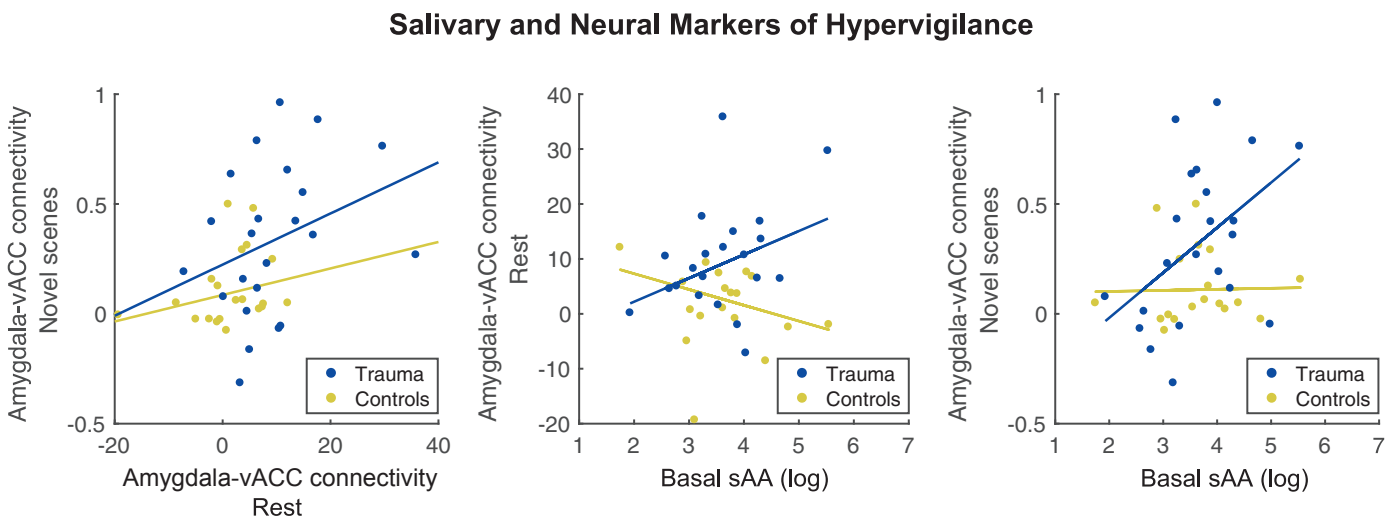

Fig. 4. Greater resting connectivity between the left amygdala and the ventral anterior cingulate cortex (vACC) is associated with greater neural alerting response to novelty (left panel) and higher basal sympathetic activity in trauma-exposed women (middle panel). Higher basal sympathetic activity is also associated with greater neural alerting response to novelty in trauma-exposed women only (right panel). Amygdala-vACC connectivity represents mean parameter estimates (unstandardized betas; arbitrary units) extracted from the vACC region.

et al., 2015), and predict individual differences in behavior (e.g., Magnuson et al., 2015), task performance (e.g., Tavor et al., 2016), and responsiveness to treatment (e.g., Drysdale et al., 2017), and the associations between resting-state connectivity and behavioral measures are a stable characteristic, as well (e.g., Touroutoglou et al., 2015).

Evidence from animal research and human neuroimaging studies suggests that the amygdala-vACC circuit is part of the innate neural alarm system, a subcortico-cortical network involved in automatic threat detection (e.g., Liddell et al., 2005). Within this network, the amygdala is thought to evaluate the motivational and affective significance of incoming sensory information (e.g., detect potential threat), and feed its affective assessments (e.g., danger signals) to the vACC (e.g., Stein et al., 2007). The vACC in turn initiates an orienting response to biologically or perceptually salient stimuli and modulates amygdala activity in a top-down manner. Neuroanatomical support for this theory comes from evidence of positive intrinsic functional coupling (e.g., Roy et al., 2009) and reciprocal excitatory connections between the vACC and the amygdala (e.g., Stefanacci and Amaral, 2002). In addition, the amygdala exerts a strong bottom-up influence on the vACC during affective processing (Stein et al., 2007), possibly relaying the affective significance of incoming stimuli. Thus, greater synchronization of amygdala and vACC activity in TE people might reflect increased stimulation of the vACC by the amygdala as part of an exaggerated neural alerting response. This interpretation is consistent with prior evidence of stronger direct influences of the amygdala on the vACC in people with PTSD during symptom provocation (Gilboa et al., 2004). Persistence of amygdala-vACC connectivity at rest suggests sustained activation of the neural alerting response even in the absence of threat, and this activation might underlie chronic trauma-related hypervigilance. Consistent with this interpretation, greater resting amygdala-vACC connectivity has been associated with perceived chronic stress (Taren et al., 2015) and faster orienting to threat (Carlson et al., 2013).

4.2. Exaggerated and persistent novelty response as a reactivity-based index of hypervigilance

In no-trauma controls, amygdala-vACC connectivity was greater in response to novel compared to familiar information. This result is consistent with the well-established role of the amygdala in novelty detection. As part of its threat detection function, the amygdala shows a fast and automatic alerting response to novel stimuli, which habituates upon repeated stimulus presentation (e.g., Weierich et al., 2010). The vACC might also be part of novelty detection circuitry, as it shows evidence of increased reactivity to novelty (e.g., Berns et al., 1997; Weierich et al., 2010) and habituation (Williams et al., 2006). Thus, an initial increase in amygdala-vACC connectivity might be part of a normative alerting response to novelty, which is followed by a decrease in connectivity as part of normative habituation.

In TE women, however, amygdala-vACC connectivity did not differ during processing of novel *vs.* familiar information and was consistently higher than in controls, suggesting an exaggerated and persistent neural alerting response. These results align with previously documented hyper-reactivity to novelty and impaired habituation in TE people, which might reflect chronic trauma-related hypervigilance (e.g., Yoon and Weierich, 2016; 2017). For example, greater trauma symptom severity is associated with heightened autonomic (e.g., Hendrickson and Raskind, 2016) and neural reactivity to novel information (e.g., van den Bulk et al., 2016). Similarly, trauma exposure and trauma-related symptoms have been associated with delayed habituation of the autonomic (e.g., Hendrickson and Raskind, 2016) and amygdala responses to affective information (e.g., Yoon and Weierich, 2017). Finally, a temperamental tendency to avoid novel stimuli and environments also has been associated with heightened amygdala reactivity to novel stimuli (Schwartz et al., 2003) and impaired amygdala habituation (e.g., Blackford et al., 2012). Thus, an indiscriminately sustained increase in amygdala-vACC connectivity during processing of novel but also familiar information might index an exaggerated and persistent neural alerting response, indicative of chronic hypervigilance.

Consistent with our hypothesis, an exaggerated neural alerting response to novelty, indexed by amygdala-vACC connectivity, was associated with greater resting amygdala-vACC connectivity, supporting the proposed role of resting connectivity as a resting-state neural marker of hypervigilance. We speculate that greater synchronization of resting amygdala and vACC activity in TE women might reflect sustained activation of the neural alerting response and underlie chronic trauma-related hypervigilance, which in turn might prime threat-detection circuitry for an exaggerated and persistent alerting response to novelty.

4.3. Elevated basal sympathetic tone as a physiological marker of chronic hypervigilance

TE women had lower basal cortisol than controls, consistent with the well-documented post-trauma HPA blunting (e.g., Galatzer-Levy

et al., 2017). However, basal cortisol was not associated with a hypervigilant neural response to either novel or familiar information, suggesting that it is not a reliable endocrine marker of trauma-related hypervigilance. Counter to previous reports of elevated basal sympathetic activity (e.g., Pole, 2007), including activity indexed by salivary alpha-amylase (sAA; e.g., Vigil et al., 2010), TE women as a group did not show greater sAA compared to controls. This result might be attributed to the fact that our TE sample was high-functioning and represented the moderate range of trauma symptoms.

However, basal sympathetic tone varied among our TE women (consistent with general individual differences and the considerable variability in symptoms among trauma survivors), such that TE women with the highest basal sympathetic activity showed a hypervigilant neural response to novelty and at rest. This result is consistent with previous associations between trauma-related hyperarousal symptoms and noradrenergic hyperactivity, indexed by increased basal sympathetic tone (e.g., Keeshin et al., 2015; Pole, 2007) and central norepinephrine neurotransmission (e.g., Hendrickson and Raskind, 2016). Similarly, trauma-related hyperarousal and re-experiencing symptoms can be induced via pharmacologic stimulation of brain norepinephrine release and respond well to treatment with drugs that block norepinephrine neurotransmission (e.g., Hendrickson and Raskind, 2016). In addition, trait hypervigilance has been associated with autonomic hyperarousal and sympathetic hyper-reactivity (e.g., Williams et al., 2009).

The association between basal sympathetic tone and amygdala-vACC connectivity during affective processing and at rest is not surprising, as the amygdala and the vACC are key regions of the central autonomic network (e.g., Beissner et al., 2013). Both the amygdala (e.g., Herman et al., 2012) and the vACC (e.g., Vogt and Derbyshire, 2009) activate the autonomic and endocrine responses to psychological stressors, such as unfamiliar situations and environments, via their projections to effector nuclei in the limbic system, hypothalamus, and brainstem. In rodents, electrical stimulation of the amygdala or the animal homolog of the vACC and the adjacent ventral medial prefrontal cortex elicits sympathetic and HPA activation, promotes anxiety-like behavior, and facilitates subsequent fear learning (e.g., Bissière et al., 2008). Conversely, lesions or transient inactivation of the amygdala or the vACC region that has direct projections to the amygdala reduce the HPA stress response (e.g., Radley et al., 2006), impair fear acquisition, and inhibit conditioned cardiovascular and behavioral responses to stress (e.g., Bissière et al., 2008). Taken together, the literature and our results suggest that elevated sympathetic tone observed in some TE people, which predicts hypervigilant responses to novelty, might stem from functional abnormalities in the amygdala-vACC circuit that underlie sustained activation of the neural alerting response.

Our results also align with our previous research (e.g., Yoon and Weierich, 2016) and other work (e.g., Keeshin et al., 2015; Vigil et al., 2010) suggesting that sAA might serve as a salivary biomarker of neural hypervigilance and autonomic hyperarousal. As a resting-state biomarker, basal sAA has a number of advantages over traditional measures of hypervigilance, such as self-report and potentiated physiological reactivity to affective stimuli. For example, basal sAA can be measured at rest without imposing additional burden on the stress system and might be more reliable and objective than self-report, as it indexes a hypervigilant brain state (i.e., exaggerated neural novelty response).

Our results also suggest that, similar to other trauma-related symptoms, the severity and duration of chronic hypervigilance varies among TE people (hence the absence of a group difference in basal sympathetic tone), such that hypervigilance might be greatest in people with elevated basal SNS activity and sustained synchronization of threat-detection circuitry. We speculate that our candidate basal markers of hypervigilance might be consistently elevated in a clinical sample of trauma survivors – a promising avenue for future work.

4.4. Limitations

Our study has several limitations. First, due to our relatively small and high-functioning TE sample with a restricted range of symptom counts, and an absence of more nuanced symptom frequency and severity data, we were not able to test the sensitivity and specificity of the proposed markers of chronic hypervigilance. It is possible that the associations identified in our non-clinical sample might be even stronger in people with diagnosed psychopathology, however such a question requires additional symptom data.

In addition, the proposed biomarkers of hypervigilance should be tested in men, given well-documented sex differences in stress reactivity (e.g., Kajantie and Phillips, 2006) and amygdala connectivity (e.g., Kilpatrick et al., 2006). Relatedly, we did not assess ovarian hormones, which are associated with fluctuations in stress reactivity (e.g., Kajantie and Phillips, 2006) and connectivity of affective neural circuitry (e.g., Peper et al., 2011). Future work should test the effects of sex hormones on the proposed markers of hypervigilance.

Finally, we used an exaggerated and persistent neural novelty response as a reactivity-based index of trauma-related hypervigilance (e.g., Yoon and Weierich, 2016; 2017). However, to assess the reliability of the proposed resting-state markers of chronic hypervigilance, future studies should test their associations with other behavioral and physiological measures of hypervigilance, such as increased visual scanning (e.g., Kimble et al., 2013) or exaggerated startle (e.g., Pole, 2007).

4.5. Conclusions and implications

Together, our results suggest that increased synchronization of threat-detection circuitry in the absence of threat and elevated basal sympathetic tone might serve as resting-state markers of chronic hypervigilance and provide complementary information about the underlying cognitive and physiological mechanisms, respectively. These results align with the neurobiological model of chronic hypervigilance, conceptualized as sustained activation of the neural alerting response, which primes threat-detection circuitry and the neuroendocrine stress systems for an exaggerated and persistent response not only to threat-relevant information, but also to salient, yet innocuous, stimuli. Given the utility of chronic hypervigilance in predicting long-term clinical outcomes (Schell et al., 2004), the identified non-invasive resting-state markers might hold diagnostic and prognostic promise in clinical settings. For example, the proposed neural and neuroendocrine biomarkers could help identify trauma survivors who are more likely to benefit from treatments aimed at reducing cognitive hypervigilance and physiological hyperarousal, respectively, as well as serving as outcome markers of treatment effectiveness.

Funding sources

This research was funded by the National Institutes of Health NCRRG12RR003037-25S3 (ARRA supplement to MRW), NIMHDMD007599 (seed grant funding to MRW), NIDADA012136 (pilot grant funding to MRW), and NINDS5R25NS080686 (MRW). The sponsor had no involvement in study design, data collection, analysis, and interpretation, article preparation, or the decision to submit the article for publication.

Contributors

Olena Kleshchova, Jenna K. Rieder, and Mariann R. Weierich were involved in study design, data collection, and data analysis. Olena Kleshchova, Jenna K. Rieder, Mariann R. Weierich, and Jack Grinband were involved in data interpretation and article preparation. All four authors reviewed and approved the final manuscript and its submission to Psychoneuroendocrinology.

Conflict of interest

Authors have no conflicts of interest.

Acknowledgments

The authors thank Seungyeon A. Yoon for her help in data collection and interpretation. The authors also thank Jonathan P. Dyke and Jojo Borja for technical assistance.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2018.11.036.

References

Beck, A.T., Steer, R.A., Ball, R., Ranieri, W.F., 1996. Comparison of Beck Depression Inventories-IA and-II in psychiatric outpatients. J. Pers. Assess. 67, 588–597.
Beckmann, C.F., Smith, S.M., 2004. Probabilistic independent component analysis for functional magnetic resonance imaging. IEEE Trans. Med. Imaging 23, 137–152.
Beckmann, C.F., Jenkinson, M., Smith, S.M., 2003. General multilevel linear modeling for group analysis in FMRI. NeuroImage 20, 1052–1063.
Beissner, F., Meissner, K., Bär, K.J., Napadow, V., 2013. The autonomic brain: an activation likelihood estimation meta-analysis for central processing of autonomic function. J. Neurosci. 33, 10503–10511.
Berns, G.S., Cohen, J.D., Mintun, M.A., 1997. Brain regions responsive to novelty in the absence of awareness. Science 276, 1272–1275.
Bissière, S., Plachta, N., Hoyer, D., McAllister, K.H., Olpe, H.R., Grace, A.A., Cryan, J.F., 2008. The rostral anterior cingulate cortex modulates the efficiency of amygdala-dependent fear learning. Biol. Psychiat. 63, 821–831.
Blackford, J.U., Allen, A.H., Cowan, R.L., Avery, S.N., 2012. Amygdala and hippocampus fail to habituate to faces in individuals with an inhibited temperament. Soc. Cogn. Affect. Neur. 8, 143–150.
Boubela, R.N., Kalcher, K., Huf, W., Kronnerwetter, C., Filzmoser, P., Moser, E., 2013. Beyond noise: using temporal ICA to extract meaningful information from high-frequency fMRI signal fluctuations during rest. Front. Hum. Neurosci. 7, 168.
Carlson, J.M., Cha, J., Mujica-Parodi, L.R., 2013. Functional and structural amygdala–anterior cingulate connectivity correlates with attentional bias to masked fearful faces. Cortex 49, 2595–2600.
Carlson, J.M., Reinke, K.S., Habib, R., 2009. A left amygdala mediated network for rapid orienting to masked fearful faces. Neuropsychologia 47, 1386–1389.
Chen, B., Xu, T., Zhou, C., Wang, L., Yang, N., Wang, Z., Dong, H.M., Yang, Z., Zang, Y.F., Zuo, X.N., Weng, X.C., 2015. Individual variability and test-retest reliability revealed by ten repeated resting-state brain scans over one month. PLoS One 10, e0144963.
Choe, A.S., Jones, C.K., Joel, S.E., Muschelli, J., Belegu, V., Caffo, B.S., Lindquist, M.A., van Zijl, P.C.M., Pekar, J.J., 2015. Reproducibility and temporal structure in weekly resting-state fMRI over a period of 3.5 years. PLoS One 10, e0140134.
Cohen, S., Kamarck, T., Mermelstein, R., 1983. A global measure of perceived stress. J. Health Soc. Behav. 24, 385–396.
Ditzen, B., Ehlert, U., Nater, U.M., 2014. Associations between salivary alpha-amylase and catecholamines – a multilevel modeling approach. Biol. Psychol. 103, 15–18.
Drysdale, A.T., Grosenick, L., Downar, J., Dunlop, K., Mansouri, F., Meng, Y., Fetcho, R.N., Zebley, B., Oathes, D.J., Etkin, A., Schatzberg, A.F., Sudheimer, K., Keller, J., Mayberg, H.S., Gunning, F.M., Alexopoulos, G.S., Fox, M.D., Pascual-Leone, A., Voss, H.U., Casey, B.J., Dubin, M.J., Liston, C., 2017. Resting-state connectivity biomarkers define neurophysiological subtypes of depression. Nat. Med. 23, 28–38.
Finn, E.S., Shen, X., Scheinost, D., Rosenberg, M.D., Huang, J., Chun, M.M., Papademetris, X., Constable, R.T., 2015. Functional connectome fingerprinting: identifying individuals using patterns of brain connectivity. Nat. Neurosci. 18, 1664–1671.
Fries, E., Hesse, J., Hellhammer, J., Hellhammer, D.H., 2005. A new view on hypocortisolism. Psychoneuroendocrinology 30, 1010–1016.
Galatzer-Levy, I.R., Ma, S., Statnikov, A., Yehuda, R., Shalev, A.Y., 2017. Utilization of machine learning for prediction of post-traumatic stress: a re-examination of cortisol in the prediction and pathways to non-remitting PTSD. Transl. Psychiat. 7, e1070.
Gilboa, A., Shalev, A.Y., Laor, L., Lester, H., Louzoun, Y., Chisin, R., Bonne, O., 2004. Functional connectivity of the prefrontal cortex and the amygdala in posttraumatic stress disorder. Biol. Psychiat. 55, 263–272.
Gold, A.L., Morey, R.A., McCarthy, G., 2015. Amygdala–prefrontal cortex functional connectivity during threat-induced anxiety and goal distraction. Biol. Psychiat. 77, 394–403.
Gray, M.J., Litz, B.T., Hsu, J.L., Lombardo, T.W., 2004. Psychometric properties of the life events checklist. Assessment 11, 330–341.
Griffanti, L., Salimi-Khorshidi, G., Beckmann, C.F., Auerbach, E.J., Douaud, G., Sexton, C.E., Zsoldos, E., Ebmeier, K.P., Filippini, N., Mackay, C.E., Moeller, S., Xu, J., Yacoub, E., Baselli, G., Ugurbil, K., Miller, K.L., Smith, S.M., 2014. ICA-based artefact removal and accelerated fMRI acquisition for improved resting state network imaging. NeuroImage 95, 232–247.
Hendrickson, R.C., Raskind, M.A., 2016. Noradrenergic dysregulation in the pathophysiology of PTSD. Exp. Neurol. 284, 181–195.
Herman, J.P., McKlveen, J.M., Solomon, M.B., Carvalho-Netto, E., Myers, B., 2012. Neural regulation of the stress response: glucocorticoid feedback mechanisms. Braz. J. Med. Biol. Res. 45, 292–298.
Hermans, E.J., van Marle, H.J., Ossewaarde, L., Henckens, M.J., Qin, S., van Kesteren, M.T., Schoots, V.C., Cousijn, H., Rijpkema, M., Oostenveld, R., Fernández, G., 2011. Stress-related noradrenergic activity prompts large-scale neural network reconfiguration. Science 334, 1151–1153.
Jacques, P.L.S., Botzung, A., Miles, A., Rubin, D.C., 2011. Functional neuroimaging of emotionally intense autobiographical memories in post-traumatic stress disorder. J. Psychiat. Res. 45, 630–637.
Jenkinson, M., Bannister, P., Brady, M., Smith, S., 2002. Improved optimization for the robust and accurate linear registration and motion correction of brain images. NeuroImage 17, 825–841.
Jin, C., Qi, R., Yin, Y., Hu, X., Duan, L., Xu, Q., Zhang, Z., Zhong, Y., Feng, B., Xiang, H., Gong, Q., 2014. Abnormalities in whole-brain functional connectivity observed in treatment-naive post-traumatic stress disorder patients following an earthquake. Psychol. Med. 44, 1927–1936.
Kajantie, E., Phillips, D.I., 2006. The effects of sex and hormonal status on the physiological response to acute psychosocial stress. Psychoneuroendocrinology 31, 151–178.
Keeshin, B.R., Strawn, J.R., Out, D., Granger, D.A., Putnam, F.W., 2015. Elevated salivary alpha amylase in adolescent sexual abuse survivors with posttraumatic stress disorder symptoms. J. Child Adolesc. Psychopharmacol. 25, 344–350.
Kelly, R.E., Alexopoulos, G.S., Wang, Z., Gunning, F.M., Murphy, C.F., Morimoto, S.S., Kanellopoulos, D., Jia, Z., Lim, K.O., Hoptman, M.J., 2010. Visual inspection of independent components: defining a procedure for artifact removal from fMRI data. J. Neurosci. Meth. 189, 233–245.
Kilpatrick, L.A., Zald, D.H., Pardo, J.V., Cahill, L.F., 2006. Sex-related differences in amygdala functional connectivity during resting conditions. NeuroImage 30, 452–461.
Kimble, M.O., Fleming, K., Bennion, K.A., 2013. Contributors to hypervigilance in a military and civilian sample. J. Interpers. Violence 28, 1672–1692.
Kriegeskorte, N., Simmons, W.K., Bellgowan, P.S., Baker, C.I., 2009. Circular analysis in systems neuroscience: the dangers of double dipping. Nat. Neurosci. 12, 535–540.
Liddell, B.J., Brown, K.J., Kemp, A.H., Barton, M.J., Das, P., Peduto, A., Gordon, E., Williams, L.M., 2005. A direct brainstem–amygdala–cortical “alarm” system for subliminal signals of fear. NeuroImage 24, 235–243.
Lieberman, M.D., Eisenberger, N.I., Crockett, M.J., Tom, S.M., Pfeifer, J.H., Way, B.M., 2007. Putting feelings into words. Psychol. Sci. 18, 421–428.
Magnuson, M.E., Thompson, G.J., Schwarb, H., Pan, W.J., McKinley, A., Schumacher, E.H., Keilholz, S.D., 2015. Errors on interrupter tasks presented during spatial and verbal working memory performance are linearly linked to large-scale functional network connectivity in high temporal resolution resting state fMRI. Brain Imaging Behav. 9, 854–867.
Nater, U.M., Rohleder, N., 2009. Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: current state of research. Psychoneuroendocrinology 34, 486–496.
Osuch, E.A., Willis, M.W., Bluhm, R., Ursano, R.J., Drevets, W.C., CSTS Neuroimaging Study Group, 2008. Neurophysiological responses to traumatic reminders in the acute aftermath of serious motor vehicle collisions using [15O]-H2O positron emission tomography. Biol. Psychiat. 64, 327–335.
Patel, R., Spreng, R.N., Shin, L.M., Girard, T.A., 2012. Neurocircuitry models of posttraumatic stress disorder and beyond: a meta-analysis of functional neuroimaging studies. Neurosci. Biobehav. Res. 36, 2130–2142.
Peper, J.S., van den Heuvel, M.P., Mandl, R.C., Pol, H.E.H., van Honk, J., 2011. Sex steroids and connectivity in the human brain: a review of neuroimaging studies. Psychoneuroendocrinology 36, 1101–1113.
Pezawas, L., Meyer-Lindenberg, A., Drabant, E.M., Verchinski, B.A., Munoz, K.E., Kolachana, B.S., Egan, M.F., Mattay, V.S., Hariri, A.R., Weinberger, D.R., 2005. 5-HTTLPR polymorphism impacts human cingulate-amygdala interactions: a genetic susceptibility mechanism for depression. Nat. Neurosci. 8, 828–834.
Poldrack, R.A., Mumford, J.A., 2009. Independence in ROI analysis: Where is the voodoo? Soc. Cogn. Affect. Neur. 4, 208–213.
Pole, N., 2007. The psychophysiology of posttraumatic stress disorder: a meta-analysis. Psychol. Bull. 133, 725–746.
Rabinak, C.A., Angstadt, M., Welsh, R.C., Kenndy, A.E., Lyubkin, M., Martis, B., Phan, K.L., 2011. Altered amygdala resting-state functional connectivity in post-traumatic stress disorder. Front. Psychiat. 2, 1–8.
Radley, J.J., Arias, C.M., Sawchenko, P.E., 2006. Regional differentiation of the medial prefrontal cortex in regulating adaptive responses to acute emotional stress. J. Neurosci. 26, 12967–12976.
Roy, A.K., Shehzad, Z., Margulies, D.S., Kelly, A.C., Uddin, L.Q., Gotimer, K., Biswal, B.B., Castellanos, F.X., Milham, M.P., 2009. Functional connectivity of the human amygdala using resting state fMRI. NeuroImage 45, 614–626.
Schell, T.L., Marshall, G.N., Jaycox, L.H., 2004. All symptoms are not created equal: the prominent role of hyperarousal in the natural course of posttraumatic psychological distress. J. Abnorm. Psychol. 113, 189–197.
Schwartz, C.E., Wright, C.I., Shin, L.M., Kagan, J., Rauch, S.L., 2003. Inhibited and uninhibited infants "grown up": adult amygdalar response to novelty. Science 300, 1952–1953.
Seeley, W.W., Menon, V., Schatzberg, A.F., Keller, J., Glover, G.H., Kenna, H., Reiss, A.L., Greicius, M.D., 2007. Dissociable intrinsic connectivity networks for salience processing and executive control. J. Neurosci. 27, 2349–2356.
Shah, L.M., Cramer, J.A., Ferguson, M.A., Birn, R.M., Anderson, J.S., 2016. Reliability and reproducibility of individual differences in functional connectivity acquired during

task and resting state. Brain Behav. 6, e00456.
Simpson, J.R., Drevets, W.C., Snyder, A.Z., Gusnard, D.A., Raichle, M.E., 2001. Emotion-induced changes in human medial prefrontal cortex: II. During anticipatory anxiety. Proc. Natl. Acad. Sci. 98, 688–693.
Smith, S.M., 2002. Fast robust automated brain extraction. Hum. Brain Mapp. 17, 143–155.
Smith, S.M., Beckmann, C.F., Andersson, J., Auerbach, E.J., Bijsterbosch, J., Douaud, G., Duff, E., Feinberg, D.A., Griffanti, L., Harms, M.P., Kelly, M., Laumann, T., Miller, K.L., Moeller, S., Petersen, S., Power, J., Salimi-Khorshidi, G., Snyder, A.Z., Vu, A.T., Woolrich, M.W., Xu, J., Yacoub, E., Uğurbil, K., Van Essen, D.C., Glasser, M.F., 2013. Resting-state fMRI in the human connectome project. NeuroImage 80, 144–168.
Spielberger, C.D., Gorsuch, R.L., Lushene, R., Vagg, P.R., Jacobs, G.A., 1983. Manual for the State-Trait Anxiety Inventory. Consulting Psychologists Press, Palo Alto, CA.
Stefanacci, L., Amaral, D.G., 2002. Some observations on cortical inputs to the macaque monkey amygdala: an anterograde tracing study. J. Comp. Neurol. 451, 301–323.
Stein, J.L., Wiedholz, L.M., Bassett, D.S., Weinberger, D.R., Zink, C.F., Mattay, V.S., Meyer-Lindenberg, A., 2007. A validated network of effective amygdala connectivity. NeuroImage 36, 736–745.
Taren, A.A., Gianaros, P.J., Greco, C.M., Lindsay, E.K., Fairgrieve, A., Brown, K.W., Rosen, R.K., Ferris, J.L., Julson, E., Marsland, A.L., Bursley, J.K., 2015. Mindfulness meditation training alters stress-related amygdala resting state functional connectivity: a randomized controlled trial. Soc. Cogn. Affect. Neur. 10, 1758–1768.
Tavor, I., Jones, O.P., Mars, R.B., Smith, S.M., Behrens, T.E., Jbabdi, S., 2016. Task-free MRI predicts individual differences in brain activity during task performance. Science 352, 216–220.
Thomason, M.E., Marusak, H.A., Tocco, M.A., Vila, A.M., McGarragle, O., Rosenberg, D.R., 2015. Altered amygdala connectivity in urban youth exposed to trauma. Soc. Cogn. Affect. Neur. 10, 1460–1468.
Touroutoglou, A., Andreano, J.M., Barrett, L.F., Dickerson, B.C., 2015. Brain network connectivity–behavioral relationships exhibit trait-like properties: evidence from hippocampal connectivity and memory. Hippocampus 25, 1591–1598.
van den Bulk, B.G., Somerville, L.H., van Hoof, M.J., van Lang, N.D., van der Wee, N.J., Crone, E.A., Vermeiren, R.R., 2016. Amygdala habituation to emotional faces in adolescents with internalizing disorders, adolescents with childhood sexual abuse related PTSD and healthy adolescents. Dev. Cogn. Neurosci. 21, 15–25.
Vigil, J.M., Geary, D.C., Granger, D.A., Flinn, M.V., 2010. Sex differences in salivary cortisol, alpha-amylase, and psychological functioning following Hurricane Katrina. Child Dev. 81, 1228–1240.
Vogt, B., Derbyshire, S., 2009. Visceral circuits and cingulate-mediated autonomic functions. Cingulate neurobiology and disease. In: Vogt, B. (Ed.), Cingulate Neurobiology and Disease. Oxford University Press, Oxford, pp. 220–235.
Warren, C.M., van den Brink, R.L., Nieuwenhuis, S., Bosch, J.A., 2017. Norepinephrine transporter blocker atomoxetine increases salivary alpha amylase. Psychoneuroendocrinology 78, 233–236.
Watson, D., Clark, L.A., Tellegen, A., 1988. Development and validation of brief measures of positive and negative affect: the PANAS scales. J. Pers. Soc. Psychol. 54, 1063.
Weierich, M.R., Wright, C.I., Negreira, A., Dickerson, B.C., Barrett, L.F., 2010. Novelty as a dimension in the affective brain. NeuroImage 49, 2871–2878.
Williams, L.M., Gatt, J.M., Schofield, P.R., Olivieri, G., Peduto, A., Gordon, E., 2009. "Negativity bias" in risk for depression and anxiety: brain–body fear circuitry correlates, 5-HTT-LPR and early life stress. NeuroImage 47, 804–814.
Williams, L.M., Kemp, A.H., Felmingham, K., Barton, M., Olivieri, G., Peduto, A., Gordon, E., Bryant, R.A., 2006. Trauma modulates amygdala and medial prefrontal responses to consciously attended fear. NeuroImage 29, 347–357.
Woolrich, M., 2008. Robust group analysis using outlier inference. NeuroImage 41, 286–301.
Woolrich, M.W., Ripley, B.D., Brady, M., Smith, S.M., 2001. Temporal autocorrelation in univariate linear modeling of FMRI data. NeuroImage 14, 1370–1386.
Xu, T., Opitz, A., Craddock, R.C., Wright, M.J., Zuo, X.N., Milham, M.P., 2016. Assessing variations in areal organization for the intrinsic brain: from fingerprints to reliability. Cereb. Cortex 26, 4192–4211.
Yoon, S.A., Weierich, M.R., 2016. Salivary biomarkers of neural hypervigilance in trauma-exposed women. Psychoneuroendocrinology 63, 17–25.
Yoon, S.A., Weierich, M.R., 2017. Persistent amygdala novelty response is associated with less anterior cingulum integrity in trauma-exposed women. Neuroimage Clin. 14, 250–259.
Zhang, Y., Brady, M., Smith, S., 2001. Segmentation of brain MR images through a hidden Markov random field model and the expectation-maximization algorithm. IEEE Trans. Med. Imaging 20, 45–57.