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Increased BNST reactivity to affective images is associated with greater α-amylase response to social stress

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Abstract

While rodent research suggests that the bed nucleus of the stria terminalis (BNST) and centromedial amygdala (CM) coordinate the hormonal stress response, little is known about the BNST's role in the human stress response. The human BNST responds to negatively valenced stimuli, which likely subserves its role in responding to threat. Thus, variation in BNST reactivity to negatively valenced stimuli may relate to differences in the stress response. We measured participants' blood oxygenated level-dependent response to affective images and salivary cortisol and α-amylase (AA) levels in response to a subsequent Trier social stress test (TSST). Greater BNST activation to emotionally evocative images was associated with a larger TSST-evoked AA, but not cortisol response. This association remained after controlling for CM activation, which was not related to the cortisol or AA response. These results suggest that the BNST response to negatively valenced images subserves its role in coordinating the stress response, a BNST role in the stress response independent from the CM, and highlight the need for investigation of the conditions under which BNST activation predicts the cortisol response. Our findings are critical for the future study of mood and anxiety disorders, as dysregulation of the stress system plays a key role in their pathogenesis.

Key words: cortisol; alpha-amylase; bed nucleus of the stria terminalis; BNST; extended amygdala; stress

Introduction

The extended amygdala is a circuit of highly interconnected subcortical structures, including the centromedial amygdala (CM), consisting of the central (CeA) and medial nucleus (MeA), and the bed nucleus of the stria terminalis (BNST). Davis *et al.* (2010) proposed that the CM—particularly the CeA—and BNST play complementary roles in regulating the response to threat, with the CeA mediating the short-term, immediate response to imminent threat (i.e. the fear response), and the BNST mediating the sustained response to potential or distant threat, marked by vigilance behaviors (i.e. the anxiety response). An important way that the BNST and CeA mediate the response to threat is via outputs to both arms of the descending stress system, the hypothalamic–pituitary–adrenal (HPA) axis and the sympathetic nervous system (SNS; Crane *et al.*, 2003; Spencer *et al.*, 2005; Chrousos, 2009; Davis *et al.*, 2010). While human research demonstrates a BNST response to stressful (Straube *et al.*, 2007; Somerville *et al.*, 2010; Alvarez *et al.*, 2011; Shackman and Fox, 2016; Pedersen *et al.*, 2017) and negatively valenced stimuli (Somerville *et al.*, 2013; Herrmann *et al.*, 2016; Pedersen *et al.*, 2016; Brinkmann *et al.*, 2018), little is known about how BNST activity relates to the hormonal stress response in humans. Better understanding the neural inputs of the human stress system is critical for

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understanding psychopathology, as dysregulation of the stress system is thought to be central to the pathogenesis of mood and anxiety disorders (Pariante and Lightman, 2008; Faravelli *et al.*, 2012).

The Davis *et al.* (2010) model was based primarily on rodent research. This research implicates the BNST in anxiety-like behavior, such as spending more time in enclosed spaces when exploring a new environment (Pêgo *et al.*, 2008) or exhibiting increased startle in a chamber filled with light (Davis *et al.*, 2010), while the CeA has been implicated in the fear response using paradigms like fear conditioning (Phillips and LeDoux, 1992; Rogan *et al.*, 1997). Rodent research has also implicated the CeA and BNST in initiating the release of corticosterone (the homologue to cortisol in rodents) in response to threat (Sullivan *et al.*, 2004). The BNST is thought to regulate corticosterone release via projections to paraventricular nucleus (PVN) of the hypothalamus, a critical node in HPA axis activation (Crane *et al.*, 2003; Spencer *et al.*, 2005). While the amygdala has only very sparse connections with the PVN (Herman *et al.*, 2003; Dedovic *et al.*, 2009), it is thought to affect the HPA-mediated stress response via connections with other regions, including the BNST. Both the CeA and BNST are thought to regulate SNS function via projections to the lateral hypothalamus and brainstem (Davis and Whalen, 2001). This suggests that biomarkers of SNS activation, such as salivary α-amylase (AA; Nater and Rohleder, 2009), are likely also related to CeA and BNST activation.

Social stressors are a common source of stress and are often used to induce stress in the laboratory. In rodents, both the CeA and BNST have been implicated in the avoidance of novel conspecifics (Gonzalez *et al.*, 1996; Khoshbouei *et al.*, 2002; Navarro *et al.*, 2004; Lungwitz *et al.*, 2012) and in defensive–submissive behaviors toward threatening conspecifics (Jasnow *et al.*, 2004; Robison *et al.*, 2009). In humans, evidence of an amygdala response to social stress is mixed. A positron emission tomography (PET) study (Rosenkranz *et al.*, 2018) found increased activation in an amygdala region of interest (ROI) during the Trier social stress test (TSST), a task commonly used to induce social stress (Kirschbaum *et al.*, 1993), relative to that during a well-matched, non-stressful control condition. However, researchers attempting to adapt the TSST to fMRI found reduced amygdala activation during stress (Pruessner *et al.*, 2008). Similarly, two studies found decreased amygdala activation during a speech preparation task (Wager *et al.*, 2009a, 2009b). Human studies on the response to social stress have not focused on the BNST. However, given that human social stressors typically involve potential threat (rather than imminent danger), the BNST may play an important role in mediating the response to social stress.

Both the amygdala and BNST respond to stressful (Straube *et al.*, 2007; Somerville *et al.*, 2010; Alvarez *et al.*, 2011; Shackman and Fox, 2016; Pedersen *et al.*, 2017) and aversive stimuli more generally, including negatively valenced images (Sergerie *et al.*, 2008; Somerville *et al.*, 2013; Herrmann *et al.*, 2016; Pedersen *et al.*, 2016; Brinkmann *et al.*, 2018). This response to negative stimuli in the BNST and amygdala may subserve their roles in detecting and responding to threat, as negatively valenced stimuli often carry signals of potential threat. As such, individuals who exhibit greater amygdala and BNST sensitivity to negatively valenced stimuli may also be more sensitive to stressors. If so, individual differences in amygdala and BNST reactivity to negative stimuli may predict individual differences in sensitivity to social stress. However, despite findings in rodent research suggesting that the BNST serves as an input to the stress system (Crane *et al.*, 2003; Spencer *et al.*, 2005; Davis *et al.*, 2010), whether individual differences in human BNST reactivity are associated with the degree of the stress response, as indexed by stress hormones, has not been investigated. Doing so would establish a connection between activation in stress-related neural circuitry and activation of the peripheral stress system and may shed light on the etiology of psychopathology, which is often accompanied by dysregulation of the peripheral stress system.

To test whether amygdala and BNST reactivity to negative stimuli is related to individual differences in the stress response, we measured the neural blood oxygen level-dependent (BOLD) response while participants viewed negative, neutral and positive images. Subsequently participants' saliva was collected prior to and at several points following a TSST, to measure individual differences in the stress response, as indexed by salivary cortisol and α-amylase. We used an ROI approach to restrict analyses to regions included in our a priori hypotheses. For the amygdala, we used a CM ROI, as both the CeA and MeA are included in the extended amygdala, and because this more inclusive ROI is more likely to include CeA activation for most participants, after accounting for spatial inaccuracies introduced by warping each participants' data to a standard template.

We predicted that greater BNST and CM activation to emotionally evocative images would be associated with larger TSST cortisol and AA responses. We expected the association between BNST and CM responses to negatively valenced images and TSST-evoked cortisol and AA responses to be stronger than the response to positive images. As the CM and BNST likely play complementary but distinct roles in the human stress response, we expected CM and BNST reactivity to negative images to account for distinct portions of variance in stress responsivity. Thus, while we expected CM reactivity to negative images to be related to stress responsivity, we hypothesized that the relationship between BNST activation to negative images and increased TSST-evoked cortisol and AA would remain significant after controlling for the CM response.

Method

Participants

The study sample included 158 healthy participants. Study protocols were approved by UW-Madison Health Sciences Institutional Review Board, and all participants provided consent and were given monetary compensation for their participation. Individuals were excluded from participation if they had used psychotropic medications or had a psychiatric disorder in the past year. Individuals with a history of bipolar or schizophrenic disorders, seizures or brain damage were also excluded. Data from two participants were excluded from analysis due to brain abnormalities, data from one participant were excluded due to fMRI artifacts associated with a dental implant, three participants were unable to complete the task due to technical difficulties, and one participant withdrew from the study prior to taking part in the fMRI task. As a result, data from 151 participants (93 females, 58 males) were included in the analysis. These participants had a mean age of 48.73 years (s.d. = 10.64).

As the data used in the current report were collected as part of a larger investigation of the effects of meditation practice on neural activity and stress responding, these 151 participants included 121 meditation-naïve participants (MNPs) and 30 long-term meditators (LTMs). Both MNPs and LTMs were included in this analysis because we expected that, while meditation experience may alter the magnitude of the stress response during the TSST, the relationship between BNST activation and stress response should be similar across these groups. As discussed later, we tested this prediction before addressing our primary

hypotheses. The effects of meditation practice on the TSST stress response (Rosenkranz *et al.*, 2016) and on the amygdala BOLD response (Kral *et al.*, 2018) in this dataset have been previously reported. In addition, voxel-wise results for negative minus neutral, positive minus neutral and the average of positive and negative minus neutral image contrasts by meditation status were conducted by Kral *et al.* (2018), with whole-brain maps available at https://neurovault.org/collections/3755.

MNPs were recruited within Madison, WI and surrounding areas using flyers and advertisements in local media and online. Recruitment materials advertised the study as investigating 'health and well-being', or the 'benefits of health wellness classes'. MNPs participated in a randomized controlled trial of mindfulness-based stress reduction (MBSR), with experimental sessions completed pre- and post-MBSR training. However, all data reported here were collected at baseline, prior to random assignment to an experimental group.

LTMs were recruited at meditation centers and through related mailing lists throughout the United States, in addition to flyers and advertisements in newspapers, similar to the recruitment strategy for MNPs. Participants qualified as LTMs if they practiced meditation at least 30 min per day for the past 3 years or more; had experience with Vipassana, concentration and compassion/loving kindness meditations; and had attended at least three intensive meditation retreats lasting 5 days or more.

Affective image task

During this task, participants viewed 72 images from the International Affective Picture Set (Lang *et al.*, 2008; IAPS) across four fMRI scan runs. Images were negative, neutral and positive in valence in equal numbers, resulting in 24 images of each type. For each condition, half of the images were social, and half were non-social. Normative valence and arousal scores on a Likert scale from 1 to 9 are as follows: negative images, valence = 2.87 (s.d. = 0.87), arousal = 5.51 (s.d. = 0.47); neutral images: valence = 5.08 (s.d. = 0.6), arousal = 3.86 (s.d. = 0.63); positive images, valence = 7.1 (s.d. = 0.47) and arousal = 5.36 (s.d. = 0.37). Valence order was pseudo-randomized and picture order was completely randomized within the task.

The task also included the presentation of neutral (male and female) faces from the Extended Multimodal Face Database (Messer *et al.*, 1999), which were presented for 500 ms after the offset of the picture in two-thirds of the trials, and appeared either 1 (8x per valence) or 3 s (8x per valence) post-picture offset. There were also eight trials in which a face did not follow the image. Trials were separated by a jittered inter-trial interval of 5–18 s. Face stimuli were included to examine questions not addressed here. Although the presentation of faces was modeled in the first level regression during imaging analysis, the resulting parameter estimates were not used in the current report.

Image acquisition

Images were acquired on a GE X750-3.0 Tesla MRI scanner device with an 8-channel head coil. Anatomical scans consisted of a high-resolution 3D T1-weighted inversion recovery fast gradient echo image (inversion time = 450 ms, 256 × 256 in-plane resolution, 256 mm FOV, 124 × 1.0 mm axial slices). Four functional scan runs were acquired for the affective image task using a gradient echo EPI sequence (64 × 64 in-plane resolution, 240 mm FOV, TR/TE/Flip = 2000 ms/25 ms/60°, 40 × 4 mm interleaved sagittal slices, and 159 3D volumes per run).

Imaging analysis

Functional images were processed using FEAT (FMRI Expert Analysis Tool) Version 6.00, part of FSL (FMRIB's Software Library) including a high-pass temporal filter of 100 s, motion correction with MCFLIRT (Jenkinson *et al.*, 2002), BET brain extraction (Smith, 2002), spatial smoothing at 5 mm FWHM and FILM pre-whitening (Woolrich *et al.*, 2001). Transformation matrices for registration were computed at the first level (within-scan run) and applied at the second level using FSL in a two-stage process where the boundary-based registration (BBR) approach (Greve and Fischl, 2009) was used to register the subject's time series data to their anatomical template. Subject's anatomical images were aligned to Montreal Neurological Institute space with a 12 DOF affine transformation using FLIRT (Jenkinson *et al.*, 2002) followed by nonlinear warping via FNIRT (Andersson, Jenkinson, 2007).

The functional data from individual subjects were analyzed using a general linear model (GLM) in three levels, where the first level (within-scan) modeled stimulus presentation with a double-gamma hemodynamic response function is defined in FSL. Each trial type was modeled with up to two regressors for each of two events; the 4 s presentation period of the IAPS image and the 0.5 s presentation of the neutral face on the two-third of trials in which a face was presented (one-third of trials did not have a face) for a total of nine regressors (faces presented 1 s after the offset of the IAPS images were modeled separately from those presented 3 s after the IAPS image). Additional regressors of no interest were included to model the 24 motion-related parameters (the standard plus extended parameters, which include the squares, derivatives and squares of derivatives). The second level combined data within-subject and across scan runs using a fixed effects modeling approach.

TSST

Subsequent to the affective image task, participants performed a modified version of the TSST (Kirschbaum *et al.*, 1993) outside the scanner. Both this task and the affective image task were administered during a 24-h lab visit, in which participants completed several tasks. The TSST consisted of a 3-min preparation period, a 5-min impromptu speech, followed by 5 min of mental arithmetic. These tasks were performed standing in front of a microphone before a panel of two (one male, one female) non-supportive and stern-looking judges and a video camera. For the speech task, participants were asked to convince the judges why they are the best candidate for their ideal job. They were given 3 min to prepare their speech after the topic was revealed but were not allowed to use their notes during the speech. If participants did not speak for the entire 5-min period, they were told that there was time remaining, to please continue. This version differs from the original only in that the speech preparation time is 7 min shorter. All participants completed the TSST between 3:00 and 5:00 pm.

Levels of two salivary stress hormones, cortisol and α-amylase provided measures of the magnitude of the stress response to the TSST. These hormones were chosen as markers of activity in the HPA axis and SNS, respectively. Participants provided samples of saliva at baseline using Salivette devices (Sarstedt, Nümbrecht, Germany). Subsequent saliva samples were collected immediately after the completion of the TSST, as well as every 10 min for the next 40 min, for a total of six saliva samples. Salivary cortisol and AA levels were measured by Dr Nicolas Rohleder and Dr Jutta Wolf at Brandeis University,

using standard assay techniques. Cortisol was measured using a commercially available luminescence immunoassay (CLIA; IBL-Hamburg, Hamburg, Germany), and AA was measured using an enzyme kinetic assay using reagents provided by Roche Diagnostics (Indianapolis, IN, USA) as previously described (Rohleder and Nater, 2009). Intra- and inter-assay coefficients of variation for cortisol were 3.42% and 4.06% and for α-amylase were 5.64% and 3.63%. Cortisol and AA area under the curve (AUC) with respect to ground were calculated as described by Pruessner *et al.* (2003) and then log transformed to normalize their distributions.

One participant did not finish the TSST, and another five did not have saliva samples collected for all time points. These participants were excluded from analysis. In addition, data from several participants had data excluded due to circumstances that invalidated the data, such as corticosteroid medication use or not providing enough saliva to obtain an accurate measurement. Four participants had both cortisol and AA data excluded, three had cortisol data excluded, and one had AA data excluded due to invalid data.

Statistical analysis

Anatomical ROIs were used to focus analyses on the specific regions involved in our hypotheses. We used BNST ROIs created by Theiss *et al.* (2017), thresholded at a 50% probability. CM ROIs were taken from the Tyszka and Pauli (2016) probabilistic atlas of the amygdala and consisted of the atlas regions labeled cortical and medial nuclei, as well as central nucleus, thresholded at 50% probability and merged together for each side. While much of the rodent literature has focused specifically on the role of the CeA in the stress response, we chose to use a CM ROI to compare with the BNST for a number of reasons. The CM and BNST are functionally connected and have a similar degree of functional diversity. Furthermore, this more inclusive ROI—which covers the majority of the dorsal amygdala—is more likely to include CeA activation for most participants, after accounting for spatial inaccuracies introduced by warping each participants' data to a standard template.

Mean percent signal change for activation in response to negative, neutral and positive images was extracted from the BNST and CM ROIs, for each side. The resulting values were entered into SPSS Statistics version 24 for further analyses. For each subject and ROI, orthogonal contrasts were created. The first contrast consisted of percent signal change for the negative image condition minus the positive condition (NEG > POS), representing valence-specific activation. The second contrast was average percent signal change for the positive and negative image conditions, minus the neutral condition (NEGPOS > NEU), representing a response to emotionally evocative images, regardless of valence. These contrast scores were entered into a series of one-sample *t*-tests to investigate CM and BNST responses to emotional images. A series of regressions was also run to determine whether BNST and CM activation are associated with the magnitude of stress responsivity, as measured by AUC for salivary AA and cortisol in response to the TSST. Age and gender were modeled as covariates in these regressions, as these variables are known to affect cortisol and AA levels.

As our primary questions of interest were focused on BNST activation, with the CM serving as a comparison, we adjusted *P*-values using Holm–Bonferroni correction for the number of comparisons within the BNST for each family of statistical tests, with the same being done for CM comparisons. All reported *P*-values are corrected, except where otherwise noted. Significant relationships involving the NEGPOS > NEU contrast were subjected to follow-up testing to determine whether effects were driven by the negative or positive condition. Holm–Bonferroni correction was also applied to these follow-up tests. Reported confidence intervals are not corrected for multiple comparisons. Outliers were detected based on Cook's *D*, using a cutoff threshold of 4/(N-P) for data points disconnected from the distribution. In most cases the removal of outliers did not affect whether a given statistical test was significant. In these cases, statistics are reported using all subjects. In cases where outlier removal did have an impact, statistics are reported both with and without the outliers included.

Voxel-wise analysis

To visualize the spatial distribution of the activation patterns driving reported effects, we supplemented our ROI-based approach, with a voxel-wise analysis. Pre-processed data were submitted to the FSL program Randomise (Winkler *et al.*, 2014) to find areas of activation significantly associated with the TSST-evoked AA and cortisol responses. This was done separately for the two condition contrasts (NEG > POS and NEGPOS > NEU), as well as for the two dependent variables (cortisol and AA AUC scores). Resulting clusters of activation were controlled for multiple comparisons using threshold-free cluster enhancement ($P < 0.05$).

Results

Response to affective images in the BNST and CM

One-sample *t*-tests for NEG > POS contrast score revealed that there were no effects for the NEG > POS contrast in the either the left, $t(150) = -0.247$, $P = 0.964$, 95% CI = [−0.0156, 0.0121], $d = -0.02$, or right BNST, $t(150) = 0.706$, $P = 0.964$, 95% CI = [−0.0086, 0.0182], $d = 0.057$. The NEGPOS > NEU contrast was significant in the left BNST, $t(150) = 4.252$, $P < 0.001$, 95% CI = [0.0119, 0.0325], $d = 0.346$, exhibiting activation to both negative *vs* neutral, $t(150) = 3.486$, $P < 0.001$, 95% CI = [0.0092, 0.0334], $d = 0.284$, and positive *vs* neutral images, $t(150) = 3.574$, $P < 0.001$, 95% CI = [0.0103, 0.0358], $d = 0.291$. While there was a trend-level effect in the NEGPOS > NEU contrast in the right BNST (uncorrected $P = 0.033$), it did not survive correction for multiple comparisons, $t(150) = 2.151$, $P = 0.099$, 95% CI = [0.0011, 0.0264], $d = 0.175$.

There was an effect for the NEG > POS contrast in both the left, $t(150) = 2.861$, $P = 0.01$, 95% CI = [0.0094, 0.0515], $d = 0.233$, and right CM, $t(150) = 2.341$, $P = 0.021$, 95% CI = [0.0045, 0.0535], $d = 0.191$. There was also an effect for the NEGPOS>NEU contrast in both the left, $t(150) = 6.514$, $P < 0.001$, 95% CI = [0.0456, 0.0853], $d = 0.53$, and right CM, $t(150) = 6.577$, $P < 0.001$, 95% CI = [0.0475, 0.0884], $d = 0.535$. Bilaterally, this effect was marked by increased responding to negative *vs* neutral (left CM: $t(150) = 7.182$, $P < 0.001$, 95% CI = [0.0585, 0.1029], $d = 0.584$; right CM: $t(150) = 7.124$, $P < 0.001$, 95% CI = [0.0596, 0.1053], $d = 0.58$), as well as positive *vs* neutral images (left CM: $t(150) = 4.366$, $P < 0.001$, 95% CI = [0.0275, 0.073], $d = 0.355$; right CM: $t(150) = 4.275$, $P < 0.001$, 95% CI = [0.0288, 0.0782], $d = 0.348$). Condition means for both the BNST and CM are presented in Figure 1.

BNST and CM activation as predictors of stress reactivity during the TSST

Peak cortisol and AA levels during the TSST were higher than at baseline (cortisol, mean difference = 13.28 nmol/L, $t(137) = 11.51$,

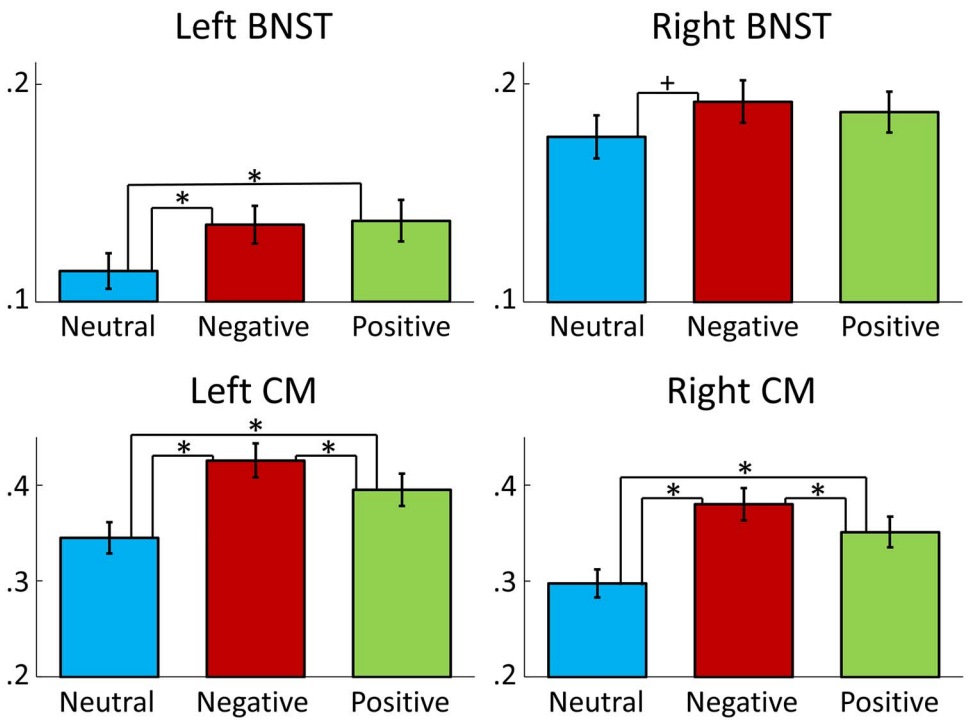

Fig. 1. Activation to negative, neutral and positive images in the BNST and CM. The left BNST responded to positive and negative (*vs* neutral) images but exhibited no difference in response between positive and negative images. Bilaterally, the CM exhibited a greater response to negative than positive and to positive than neutral images. $*P < 0.05$ after Holm–Bonferroni correction. $^{+}P < 0.05$ before correction.

$P < 0.001$; AA, mean difference = 104.05 U/ml, t(139) = 12.2, $P < 0.001$), indicating that the TSST was successful in inducing a stress response. We examined whether activation of the BNST and CM during the presentation of emotionally evocative images was related to the stress response during a subsequent TSST. We did so with a series of regressions where the NEG > POS and NEGPOS > NEU contrast scores from BNST and CM ROIs, meditation group status and their interaction were entered as predictors and AUC scores for TSST-evoked salivary cortisol and AA were entered as dependent variables. As predicted, meditation status did not interact with BNST or CM activation to affect either the cortisol or AA response in any of these regressions. As such, this variable was dropped from the models in these analyses. Holm–Bonferroni correction was applied for the eight main comparisons (2 contrasts × 2 stress biomarkers × 2 hemispheres) within the BNST and CM, respectively.

A trend-level positive association was observed between the NEG > POS contrast in the left BNST and salivary cortisol response (uncorrected $P = 0.046$), but this effect did not survive correction for multiple comparisons, $ß = –0.166$, $t = –2.01$, $P = 0.276$, $pr = –0.171$. The NEG > POS contrast in the right BNST was unrelated to the salivary cortisol response, $ß = –0.023$, $t = –0.283$, $P = 1$, $pr = –0.024$. The BNST response for the NEGPOS>NEU contrast was also unrelated to salivary cortisol response (left BNST, $ß = 0.028$, $t = 0.343$, $P = 1$, $pr = 0.03$; right BNST, $ß = 0.096$, $t = 1.181$, $P = 1$, $pr = 0.101$). Similarly, neither the CM response to NEG > POS (left CM, $ß = -0.017$, $t = –0.204$, $P = 1$, $pr = –0.018$; right CM, $ß = 0.027$, $t = 0.334$, $P = 1$, $pr = 0.029$) nor the CM response to NEGPOS > NEU was related to the salivary cortisol response (left CM, $ß = 0.085$, $t = 1.044$, $P = 1$, $pr = 0.09$; right CM, $ß = -0.017$, $t = –0.209$, $P = 1$, $pr = –0.018$).

The NEGPOS > NEU contrast, in both the left and right BNST, was related to the AA response to the TSST (left BNST, $ß = 0.263$, $t = 3.432$, $P = 0.006$, $pr = 0.282$; right BNST, $ß = 0.245$, $t = 3.193$, $P = 0.014$, $pr = 0.264$; see Figure 2). Follow-up tests revealed that, on both sides, the BNST response to both negative *vs* neutral images (left BNST, $ß = 0.237$, $t = 3.052$, $P = 0.006$, $pr = 0.253$; right BNST, $ß = 0.222$, $t = 2.878$, $P = 0.01$, $pr = 0.24$) and positive *vs* neutral images (left BNST, $ß = 0.202$, $t = 2.577$, $P = 0.011$, $pr = 0.216$; right BNST, $ß = 0.207$, $t = 2.667$, $P = 0.01$, $pr = 0.223$) was positively related to the AA response. When negative *vs* neutral and positive *vs* neutral contrasts were simultaneously used to predict AA, a trend-level association for the negative *vs* neutral contrast remained on the left ($ß = 0.187$, $t = 2.251$, $P = 0.052$, $pr = 0.19$), but not right side ($ß = 0.156$, $t = 1.709$, $P = 0.18$, $pr = 0.146$), while the positive *vs* neutral was no longer related to the AA response on either side (left BNST, $ß = 0.132$, $t = 1.578$, $P = 0.117$, $pr = 0.135$; right BNST, $ß = 0.123$, $t = 1.342$, $P = 0.182$, $pr = 0.115$).

In contrast, the CM response to NEGPOS > NEU was not related to the AA response (left CM, $ß = 0.119$, $t = 1.505$, $P = 1$, $pr = 0.128$; right CM, $ß = 0.078$, $t = 0.986$, $P = 1$, $pr = 0.084$). The TSST-evoked AA response was not related to NEG > POS contrast in either the BNST (left, $ß = 0.026$, $t = 0.317$, $P = 1$, $pr = 0.027$; right, $ß = 0.042$, $t = 0.522$, $P = 1$, $pr = 0.045$) or the CM (left, $ß = 0.077$, $t = 0.977$, $P = 1$, $pr = 0.083$; right, $ß = 0.049$, $t = 0.616$, $P = 1$, $pr = 0.053$,).

BNST activation is associated with AA TSST reactivity after controlling for CM activation

To test whether BNST NEGPOS > NEU contrast scores is associated with AA TSST responsivity over and above CM NEGPOS > NEU contrast scores, we ran regressions for each side using BNST NEGPOS > NEU contrast scores to predict AA reactivity, while controlling for the NEGPOS > NEU contrast in the ipsilateral CM. Age and gender were also controlled for in these regressions.

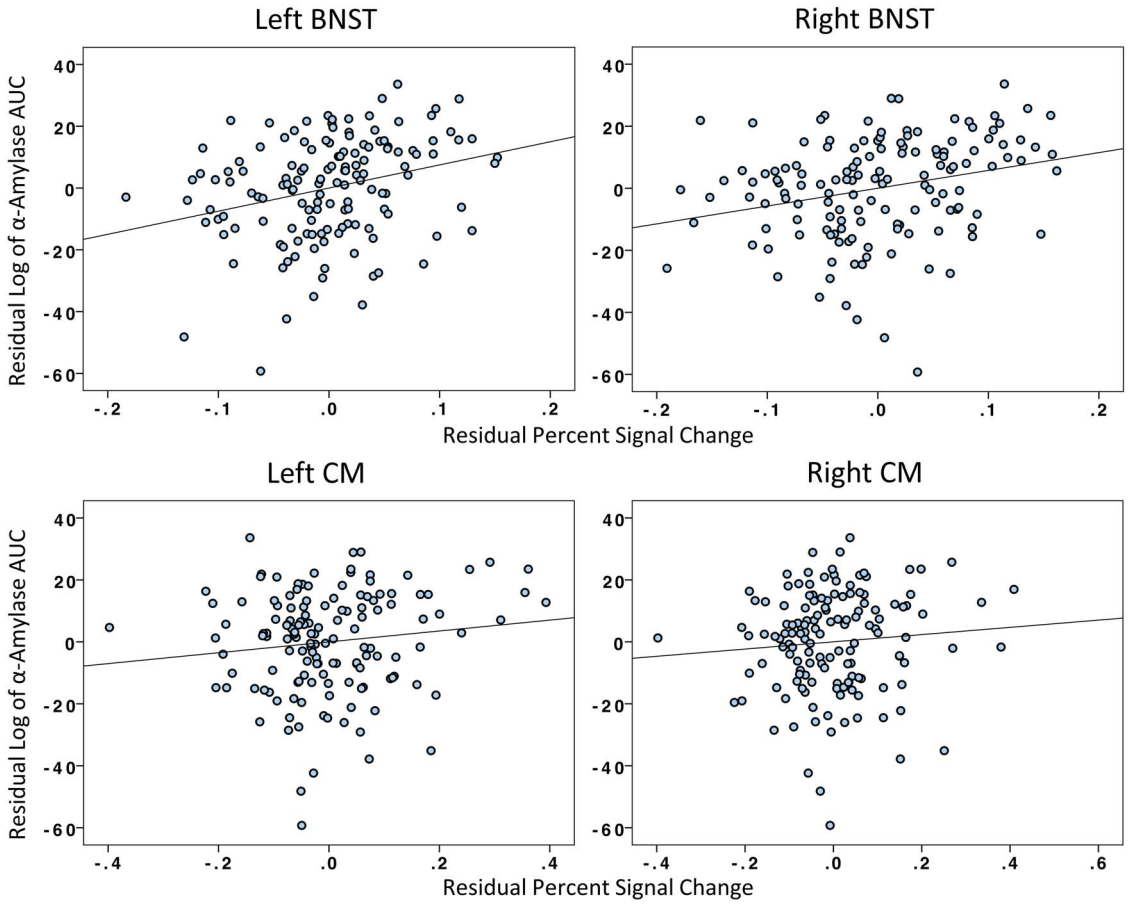

Fig. 2. Partial regression plots demonstrating that the NEGPOS > NEU contrast scores predict increased TSST-evoked salivary AA in both left, $\beta = 0.263$, $t = 3.432$, $P = 0.006$, $pr = 0.282$, and right BNST, right BNST, $\beta = 0.245$, $t = 3.193$, $P = 0.014$, $pr = 0.264$, controlling for age and gender. In contrast, CM NEGPOS > NEU scores do not predict TSST-evoked salivary AA. BNST NEGPOS > NEU scores continue to predict TSST-evoked salivary AA after controlling for NEGPOS>NEU scores in the ipsilateral CM (left BNST, $\beta = 0.266$, $t = 3.049$, $P = 0.006$, $pr = 0.254$; right BNST, $\beta = 0.244$, $t = 3.016$, $P = 0.006$, $pr = 0.251$).

Holm–Bonferroni correction was applied for the two main comparisons.

Bilateral BNST NEGPOS > NEU contrast scores remained significant predictors of AA TSST reactivity when controlling for CM NEGPOS > NEU contrast scores (left BNST, $\beta = 0.266$, $t = 3.049$, $P = 0.006$, $pr = 0.254$; right BNST, $\beta = 0.244$, $t = 3.016$, $P = 0.006$, $pr = 0.251$). Follow-up regressions demonstrated that the BNST response to both negative *vs* neutral (left BNST, $\beta = 0.217$, $t = 2.482$, $P = 0.028$, $pr = 0.209$; right BNST, $\beta = 0.213$, $t = 2.649$, $P = 0.018$, $pr = 0.222$) and positive *vs* neutral images (left BNST, $\beta = 0.216$, $t = 2.429$, $P = 0.028$, $pr = 0.205$; right BNST, $\beta = 0.214$, $t = 2.623$, $P = 0.018$, $pr = 0.22$) were associated with AA responsivity when controlling for the corresponding contrast scores in the CM. However, in the left BNST the effect of negative minus neutral activation predicting AA reactivity, while controlling for negative minus neutral CM activation was no longer significant after the removal of two outliers, $\beta = 0.136$, $t = 1.495$, $P = 0.137$, $pr = 0.129$.

Voxel-wise analysis

We supplemented ROI analyses with a voxel-wise approach to visualize the spatial distribution of reported effects. There were no regions where the NEG > POS contrast was significantly related to TSST-evoked cortisol or AA. There were also no regions where NEGPOS > NEU contrast was significantly related to TSST cortisol response. However, there was a cluster in the basal forebrain representing a significant relationship between NEGPOS > NEU activation and TSST-evoked AA. This cluster included distinct peaks centered on left and right BNST, respectively (Figure 3). This cluster also extended into the left nucleus accumbens, as well as the fornix and bilateral superior thalamus. While there was no relation between NEGPOS > NEU and TSST-evoked AA in the CM, there was a cluster of activation centered on the parahippocampal gyrus that extended into the margins of the basolateral amygdala. Figure 3 depicts this activation in the BNST and amygdala, whereas a statistical map for this contrast across the entire brain can be found in the supplemental materials.

Discussion

To examine the relationship between BNST activity and stress responsivity, we investigated the relationship between individual differences in BNST reactivity to emotionally evocative images and the magnitude of TSST-evoked stress hormone release. We predicted that greater BNST activation to emotionally evocative images would be associated with larger TSST-evoked cortisol and AA responses, and that the BNST response to negative images would be more strongly associated with TSST-evoked cortisol and AA responses than the response to positive images. While we expected CM reactivity to negative images to be associated with TSST-evoked cortisol and AA, we

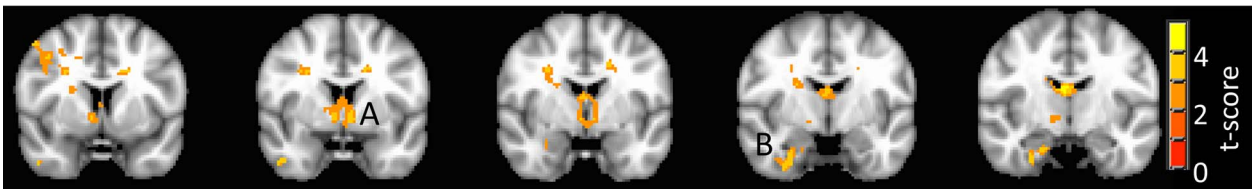

Fig. 3. Voxels of NEGPOS > NEU activation that are associated with the magnitude of the TSST-evoked AA response, controlling for age and gender. Clusters were controlled for multiple comparisons using threshold-free cluster enhancement ($P < 0.05$). Activation included distinct peaks centered on left and right BNST, respectively (A). While there was no relation between NEGPOS > NEU and TSST-evoked AA in the CM, there was a cluster of activation centered on the left parahippocampal gyrus that extended into the margins of the basolateral amygdala (B).

predicted that BNST reactivity to negative images would be associated with larger TSST-evoked cortisol and AA responses, even after controlling for CM reactivity.

We first tested whether the CM and BNST exhibited a response to emotionally evocative images. The CM had a greater response to negative *vs* neutral and positive *vs* neutral images. The CM also had a greater response to negative *vs* positive images. These observations are in accord with a meta-analysis which found that the amygdala responds to stimuli of both positive and negative valence but that it may also exhibit a larger response to some types of negative stimuli in comparison to positive, with fear and disgust stimuli eliciting more activation than happy or unspecified positive stimuli (Costafreda *et al.*, 2008). In contrast to our CM results, we found that the left BNST had a greater response to both negative *vs* neutral and positive *vs* neutral images, with no difference for negative *vs* positive. While past research has demonstrated that the BNST is involved in processing threat and reward (Lebow and Chen, 2016), little research has investigated the BNST response to positive and negative stimuli within the same study. Our results suggest that the BNST exhibits a response that is similar in magnitude for positive and negative stimuli, while the CM preferentially responds to negative stimuli. This does not necessarily imply that the BNST does not encode valence-specific information. While little is known about the functional significance of individual BNST subregions in humans, rodent research suggests functional diversity across BNST nuclei (Lebow and Chen, 2016). For example, a recent study suggests that neurons expressing corticotropin-releasing factor, located primarily in the lateral BNST, are involved in threat responding, while cholecystokinin-expressing neurons in the medial BNST may be more heavily involved in reward processes (Giardino *et al.*, 2018). Further research is needed to understand how different types of affective stimuli and stressors may be encoded by the BNST.

Our primary aim was to test whether individual differences in the BNST BOLD response to emotionally evocative stimuli is associated with the magnitude of the TSST-evoked stress response, as indexed by AA and cortisol. We found that greater activation to emotionally evocative images in both the left and right BNST predicted a larger TSST-evoked AA response. Additionally, BNST reactivity to emotional images predicted the TSST-evoked AA response over and above CM reactivity. As salivary AA levels are related to activation of the SNS, these findings indicate greater SNS stress activation in participants who exhibit greater BNST sensitivity to emotional stimuli. This suggests overlap between the BNST response to emotionally evocative images and its role in the stress response. We posit that the BNST response to emotionally evocative images may subserve its role in detecting and responding to threat. This finding is consistent with rodent research demonstrating that the BNST serves as an input to the stress system (Crane *et al.*, 2003; Spencer *et al.*, 2005) and that the BNST regulates the SNS (Davis *et al.*, 2010). While past studies have demonstrated that the human BNST responds to stressful stimuli (Straube *et al.*, 2007; Somerville *et al.*, 2010; Alvarez *et al.*, 2011; Shackman and Fox, 2016; Pedersen *et al.*, 2017) and negatively valenced images (Somerville *et al.*, 2013; Pedersen *et al.*, 2016), this is the first study, to our knowledge, demonstrating that human BNST activation is related to individual differences in the stress response as indexed by stress biomarkers.

Although we found that the BNST response to emotionally evocative images was associated with a greater AA TSST response, our prediction that BNST activation would be associated with the cortisol response was not supported. Past research suggests that viewing negatively valenced images provokes an AA response, but not a cortisol response (van Stegeren *et al.*, 2006). It may be that a more challenging or longer duration stimulus is needed to provoke a cortisol response (van Stegeren *et al.*, 2006). The anterior division of the BNST appears to be particularly involved in regulating the SNS (Crestani *et al.*, 2013). Thus, the emotionally evocative images used in the current study may have elicited activation from a subset of cells in the BNST that overlaps more with those that mediate the AA response to the TSST, than with those that would mediate the cortisol response. If this is the case, individual differences in BNST activation to a more challenging stimulus may be a more relevant predictor of the cortisol response. Future research should investigate whether BNST reactivity to a more challenging or more enduring stressor is associated with a larger cortisol response to stress.

We also predicted that BNST reactivity to negative images would predict the magnitude of the stress response more strongly than BNST reactivity to positive images. We found mixed evidence for this hypothesis. When controlling for positive *vs* neutral activation, there was a trend-level association between negative *vs* neutral activation and the AA response in the left, but not right BNST. The BNST has been implicated in both threat and reward processing (Jalabert *et al.*, 2009; Lebow and Chen, 2016), and populations of cells that differentially respond to threat and reward likely exist (Daniel and Rainnie, 2016; Ch'ng *et al.*, 2018). Similarly, rodent research suggests that different BNST subregions may play different roles in stress responding (Lebow and Chen, 2016). For example, activation of the anteroventral BNST promotes the HPA axis stress response, while posterior BNST activation has the opposite effect (Herman *et al.*, 2005). Furthermore, while positive affect can be accompanied by SNS activation (Kreibig, 2010), it is unclear whether the BNST plays a role in regulating the SNS during positive states. Our results highlight the need for further research on the possible role of the BNST in mediating the physiological response to positive stimuli. Additionally, high-resolution imaging and perhaps advancements in neuroimaging methods are needed to detail the complex role the human BNST likely plays in the stress response.

In contrast to the BNST, we found no relationship between CM reactivity and either the cortisol or AA response to the TSST. One possible explanation for this is that the BNST may be more central in mediating the response to social stress than the CM. The Davis *et al.* (2010) model predicts that the BNST mediates the response to sustained and uncertain threat (i.e. the anxiety response), while the CeA mediates the response to more immediate danger (i.e. the fear response). While social stress is a complex stimulus that likely elicits some mixture of these responses, it may provoke anxiety more heavily than fear. We expected activation to negatively valenced stimuli in these regions to associate with the magnitude of the stress response, based on the premise that the response to negative stimuli in these regions may subserve their role in detecting and responding to threat. Based on this framework, if the BNST is more central in responding to social threat than the CM, BNST activation to emotionally evocative stimuli may be a more relevant predictor of stress responsivity to social threat. Further research is needed to clarify the roles of the CM and BNST in the response to social stress.

Our results are relevant to future research on psychopathology, as dysregulation of the stress system is thought to be critical in the pathogenesis of mood and anxiety disorders (Pariante and Lightman, 2008; Faravelli *et al.*, 2012). In addition to dysregulation of the HPA axis, these disorders are marked by dysregulation of the SNS response to stress and altered levels of AA (van Veen *et al.*, 2008; Nater and Rohleder, 2009; Ishitobi *et al.*, 2010; Veen *et al.*, 2013). While the amygdala has been heavily studied in relation to psychopathology (Anand and Shekhar, 2003; Drevets, 2003), the role of the BNST has been largely overlooked in human research. This is the first study to establish a correlation between activation in the human BNST and the hormonal stress response. We found no evidence for a similar effect in the CM. These results are consistent with the Davis *et al.* (2010) model, which suggests that the BNST may mediate the stress response to stimuli that are sustained, distant or unpredictable, rather than those that pose an immediate danger. Further research on the role of the human BNST in the stress response may prove critical in understanding the dysregulation of the stress system that accompanies mood and anxiety disorders (Pariante and Lightman, 2008; Faravelli *et al.*, 2012).

Supplementary data

Supplementary data are available at SCAN online.

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Conflicts of interest

Dr Richard J. Davidson is the founder and president and serves on the board of directors for the non-profit organization, Healthy Minds Innovations, Inc. In addition, Dr Davidson served on the board of directors for the Mind & Life Institute from 1992 to 2017. No donors, either anonymous or identified, have participated in the design, conduct or reporting of research results in this manuscript.

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