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Original article

The effects of stimulus novelty and negativity on BOLD activity in the amygdala, hippocampus, and bed nucleus of the stria terminalis

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Abstract

The amygdala responds to stimulus novelty, which may correspond to an evaluation of novel stimuli for potential threat, and trait anxiety may modulate this response. The bed nucleus of the stria terminalis (BNST) may also be sensitive to novelty as it responds to both uncertainty and threat. If so, a BNST novelty response may also be affected by trait anxiety and interact with stimulus negativity. We presented participants with novel and repeated negative and neutral images while measuring brain activity via fMRI, and assessed participants' self-reported trait anxiety. We expected to replicate past findings of novelty responses in the hippocampus and amygdala that are independent of stimulus negativity. We also expected BNST novelty-sensitivity and that trait anxiety would predict greater sensitivity to both novelty and negativity in the amygdala and BNST, but not the hippocampus. Our *a priori* analyses replicated past findings of a novelty response that was independent of valence in the hippocampus and amygdala. The BNST exhibited a novelty response for negative, but not neutral, images. Trait anxiety did not modulate the response to novelty or negativity in any of the ROIs investigated. Our findings suggest that the BNST plays a role in the detection of novelty.

Key words: novelty; bed nucleus of the stria terminalis; BNST; amygdale; fMRI; BST

Introduction

The detection of novelty is an important function of the brain, as novel stimuli represent a potential source of reward or threat that requires evaluation. While past research has established that the hippocampus (Tulving *et al.*, 1996; Grunwald *et al.*, 1998; Menon *et al.*, 2000; Daselaar *et al.*, 2006; Kirwan *et al.*, 2009; Lever *et al.*, 2010) and amygdala (Schwartz *et al.*, 2003; Wright *et al.*, 2003; Wright *et al.*, 2008; Blackford *et al.*, 2010; Balderston *et al.*, 2011; Ousdal *et al.*, 2014) are involved in novelty detection, this has not been examined in the bed nucleus of the stria terminalis (BNST). Given that the BNST plays a role in vigilance toward threat (Somerville *et al.*, 2010) and works with the amygdala to regulate the expression of anxious behaviors in rodents (Pêgo *et al.*, 2008), this component of the extended amygdala (Davis and Whalen, 2001) may also play a role in novelty detection.

Early studies on novelty detection in humans focused on the hippocampus, showing preferential recruitment for novel stimuli (Tulving *et al.*, 1996; Grunwald *et al.*, 1998; Menon *et al.*, 2000; Daselaar *et al.*, 2006; Kirwan *et al.*, 2009; Lever *et al.*, 2010). However, other studies have found that the amygdala is also activated in response to novel faces (Schwartz *et al.*, 2003; Wright *et al.*, 2003; Wright *et al.*, 2008; Balderston *et al.*, 2013; Ousdal *et al.*, 2014), images of humans (Balderston *et al.*, 2011) and objects (Blackford *et al.*, 2010). Amygdala responses to

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novelty are compatible with conceptualizations of the amygdala that emphasize its role in vigilance toward signals of threat or reward (Davis and Whalen, 2001). Balderston *et al.* (2011; 2013) posited that while the hippocampus plays a general role in novelty detection regardless of stimulus type or context, the amygdala responds to novelty as part of its role in the evaluation of potential threat, and therefore responds to novelty only with stimuli that are biologically relevant, or when primed by recent signals of threat. Surprisingly, however, once the amygdala has been primed by recent signals of threat, it seems to respond to novelty independent of stimulus negativity (Balderston *et al.*, 2013), suggesting that once threat has been detected in the environment, the amygdala may become sensitive to the novelty of all types of stimuli, regardless of whether the stimulus is neutral or negatively-valenced.

Increased amygdala reactivity to negatively-valenced stimuli in participants with high trait anxiety is well documented (Etkin *et al.*, 2004; Stein *et al.*, 2007; Fakra *et al.*, 2009; Hariri, 2009; Hariri *et al.*, 2009; Laeger *et al.*, 2012), and is likely related to the hypervigilance to threat seen in anxiety (see Cisler and Koster, 2010). If the amygdala response to novelty reflects an evaluation of novel stimuli for potential threat, this response may vary with trait anxiety. The amygdala responds to uncertainty (Sarinopoulos *et al.*, 2010; Williams *et al.*, 2015), and uncertainty is related to both anxiety (Grupe and Nitschke, 2013) and novelty, as novel stimuli require evaluation for potential threat due to the inherent uncertainty of their meaning or significance. Rodent research has implicated the amygdala in neophobic behaviors (Hughes, 2007), including avoidance of novel conspecifics (Gonzalez *et al.*, 1996; Navarro *et al.*, 2004). Additionally, neuroimaging studies have found increased amygdala reactivity toward novelty in participants high in inhibited temperament (Schwartz *et al.*, 2003; Blackford *et al.*, 2011; Blackford *et al.*, 2013)–a personality trait that overlaps with trait anxiety and is a risk factor for anxiety disorders (Fox *et al.*, 2005; Degnan *et al.*, 2010). These findings suggest that trait anxiety may be associated with modulation of amygdala reactivity to novel stimuli.

Although the BNST is under-studied in humans, this is another area that could be expected to exhibit an anxiety-modulated novelty response. Like the amygdala, the BNST is sensitive to threat (Alvarez *et al.*, 2011; Somerville *et al.*, 2013), and is associated with hypervigilant threat monitoring in high trait anxiety (Somerville *et al.*, 2010). The BNST is also activated by conditions of uncertainty (Somerville *et al.*, 2013), and exhibits increased reactivity to uncertainty in patients with generalized anxiety disorder (Yassa *et al.*, 2012), which suggests that novelty–which is accompanied by uncertainty–may also elicit a BNST response. Additionally, the BNST and amygdala-BNST connectivity have been implicated in anxiety-like behaviors in rodents (Pêgo *et al.*, 2008; Cai *et al.*, 2012), including avoidance of novel conspecifics (Khoshbouei *et al.*, 2002; Lungwitz *et al.*, 2012). Given these findings, the BNST may play a role in the detection of novelty, and this sensitivity toward novelty may be modulated by trait anxiety.

To test how trait anxiety interacts with stimulus novelty to affect activation in the hippocampus, amygdala and BNST, we presented participants with novel and repeated images while measuring brain activity via fMRI, and assessed participants' self-reported trait anxiety. We also examined how stimulus negativity interacts with novelty and trait anxiety. Thus, half of the images we presented were neutral and half were negatively-valenced.

We expected to replicate past studies showing a novelty effect in the hippocampus (Tulving *et al.*, 1996; Grunwald *et al.*, 1998; Menon *et al.*, 2000; Daselaar *et al.*, 2006; Kirwan *et al.*, 2009; Lever *et al.*, 2010) and amygdala (Schwartz *et al.*, 2003; Wright *et al.*, 2003; Wright *et al.*, 2008; Blackford *et al.*, 2010; Balderston *et al.*, 2011; Balderston *et al.*, 2013; Ousdal *et al.*, 2014). Based on Balderston *et al.*'s (2011; 2013) findings, we predicted no interaction between novelty and stimulus negativity in the hippocampus and/or amygdala.

We predicted that the BNST would be sensitive to stimulus novelty. We did not have strong predictions for how this effect may interact with stimulus negativity. We reasoned that because the BNST plays a role in threat detection (Somerville *et al.*, 2010; Alvarez *et al.*, 2011), a BNST response to novelty may be dependent on stimulus negativity. However, given that the same argument could be made for the amygdala, and that the amygdala novelty response does not appear to be dependent on stimulus negativity (Balderston *et al.*, 2011; Balderston *et al.*, 2013), we anticipated that this may also be the case for the BNST.

Based on the hypothesis that the hippocampus exhibits a general novelty response, while the amygdala and BNST novelty responses reflect evaluation of novel stimuli for threat, we predicted that the amygdala and BNST would show greater novelty responses in participants with high trait anxiety, while the hippocampus novelty response would not be modulated by trait anxiety. Additionally, based on past studies, we also predicted participants high in trait anxiety would have greater amygdala (Etkin *et al.*, 2004; Fakra *et al.*, 2009; Hariri *et al.*, 2009; Stein *et al.*, 2007; Laeger *et al.*, 2012) and BNST (Yassa *et al.*, 2012) reactivity to stimulus negativity.

Materials and methods

Participants

One hundred and eleven participants underwent fMRI scanning at the Medical College of Wisconsin. Seventeen participants were excluded from data analysis due to excessive movement artifacts, and one participant was excluded from analysis because they did not complete the trait anxiety measure. Our final sample consisted of 93 participants (62 female) with a mean age of 22.2 (SD = 3.96). Participants provided informed consent and were given monetary compensation for participation.

Trait anxiety measure

Participants completed the State-Trait Anxiety Inventory (STAI-Trait), a 20-item measure designed to evaluate trait anxiety (Spielberger *et al.*, 1970). The STAI-Trait has high internal consistency ($\alpha = 0.89$) and test-retest reliability ($r = 0.88$; Barnes *et al.*, 2002). Participants' STAI-Trait scores ranged from 21 to 66 ($m = 39.3$, SD = 11.09).

Task design

The task used a 2 x 2 factorial event-related design, with participants viewing a series of images that varied along the factors of Novelty (novel *vs* repeated) and Negativity (negative *vs* neutral). Ten negatively-valenced (half human, half non-human scenes) and ten neutral (also half including humans) images were presented. For each participant one human and one non-human image of each stimulus negativity type was randomly selected to be repeated five times (in addition to initial presentation), and the rest of the images were presented only once. The novel conditions consisted of the presentation of the four non-repeating images, as well as the initial presentations of the images selected to be repeated for a given stimulus negativity

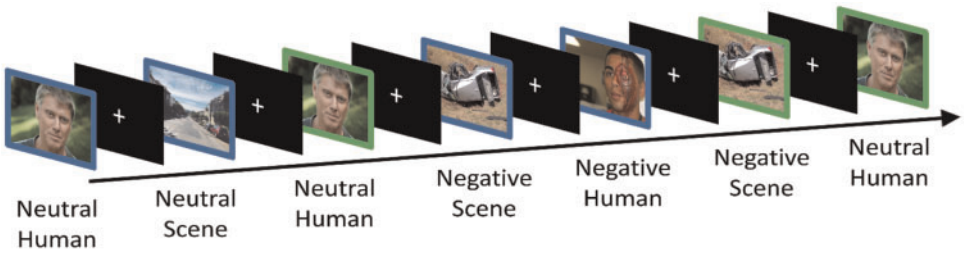

Fig. 1. Task design. Participants were shown novel (blue) and repeated (green) images that were either negatively-valenced or neutral. Images were presented for 3 s each and were followed by a jittered three to 7 s ITI. Note that images that were repeated were counted as novel on their first presentation and counted as repeated on all subsequent presentations. Due to the IAPS user agreement, photos from the public domain have been used in this figure, rather than images used in the study.

type. The repeated conditions consisted of the five repetitions of the repeated images with the initial presentation excluded.

Images were taken from the International Affective Picture System (IAPS; Lang *et al.*, 2008). Neutral images included images of healthy individuals with their faces visible, and scenes, such as suburban neighborhoods or living spaces. Negatively-valenced images included mutilated bodies, vehicular accidents, a dirty toilet, pictures of dead animals and a KKK ceremony. Valence and arousal ratings from the IAPS norms were used to select negatively-valenced *vs* neutral images. Stimuli intended to elicit negative emotional responses had a mean valence of 1.88 (SD = 0.24) and arousal of 6.58 (SD = 0.18). Neutral images had a mean valence of 5.22 (SD = 0.18) and arousal of 3.31 (SD = 0.17).

All images were presented in a single run for 3 s each, with a jittered intertrial interval of 3–7 s. The order of trial types was selected to minimize collinearity between regressors in the fMRI analysis. Thus, the same trial type order was used for each participant. (Figure 1) depicts the task design.

MRI data acquisition

MRI data were acquired on a 3 Tesla General Electric (GE Healthcare, Waukesha, WI) scanner. High-resolution T_1-weighted whole-brain anatomical images were acquired using a spoiled gradient-recalled echo sequence (inversion time/repetition time/echo time/flip angle/field of view/matrix/slice thickness: 450 ms/8.2 ms/3.2 ms/12°/240 mm/256 × 224/1 mm). Whole-brain functional scans were obtained using a T_2^*-weighted echo-planar image (EPI) sequence (repetition time/echo time/flip angle/number of excitations/field of view/matrix: 2000 ms/25 ms/77°/1/240 mm/64 × 64; 41 × 3.5-mm sagittal slices; gap: 0 mm; 166 volumes). To ensure that participants were paying attention to the images, they were instructed to press a button each time an image was presented.

Anatomical ROIs

Because our hypotheses concerned how the amygdala, hippocampus and BNST respond to novelty, our primary analysis focused on anatomically defined regions of interest (ROIs) for these regions. The supplement presents areas of significant activation emerging from whole brain analyses.

Amygdala and hippocampus ROIs were created for each participant using Freesurfer image analysis suite's subcortical segmentation (v. 5.0, Martinos Center for Biomedical Imaging, Harvard-MIT, Boston, USA; Fischl *et al.*, 2002). Volumes generated by Freesurfer were aligned to native space using AFNI. These ROIs were then visually inspected to ensure accurate segmentation and alignment. BNST ROIs were traced by hand in AFNI using the anatomical boundaries detailed by Avery *et al.* (2014; see Figure 2). On average, the left BNST ROI had a volume of 55.53 mm^3 and had a relative volume (absolute volume/intracranial contents*100) of .00367%, while the right BNST ROI had a volume of 56.1 mm^3 and had a relative volume of 0.00374%.

FMRI data analysis

FMRI data were analyzed using the Analysis of Functional NeuroImages software package (AFNI; Cox, 1996). The first 3 volumes were discarded to allow for scanner equilibration and volumes with excessive motion were censored. Remaining EPI volumes were slice time corrected, motion corrected, aligned to the anatomical volume, and converted to percent signal change. Single subject BOLD responses at stimulus onset were modeled using GLM and a 14 s tent function, with eight tents. Regressors for each of the four condition types (novel neutral, repeated neutral, novel negative and repeated negative) were included, as well as nuisance regressors for low-frequency drift (linear, quadratic and cubic) and motion (L/R, A/P, S/I, roll, pitch, yaw, and their derivatives). An average of tents 3–6 (representing peak activation) for each condition type was computed for each voxel. For each ROI, a weighted-average of this value for every voxel–with percent overlap between a given functional voxel and the high resolution ROI mask serving as the weight–within that ROI was extracted for statistical analysis.

Statistical analysis

For each participant average percent signal change was extracted for each condition for each ROI. Observations greater than 3.5 standard deviations from the mean for that trial type were considered outliers and replaced with the nearest non-outlier value. Outliers made up 0.6% of observations. A linear mixed model was used to analyze the data, with ROI (amygdala *vs* hippocampus *vs* BNST), Novelty (novel *vs* repeated), Negativity (negative *vs* neutral), Anxiety (STAI-trait scores), and Side (left *vs* right) as fixed factors and Participant as a random factor. In the initial model, parameters were estimated for all fixed factors, as well as interactions between them. ROI was included as a fixed factor in this model to test whether activation patterns significantly differed across the three ROIs.

Because experiments using a similar task found that the responses to novelty and negativity in the hippocampus and amygdala were not lateralized, we first tested whether removing Side from the model improved fit, as measured by second order Akaike information criterion (AICc). Intercept-only models were used for these comparisons. Once the set of fixed effects was determined, inclusion of random effects was determined using the process described by Bates *et al.*, (2015), by beginning with the maximal set of random effects, eliminating correlation parameters between random effects if the model failed to

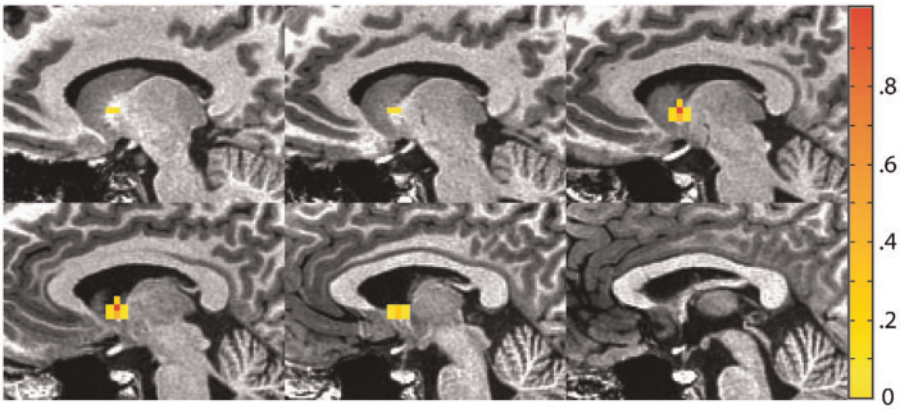

Fig. 2. Example of fMRI voxels inculed in BNST ROI. A weighted averages of BOLD activity was extracted with each fMRI voxel weighted by proportion of overlap with the anatomical BNST ROI that was created using the boundaries detailed by Avery *et al.*, (2014). Voxel color denotes weight used in weighted-average.

converge, and removing non-significant random effects (as determined by likelihood ratio tests) when principle components analysis suggested over-parameterization of the model (via the R package RePsychLing; Baayen *et al.*, 2015).

Models were estimated using the R package lme4 (Bates *et al.*, 2015). Significance of main and interaction effects were assessed using Type II Wald Chi-squared tests using the R package car (Fox and Weisberg, 2011). Follow-up tests were obtained using general linear hypothesis tests with the R package phia (De Rosario-Martinez, 2015).

Because one goal of the study was to test *a priori* predictions about how activity in each of the selected ROIs respond to novelty, negativity and trait anxiety, we also created a model with Side (left *vs* right), Novelty (novel *vs* repeated), Negativity (negative *vs* neutral) and Anxiety (STAI-trait scores) as fixed factors and Participant as a random factor, for each ROI (BNST, amygdala and hippocampus).

Results

Full model

We started by fitting a model with ROI, Novelty, Negativity, Anxiety and Side as fixed factors, and then tested whether excluding Side led to better model fit, as measured by AICc. The full model had worse fit (AICc = –678.5) than the same model with Side removed (AICc = –719), thus our final model involved the fixed factors of ROI, Novelty, Negativity and Anxiety, with Participant as a random factor. As the model did not converge using the maximal set of random effects, correlations between random effects were set to zero. Next, random effects with smallest variances were removed iteratively starting with high-order interactions when PCA suggested over-parameterization, using likelihood ratio tests at each step to check that parameter removal did not result in a significant decrease in model fit. This procedure resulted in random slopes for Novelty, Negativity, ROI, Anxiety, Novelty x Negativity, Novelty x Anxiety and Novelty x Negativity x Anxiety. When correlation parameters were added between these random effects, the model again failed to converge. As such, correlations between parameters were excluded from the model.

In this model, novel images elicited more activity than repeated images, $X^2(1) = 79.91$, $P < 0.001$ and negative images elicited more activity than neutral images, $X^2(1) = 45.65$, $P < 0.001$. There was also a significant Novelty x Negativity interaction, $X^2(3) = 7.88$, $P = 0.005$, with a greater effect of Novelty for negative, $b = 0.094$, $X^2(1) = 79.9$, $P < 0.001$, than for neutral images, $b = 0.053$, $X^2(1) = 18.04$, $P < 0.001$. There was also a significant ROI x Anxiety effect, $X^2(2) = 8.06$, $P < 0.018$. Follow-up tests suggested a trend toward a less negative slope of Anxiety in the hippocampus ($b = –0.0004$) than the BNST ($b = –0.004$), $X^2(1) = 5.27$, $P = 0.065$, with no differences in the slope of anxiety between either the amygdala and BNST, or the amygdala and hippocampus ($ps > 0.34$; Holm–Bonferroni corrected). There were no other significant effects involving Anxiety or ROI ($ps > 0.1$). Condition means for each ROI can be seen in (Figure 3).

Results by ROI

Amygdala. To test *a priori* predictions and replicate past findings that the amygdala exhibits independent effects of novelty and stimulus negativity, we created a mixed linear model with Side (left *vs* right), Novelty (novel *vs* repeated), Negativity (negative *vs* neutral) and Anxiety (STAI-trait scores) as fixed factors and Participant as a random factor using amygdala activity as the dependent variable.

As in the full model, we first tested whether Side improved model fit. The model including Side as a factor had worse fit (AICc = 9.1) than the same model with Side removed (AICc = –5.2). As such, Side was not included in the model used for estimating effects. The model converged only after setting correlations between random effects to zero, after which PCA indicated over-parameterization. After removing several non-significant random effects, likelihood ratio tests suggested a loss of fit from removing additional parameters. The result was a model including random slopes for Novelty, Negativity, Anxiety, Novelty x Negativity and Novelty x Anxiety.

In the amygdala, novel images elicited more activity than repeated images, $X^2(1) = 27.66$, $P < 0.001$, and negative images elicited more activity than neutral images, $X^2(1) = 17.67$, $P < 0.001$. There was no interaction between Novelty and Negativity in the amygdala, $X^2(1) = 2.03$, $P = 0.15$, and no effects involving Anxiety ($ps > 0.23$).

Hippocampus. We also created a mixed linear model with Side (left *vs* right), Novelty (novel *vs* repeated), Negativity (negative *vs* neutral) and Anxiety (STAI-trait scores) as fixed factors and Participant as a random factor using hippocampus activity as the dependent variable, in order to test our *a priori* predictions and replicate past findings that the hippocampus would exhibit a novelty response that did not depend on stimulus negativity. Model fit was worse when Side was included (AICc = –901.8) than when it was removed (AICc = –916.3). As a

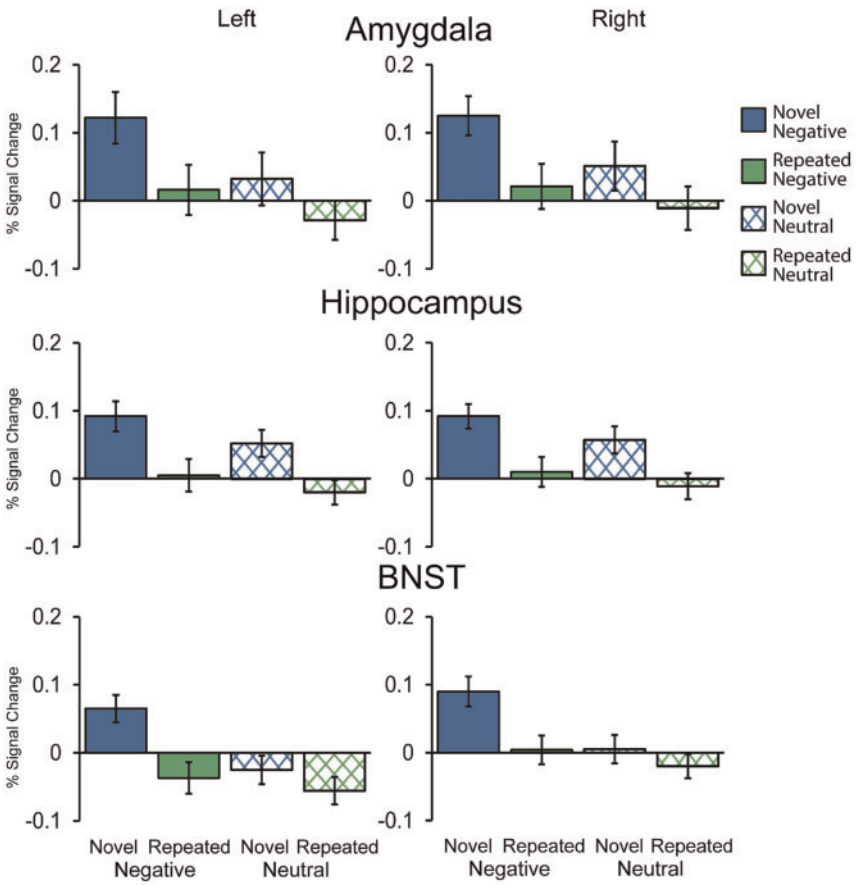

Fig. 3. BOLD response for novel (blue) and repeated (green), negatively-valenced (solid) and neutral images (cross-hatched) for left and right amygdalae, hippocampi and BNST. Error bars depict standard error. BNST= bed nucleus of the stria terminalis.

result Side was removed from the model. The model converged only after setting correlations between random effects to zero. Removing non-significant effects, as guided by PCA, resulted in the inclusion of random slopes for Novelty, Negativity, Anxiety and Novelty x Negativity.

The hippocampus exhibited a greater response toward novel than repeated images, $X^2(1) = 66.06$, $P < 0.001$, as well as a greater response toward negative than to neutral images, $X^2(1) = 15.79$, $P < 0.001$. There was no interaction between Novelty and Negativity in the hippocampus, $X^2(1) = 0.87$, $P = 0.35$. There were also no significant effects involving Anxiety, ($ps > 0.35$).

BNST. To test our *a priori* hypotheses that the BNST would exhibit sensitivity to novelty and stimulus negativity, and that novelty-sensitivity in the BNST would be modulated by trait anxiety, we created a mixed linear model with Side (left *vs* right), Novelty (novel *vs* repeated), Negativity (negative *vs* neutral) and Anxiety (STAI-trait scores) as fixed factors and Participant as a random factor using BNST activity as the dependent variable. The model (AICc = –603.8) had better fit when Side was removed as a factor (AICc = –608.7). The model converged only after setting correlations between random effects to zero. PCA mixed with likelihood ratio tests suggested that the maximal random effects structure was over-parameterized. Iterative reduction of the model led to the inclusion of random slopes for Novelty and Negativity only.

This model revealed a greater response toward novel than repeated images, $X^2(1) = 36.56$, $P < 0.001$, and a greater response toward negative than neutral images, $X^2(1) = 29.57$, $P < 0.001$. There was also a significant interaction between Novelty and Negativity, $X^2(1) = 10.63$, $P = 0.001$, with a greater effect of Novelty for negative, $b = 0.09$, $X^2(1) = 43.31$, $P < 0.001$ than for neutral images, $b = 0.03$, $X^2(1) = 3.88$, $P = 0.049$ (Holm–Bonferroni corrected). There was also a significant main effect of Anxiety in the BNST, $X^2(1) = 5.58$, $P = 0.02$, with increasing anxiety associated with less BNST activation ($b = –0.003$). There were no other effects involving Anxiety in the BNST ($ps > 0.16$).

Effects of novelty are not due to habituation

Our results replicate past findings of a greater BOLD response to novel stimuli in the amygdala and hippocampus (Balderston *et al.*, 2011), and extend this finding to the BNST. However, the effects we have reported involve a comparison of novel stimuli against stimuli repeated several times. This design does not allow us to test if these effects were due specifically to novelty (i.e. an initial decline in BOLD response from the first to second presentation of a stimulus, followed by a similar BOLD response for every repetition thereafter) as opposed to habituation (i.e. a gradual decline across repetitions).

To investigate BOLD responses across multiple repetitions of the same stimulus, we ran an analysis similar to the one we described previously, but including a regressor for each individual trial, rather than each trial type. We did so using the least squares–separate method described by Mumford *et al.*, (2012). Estimation of BOLD activation for each trial involves introducing many more regressors into the model. To reduce the number of regressors in our model and minimize issues due

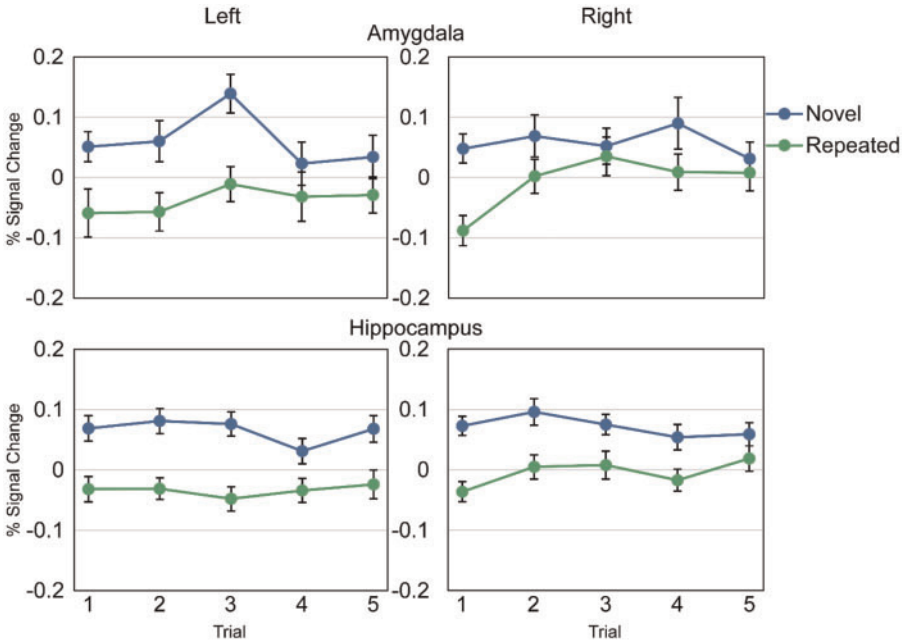

Fig. 4. BOLD activation for novel (blue) and repeated (green) images plotted by trial number for left and right hippocampi and amygdalae. Analysis revealed no novelty by trial interaction, suggesting true novelty effects, not habituation.

collinearity, we used a gamma function (which uses one regressor per stimulus of interest), rather than using a tent function (which uses several regressors for each stimulus, one for each tent pole). Regression failed for 6 participants, due to collinearity issues arising from the censoring of EPI volumes containing excessive motion, resulting in 87 participants being used in this analysis. Observations greater than 3.5 standard deviations from the mean for that trial type were considered outliers and replaced with the nearest non-outlier value. Outliers made up 1.4% of observations.

We fitted this data to a linear mixed model including Trial as a factor (ROI x Novelty x Negativity x Side x Trial). The Trial factor in this model represents the order of presentation for that condition type. Because two images were presented six times within each negativity condition, each presentation of these two images after the first (the second through sixth) were averaged together for both repeated neutral and repeated negative images. For novel images, the five trial conditions consisted of the five presentations of each set of novel images (including the first presentation of each to be repeated image) that were matched for content with the repeated images averaged together. Thus, for each novelty-negativity condition type, Trial consisted of the order of five presentations of humans and five presentations of scenes, with the repeated condition consisting of the same image, and the novel condition consisting of presentations of different images. If habituation were driving our findings, this would manifest itself in a Novelty x Trial interaction, with the BOLD response to novel images remaining steady throughout each presentation, but the BOLD response to repeated images decreasing with each subsequent repetition. In a true novelty effect, however, there would be a difference in BOLD response for novel and repeated images (i.e. a main effect of Novelty), but the response for repetitions beyond the second would be similar to each other (i.e. no Novelty x Trial interaction).

Before examining the individual effects within this model, we first sought to create a parsimonious model by testing whether the inclusion of Side led to better model fit. When Side was excluded from this model the fit was better (AICc = 8015.8), than when Side was included (AICc = 8150.9). As such, we included only ROI, Novelty, Negativity and Trial as fixed factors in this model.

As the model did not converge with the maximal random factor structure, correlation parameters between random slopes were set to zero. The model still did not converge, so third-order, second-order and first-order interactions were removed iteratively, which did allow the model to converge. As a result, our final model contained random slopes for main effects, but no random slopes for interactions effects, and no correlation parameters.

In this model, there was no significant main effect of Trial, $X^2(4) = 7.2$, $P = 0.13$. There was a significant Novelty x Trial effect, $X^2(4) = 10.35$, $P = 0.03$. For pairwise comparisons between Trial types within Novelty, there was a significantly higher response to repeated images on the fifth *vs* the first repetition (excluding the initial presentation), $X^2(1) = 11.34$, $P = 0.015$, as well as a possible trend toward a greater response to repeated image on the third *vs* the first repetition, $X^2(1) = 7.86$, $P = 0.1$ (Holm–Bonferroni corrected). These were the only significant pairwise comparisons, even without correction for multiple comparisons (all other $ps > 0.09$, uncorrected). As these effects run in the opposite direction as would be expected for a habituation effect, this suggests that sensitivity to novelty in our ROIs was not due to habituation. The means for novel and repeated images by trial for the amygdala and hippocampus can be seen in (Figure 4) and in (Figure 5) for the BNST.

There was also a significant Trial x Negativity interaction, $X^2(4) = 22.28$, $P < 0.001$. Pairwise comparisons for Trial within Negativity types revealed a greater response to negative images on the second *vs* first trial, $X^2(1) = 10.18$, $P = 0.03$, as well as a trend toward a greater response for negative images on the second *vs* the fourth trial, $X^2(1) = 8.21$, $P = 0.08$ (Holm–Bonferroni corrected). None of the other pairwise comparisons between Trial conditions within Negativity types were significant

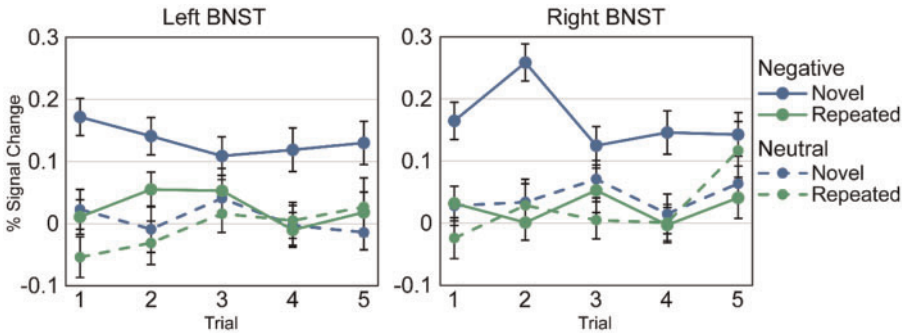

Fig. 5. BOLD activation for novel (blue) and repeated (green) negatively-vzalenced (solid) and neutral (dashed) images plotted by trial number for left and right BNST. While the bilateral novelty effect in the BNST applies only to negative images, analysis suggests that the greater BOLD response to novel *vs* repeated negative images is not due to habituation. BNST = bed nucleus of the stria terminals.

(*ps* > 0.09, Holm–Bonferroni corrected). There were no other interactions involving Trial (*ps* > 0.23).

There was also a significant Novelty x Negativity x ROI interaction within this analysis, $X^2(2) = 7$, $P = 0.03$, with pairwise comparisons between novel and repeated conditions within ROI and Negativity showing significant differences for both negative and neutral images for the hippocampus and amygdala (*ps* < 0.004), while the BNST exhibited a novelty response for negative, $X^2(1) = 47$, $P < 0.001$, but not neutral images, $X^2(1) = 0.9$, $P = 0.344$ (Holm–Bonferroni corrected). Although this interaction was not significant in the main analysis, it may be that this interaction became significant once the variance due to Trial was accounted for in the model.

Discussion

This study adds to the body of research demonstrating novelty-sensitivity in both the hippocampus (Tulving *et al.*, 1996; Grunwald *et al.*, 1998; Menon *et al.*, 2000; Daselaar *et al.*, 2006; Kirwan *et al.*, 2009; Lever *et al.*, 2010; Balderston *et al.*, 2011; Balderston *et al.*, 2013) and amygdala (Schwartz *et al.*, 2003; Wright *et al.*, 2003; Wright *et al.*, 2008; Blackford *et al.*, 2010; Balderston *et al.*, 2011; Balderston *et al.*, 2013; Ousdal *et al.*, 2014), and supports findings that this effect is not due to habituation across multiple repetitions (Balderston *et al.*, 2011). Our results also support Balderston *et al.*'s (2011; 2013) unexpected finding that the effects of novelty and stimulus negativity do not interact in the amygdala, as well as the hippocampus, although the independence of these variables was not strong enough to distinguish amygdala and hippocampus activity from BNST activity. That is, when ROI was included as a factor, there was a novelty by negativity interaction that did not interact with ROI.

Although past studies on the neural response to novelty have not focused on the BNST, we predicted a BNST novelty response based on its close relationship to the amygdala (Davis and Whalen, 2001), its sensitivity to uncertainty (Yassa *et al.*, 2012; Somerville *et al.*, 2013), and its role in anxious behaviors (Pêgo *et al.*, 2008; Khoshbouei *et al.*, 2002; Lungwitz *et al.*, 2012) and disorders (Yassa *et al.*, 2012). Our results indicate a novelty effect in the BNST that is not due to gradual habituation across multiple repetitions. Given the interconnections between BNST and amygdala (Avery *et al.*, 2014; Krüger *et al.*, 2015), these areas likely play related roles in novelty detection.

The omnibus analysis indicated that there was no Novelty x Negativity x ROI interaction, suggesting similar responses to novel and negative stimuli across ROIs. However, the *a priori* ROI specific analysis, and the analysis that included Trial as a factor, suggested that the BNST may be particularly sensitive to novel negative stimuli. The ability to detect novelty in a valence-specific manner is likely important, as sources of potential threat that have not been previously encountered may be especially important to evaluate and respond to.

Many human BNST studies have emphasized its role in responding to periods of prolonged threat anticipation, as opposed to transient threat (Somerville *et al.*, 2010; Alvarez *et al.*, 2011; Yassa *et al.*, 2012; Somerville *et al.*, 2013). We have found BNST activation in response to discrete, briefly presented stimuli. It should be noted, however, that our experiment presented negative and neutral images within a single block, with no cues to signal the negativity of upcoming images. As this design involves uncertainty about the negativity of upcoming images, it may have elicited anxious anticipation. Thus, while our results do suggest that the BNST is sensitive to the negativity of discrete stimuli, this may only occur in the context of anxious anticipation. These results differ from Alvarez, *et al.* (2011), who found BNST activation during signals of sustained, but not transient threat, during anxious anticipation. On the other hand, (Somerville *et al.*, 2013) found activation to briefly presented stimuli in a context of anxious anticipation in a cluster that contained BNST, but–in contrast to our findings–this area was not sensitive to stimulus negativity.

One difference between many past human BNST studies and the current study is the use of a whole brain approach in analyzing the BOLD signal as opposed to creating BNST ROIs based on individual participant anatomy. As the BNST is a small area, the blurring and correction for multiple comparisons that accompany the whole brain approach likely reduces power in detecting BNST signals. On the other hand, given the small size of the BNST, our approach of using anatomical BNST ROIs is accompanied by other possible pitfalls. While our ROIs were drawn on high resolution anatomical images with 1 mm isometric voxels, these ROIs were applied to 3.5 mm x 3.75 mm x 3.75 mm fMRI voxels. This down-sampling of resolution may have caused us to capture activity from surrounding areas in our ROIs. We constructed conservative BNST ROIs to minimize this possibility. Additionally, much of the BNST is bounded by ventricle and white matter and partial volume effects at these boundaries would be more likely to result in loss of power than a type I error. Nevertheless, it is possible that the findings we have reported for our BNST ROIs were driven by activity in surrounding areas. While our results are broadly consistent with previous results and with theory on BNST function, high resolution imaging is needed to confirm the results of the current study and to clarify the circumstances under which BNST is sensitive to discrete negatively-valenced stimuli.

It should also be noted that we did not manipulate stimulus valence independent of stimulus arousal and did not include positively-valenced stimuli in our design. As such, we cannot distinguish whether our findings involving stimulus negativity were due to valence, or to stimulus arousal or whether the neural novelty response differs for positive *vs* neutral or negative stimuli. Future studies are needed to investigate these issues.

It should also be noted that images were not matched for visual features across conditions and one trial order was used for all participants. Whole brain analysis did reveal greater visual activation for both novel *vs* repeated and negative *vs* neutral images (see supplement for more information), which could suggest visual differences in stimuli across conditions or order effects driving activation in these areas. However, differences in visual cortex activity do not necessarily imply differences in visual features across conditions (McGann, 2015). In fact, past studies have also found greater activation in visual areas for novel stimuli (Tulving *et al.*, 1996; Menon *et al.*, 2000; Schwartz *et al.*, 2003; Ousdal *et al.*, 2014). Similarly, reciprocal connections between amygdala and visual cortex have been posited as one mechanism for increased visual cortex activation for negative images which may correspond to increased attention to and processing of salient stimuli (Vuilleumier, 2005). It is possible that this same connectivity is involved in processing novel stimuli. Thus, activation in visual areas for these conditions are not unusual, and do not necessarily imply that our results were driven by these study design limitations.

In addition to investigating the response to novelty and stimulus negativity in the amygdala, hippocampus and BNST, a major aim of the current study was to assess how trait anxiety interacts with these variables. We predicted that trait anxiety would modulate amygdala and BNST responses toward both novelty and stimulus negativity. Contrary to these predictions, the only significant effect of anxiety involved a marginal trend toward anxiety predicting less activity in the BNST than in the hippocampus. With respect to emotional valence, while past studies have found that trait anxiety modulates amygdala reactivity to negativity (Etkin *et al.*, 2004; Stein *et al.*, 2007; Fakra *et al.*, 2009; Hariri *et al.*, 2009; Laeger *et al.*, 2012), we found no evidence for this. There are several possible reasons for this discrepancy. The majority of past studies investigating the relationship between trait anxiety and amygdala reactivity toward negativity have used facial expressions as stimuli (Etkin *et al.*, 2004; Stein *et al.*, 2007; Fakra *et al.*, 2009; Hariri *et al.*, 2009), while we used IAPS images, only half of which depicted humans. Additionally, Etkin *et al.* (2004) found that trait anxiety modulated amygdala reactivity only for masked faces. Similarly, Dickie and Armony (2008) reported increased amygdala reactivity for an unattended *vs* attended face contrast in female participants. Thus, anxiety differences in amygdala reactivity to negativity may be more pronounced when the stimulus is unattended. Furthermore, another study found that the correlation between trait anxiety and amygdala reactivity only exists for participants who are low in perceived social support (Hyde *et al.*, 2011), and a study using an adolescent sample found that amygdala reactivity to facial expressions only correlated with social dimensions of trait anxiety (Killgore and Yurgelun-Todd, 2005). Thus, the current study's lack of evidence for trait anxiety modulation of amygdala reactivity could be due to the stimuli used, or characteristics of the participants. Further research is needed to examine the circumstances under which trait anxiety modulates amygdala reactivity to negatively-valenced stimuli.

Our results also suggest that anxiety may not play a significant role in modulating the novelty response in either the amygdala or BNST. If the relationship between amygdala reactivity to negativity and trait anxiety applies only to some stimulus types, this may also apply to amygdala reactivity to novelty. Studies that have investigated altered amygdalar novelty responses in inhibited temperament have focused exclusively on novel *vs* repeated faces (Schwartz *et al.*, 2003; Blackford *et al.*, 2011; Blackford *et al.*, 2013). Additionally, Balderston *et al.* (2011) found an amygdala novelty response toward humans, but not scenes. This finding led them to posit that, except when the amygdala has been primed by prior threat (Balderston *et al.*, 2013), the amygdala novelty response is dependent on stimulus content, more specifically that the amygdala only responds toward novelty for stimuli that are biologically salient. Although the finding that the amygdala responds to novel objects provides a contrast to this hypothesis (Blackford *et al.*, 2010), it is possible that the novelty responses of the amygdala and BNST are modulated by trait anxiety, but only for certain classes of stimuli. Because we used both images of humans and scenes, but did not design our study in a way that would allow us to include this as a factor in our analysis, we were unable to test this hypothesis. If anxiety does modulate the novelty response of only certain classes of stimuli, this would result in decreased sensitivity in our design to detect trait anxiety modulation of novelty responses.

While our results highlight the need for further research on amygdala reactivity and trait anxiety, the current study adds to the existing research demonstrating novelty effects in the amygdala (Schwartz *et al.*, 2003; Wright *et al.*, 2003; Wright *et al.*, 2008; Blackford *et al.*, 2010; Balderston *et al.*, 2011; Balderston *et al.*, 2013; Ousdal *et al.*, 2014) and hippocampus (Tulving *et al.*, 1996; Grunwald *et al.*, 1998; Menon *et al.*, 2000; Daselaar *et al.*, 2006; Kirwan *et al.*, 2009; Lever *et al.*, 2010) that are not due to habituation (Balderston *et al.*, 2011). We have extended this research by showing that the BNST exhibits a novelty response that interacts with stimulus negativity. This finding suggests that, under certain circumstances, the BNST does exhibit sensitivity toward discrete negative stimuli, and that the BNST may contribute to the extended amygdala's ability to detect and coordinate the response toward novel stimuli.

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