

Amygdala Task-Evoked Activity and Task-Free Connectivity Independently Contribute to Feelings of Arousal

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Abstract: Individual differences in the intensity of feelings of arousal while viewing emotional pictures have been associated with the magnitude of task-evoked blood-oxygen dependent (BOLD) response in the amygdala. Recently, we reported that individual differences in feelings of arousal are associated with task-free (resting state) connectivity within the salience network. There has not yet been an investigation of whether these two types of functional magnetic resonance imaging (MRI) measures are redundant or independent in their relationships to behavior. Here we tested the hypothesis that a combination of task-evoked amygdala activation and task-free amygdala connectivity within the salience network relate to individual differences in feelings of arousal while viewing of negatively potent images. In 25 young adults, results revealed that greater task-evoked amygdala activation and stronger task-free amygdala connectivity within the salience network each contributed independently to feelings of arousal, predicting a total of 45% of its variance. Individuals who had both increased task-evoked amygdala activation and stronger task-free amygdala connectivity within the salience network had the most heightened levels of arousal. Task-evoked amygdala activation and task-free amygdala connectivity within the salience network were not related to each other, suggesting that resting-state and task-evoked dynamic brain imaging measures may

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provide independent and complementary information about affective experience, and likely other kinds of behaviors as well. *Hum Brain Mapp* 35:5316–5327, 2014. © 2014 Wiley Periodicals, Inc.

Key words: amygdala; task-evoked activation; resting-state connectivity; individual differences; feelings of arousal

INTRODUCTION

The amygdala plays a fundamental role in affective experience. Studies of humans with brain lesions demonstrate the importance of the amygdala for affective behavior (Feinstein, et al., 2011; but see also [Feinstein, et al., 2013]). Research with healthy individuals has shown that the amygdala processes biologically relevant stimuli [Adolphs, 2008; Davis and Whalen, 2001], especially under conditions of uncertainty, ambiguity, or novelty. In one of the most widely replicated observations in functional neuroimaging, the amygdala's task-evoked hemodynamic response is greater while processing affectively potent stimuli of various types than while processing neutral stimuli [Breiter et al., 1996; Hsu et al., 2005; Lewis et al., 2007; Morris et al., 1996; Wright et al., 2006]; for a meta-analytic review see [Kober et al., 2008]. Amygdala activation is consistently observed during the experience of emotions such as anger, sadness, fear, disgust, and happiness [Lindquist et al., 2012; Wilson-Mendenhall et al., 2013], and the magnitude of amygdala activation relates to individual differences in the intensity of feelings of arousal during affective tasks [Barrett et al., 2007; Colibazzi et al., 2010; Gerdes et al., 2010; Phan et al., 2004; Wilson-Mendenhall et al., 2013].

A growing body of research also indicates that individual differences in the intensity of negative affect is associated with the strength of task-free connectivity between particular structures such as the insula and other structures that compose the "salience" network [Seeley et al., 2007; Touroutoglou et al., 2012]. Tract-tracing and recent resting-state functional connectivity studies demonstrate that dorsal portions of the amygdala (including the central nucleus) are connected within the salience network [Bickart et al., 2012; Hayes and Northoff, 2011; McDonald 1991b; Ongur and Price, 2000; Price, 2007; Saleem et al., 2008; Seeley et al., 2007; Zhou et al., 2010], which subserves affective responses to salient, ambiguous, or novel negative stimuli.

Despite the importance of these observations, a critical question remains unanswered. Are these two types of functional MRI measures redundant or independent in their relationships to individual differences in affective behavioral responses? Moreover, this question extends well beyond emotion: to our knowledge, there is not yet a study of whether a combination of task-evoked and task-free functional neuroimaging measures predicts individual differences in any type of behavior including cognitive functions.

In this article, we examined in a single experiment the contributions of task-evoked amygdala activation and task-free dorsal amygdala connectivity within the salience

network in predicting individual differences in negative affective experience. Specifically, we hypothesized that the dorsal amygdala's intrinsic connectivity within the salience network would predict individual differences in negative affective experience independently of the contributions of task-evoked amygdala activation.

To address our hypotheses, we analyzed both resting state and affective reactivity task scans in 25 participants who rated their feelings of arousal while viewing affectively potent images. Affectively potent stimuli such as high arousal negative images can evoke an affective experience that is accompanied by physiological arousal [Russell and Barrett, 1999]. To measure task-evoked amygdala activation, we calculated the percent BOLD signal change in the amygdala for high arousal negative images (vs. fixation). To assess task-free connectivity of the dorsal amygdala within the salience network, we used resting state functional connectivity analysis. Using multiple linear regression analysis, we show that individual differences in feelings of arousal arise independently from task-evoked amygdala activation and task-free dorsal amygdala connectivity within the salience network.

MATERIALS AND METHODS

Participants

Twenty-five young adults ranging in age from 19 to 32 (mean age = 24.36 years, SD = 3.12; 9 men) participated in this study, which involved the collection of resting-state blood oxygenation-level dependent (BOLD) data as well as task-evoked BOLD data. The task performed in the scanner was an affective reactivity task in which participants rated their arousal on-line as they viewed affectively potent images from the International Affective Picture Set (IAPS; [Lang et al., 1997]). All participants were right-handed, native English speakers and had normal or corrected-to-normal vision. No participant reported a history of neurological or psychiatric disorders. Other analyses of data collected as part of a larger project on age-related differences in affect and novelty processing have previously been published [Andreano et al., 2013; Moriguchi et al., 2011; Weierich et al., 2010].

Behavioral Data Acquisition

The affective reactivity task included 132 full-color images, selected from the IAPS [Lang et al., 1997]. Of these 132 images, equal numbers of each of three levels of

arousal (high, mid, and low) and types of valence (negative, neutral, and positive) were included, creating six combinations of arousal and valence (high arousal negative, high arousal positive, mid arousal negative, mid arousal positive, mid arousal neutral and low arousal neutral). The affective reactivity task was run using E-Prime experimental software (Psychology Software Tools, Pittsburgh, PA) on a PC, from which images were projected onto a screen in the magnet bore. Participants viewed images via a mirror mounted on the head coil. The task consisted of five event-related fMRI runs, the first of which was a familiarization run where participants were familiarized to two images in each stimulus category; during the latter four test runs, participants viewed these familiarized images randomly intermixed with 120 novel images that were shown only once (20 novel images in each of the six valence-arousal combinations). Each run contained 100 events, of which 40 were fixation events and 60 were stimulus events. Each run was 340 s in length and each image was presented for 3.5 s, with a stimulus onset asynchrony that varied from 4 to 16 s. The fixation lasted 2 s.

Participants used a three-button response to rate how aroused each image made them feel (1 = low, 2 = mid, 3 = high). As in previous work [Touroutoglou et al., 2012], the subjective arousal ratings were then averaged to create composite measures of feelings of arousal for use in behavioral correlation analyses. Specifically, for each participant, ratings of subjective arousal in response to all of the novel and familiarized images that were rated as high arousal negative based on normative data were averaged to create a composite measure of feelings of arousal in response to negative images. The same procedure was performed for high arousal positive images.

MRI Data Acquisition and Processing Procedures

Structural and functional MRI data were acquired using a Siemens Magnetom Trio Tim 3T whole body high-speed imaging device equipped for echo planar imaging (EPI) (Siemens Medical Systems, Iselin, NJ) with a 12 channel gradient head coil. Head motion was minimized using head restraints, including a pillow and foam padding. Noise was attenuated with ear plugs. High resolution 3D MPRAGE sequences (TR/TE/flip angle = 2.53 s/3.39 ms/7°) with an in-plane resolution of $1.3 \times 1.0 \text{ mm}^2$ and 1.3-mm slice thickness were collected for spatial normalization and for positioning the slice prescription of the subsequent sequences. Next, T1-EPI (TR/TE/flip angle = 10 s/39 ms/90°) and T2-weighted (TR/TE/flip angle = 5.21 s/94 ms/150°) sequences were collected (using the same geometry as the T2*-weighted functional images) to assist in registration of the functional data to the high-resolution anatomical scan.

Whole brain task-evoked fMRI data were acquired using a gradient-echo T2*-weighted sequence (TR/TE/flip angle = 2 s/30 ms/90°; 33 coronal slices angled perpendic-

ular to the AC/PC line), with a slice thickness of 5 mm, for a voxel size of $3.12 \times 3.12 \times 5 \text{ mm}^3$, interleaved acquisition order and foot-to-head phase encoding. Prior to each scan, four time points were acquired and discarded to allow longitudinal magnetization to reach equilibrium.

Whole-brain resting state fMRI data were acquired with echo-planar sequence (TR = 2,000 ms; TE = 30 ms; FA = 90°; $3.1 \times 3.1 \times 5.0 \text{ mm}^3$ voxels, 33 slices). The data involved one ($n = 5$) or two ($n = 20$) runs of 128 time points. During the resting state fMRI runs, participants were instructed to keep their eyes open.

Because task-evoked fMRI data and task-free (or resting state) fMRI data are two different types of fMRI measures of brain activity, we processed them independently using procedures optimized for each data type. We used the optimal and standard processing procedures for each type of data as previously published by our lab [for the task-evoked fMRI see Andreano et al., 2013; Moriguchi et al., 2011; Weierich et al., 2010; for the task-free fMRI see Bickart et al., 2012; Touroutoglou et al., 2012].

Task-Evoked fMRI Analysis

Preprocessing of the task-evoked fMRI data involved a series of steps, using the standard processing stream of the Martinos Center for Biomedical Imaging (<http://surfer.nmr.mgh.harvard.edu>). Task-evoked fMRI data were motion corrected and inspected for gross motion. Slices were discarded if the total motion vector exceeded 5 mm. Data in each run were intensity normalized and spatially smoothed (full-width halfmaximum = 8 mm) using a 3D Gaussian filter. To remove temporal autocorrelation noise, we also included polynomial drift correction with two nuisance regressors to account for low-frequency drift and whitening based on a single autocorrelation function estimated across all brain voxels [Burock and Dale, 2000]. Following preprocessing, task fMRI images for each participant were registered to that participant's anatomical image in native space (a 3D T1-weighted volume created by averaging that participant's two high-resolution 3D MPRAGE images together). We estimated the duration of the hemodynamic response to be 20 s. Task fMRI data for each condition were modeled using a finite impulse response (FIR) model beginning at 4 s prestimulus and utilizing 2-s bins. We included the 4 s prestimulus in our FIR model to make sure that the response to a previous stimulus had returned to baseline.

To measure the magnitude of task-evoked amygdala activation, we used an anatomically based approach to conduct an ROI analysis of task-evoked fMRI data from the amygdala, using FSFAST (<http://surfer.nmr.mgh.harvard.edu>). We applied FreeSurfer's automated subcortical segmentation method to the native 3D MP-RAGE structural images for each subject to create an anatomically-defined amygdala ROI in each hemisphere of each individual [Fischl et al., 2002]. Each ROI was visually inspected;

in this dataset, no errors were identified. Using FsFast's *func2roi* command, the ROIs were used to extract mean BOLD signal within each ROI. We applied an all vs. fixation contrast (all stimuli of all types of arousal and valence vs. fixation) to each amygdala ROI. The data were then extracted from a conjunction of the anatomical label and the all vs. fixation contrast, such that only those voxels within the label that responded to images relative to fixation at $P < 0.05$ were included. Finally, we extracted signal for the novel high arousal negative vs. fixation contrast from that same functional-anatomical ROI. These condition-specific estimates were then used to calculate percent signal change, in this case, for high arousal novel negative stimuli vs. fixation. Signal change estimates from other conditions have been reported in previous papers from our lab [Andreano et al., 2013; Moriguchi et al., 2011; Weierich et al., 2010]. As in our previous work [Moriguchi et al., 2011; Weierich et al., 2010], the percent signal change was calculated for the time window corresponding to 6–8 s post-stimulus. Thus, for each individual, BOLD percent signal change was measured from the voxels within the combined functional, anatomically defined amygdala ROIs (one in each hemisphere) in which there was activation in the high arousal novel negative condition vs. fixation.

Task-Free fMRI Analysis

Preprocessing of the resting state fMRI data involved a series of previously established resting state functional connectivity MRI (rs-fcMRI) procedures [Biswal et al., 1995; Van Dijk et al., 2010; Vincent et al., 2007] including: (1) removal of the four volumes to allow for T1 equilibration effects, (2) slice timing correction (SPM2, Wellcome Department of Cognitive Neurology, London, UK), and (3) head motion correction (FMRIB, Oxford, UK). Data were normalized to the Montreal Neurological Institute (MNI) atlas space (SPM2, Wellcome Department of Cognitive Neurology, London, UK) and re-sampled to 2-mm cubic voxels. A low-pass temporal filter removed frequencies higher than 0.08 Hz. Data were spatially smoothed using a 6 mm full-width half-maximum Gaussian filter. Sources of spurious variance and their temporal derivatives were removed through linear regression including: (1) six parameters obtained by rigid-body correction of head motion correction, (2) the signal averaged over the whole brain, (3) the signal averaged over the ventricles, and (4) the signal averaged over the deep cerebral white matter.

To examine the task-free functional connectivity of the dorsal amygdala, we used whole brain seed-based rs-fcMRI analysis. A hypothesis-driven approach was taken for this analysis, employing a seed region in the dorsal amygdala (MNI coordinates: $-22, -4, -12$) as previously identified in Bickart et al. [2012]. This region of the amygdala is connected with a large-scale network that we originally referred to as an “aversion” network [Bickart et al., 2012], the topography of which corresponds very closely

to the so-called salience network [Seeley et al., 2007]. A spherical ROI was used to interrogate the connectivity strength within this network. For discriminant validity analyses, two additional spherical ROIs were used in the medial amygdala (MNI coordinates: $-14, -4, -20$) and ventrolateral amygdala (MNI coordinates: $-28, -4, -22$) whose connectivity networks are preferentially involved in affiliative and perceptual aspects of social perception, respectively [Bickart et al., 2012]. As identified in Bickart et al. [2012], each of the three spherical ROIs had a 2-mm radius to satisfy two criteria: (a) to be located within the Harvard-Oxford probabilistic map of the amygdala at 25% or greater probability; and (b) to be separable from one another in space. Each amygdala seed ROI was used to generate a whole-brain map of Fisher's r -to- z correlation coefficients [$z(r)$] values. Using the three corresponding large-scale amygdala network map masks as defined independently in a separate sample [Bickart et al., 2012], the strength of task-free connectivity between each of the three amygdala subregions and the rest of its respective large-scale network was calculated. We calculated the correlation between low-frequency BOLD signal fluctuations within each amygdala seed ROI and the mean of the voxel activity in its respective amygdala network mask. This resulted in three amygdala task-free connectivity strength, $z(r)$, values for each participant: (a) dorsal amygdala task-free connectivity strength, $z(r)$, values, (b) medial amygdala task-free connectivity strength, $z(r)$, values and (c) ventrolateral amygdala task-free connectivity strength, $z(r)$, values. The dorsal amygdala task-free connectivity strength represented the connectivity strength of interest as contributing to salience processing.

Brain-Behavior Regression Analyses

We examined the independent and interactive contributions of task-evoked amygdala activation and task-free dorsal amygdala connectivity within the salience network to feelings of arousal in the following way. We conducted a linear regression analysis using both task-evoked BOLD amygdala activation (percent signal change in response to high arousal negative novel images versus fixation) and task-free dorsal amygdala connectivity strength as independent variables and the arousal ratings while viewing high arousal negative (or positive) images as the dependent variable. We performed this analysis to determine whether both neural measures contributed redundantly or synergistically to the prediction of individual differences in behavior. Brain-behavior analyses were conducted using PASW Statistics 18, Release Version 18.0.0 (SPSS, 2009, Chicago, IL, www.spss.com). Results were considered statistically significant at $P < 0.05$.

To control for participants' tendency to rate images highly across negative and neutral images, we also tested whether our two fMRI measures of interest predicted variance in the intensity of mean difference score of ratings

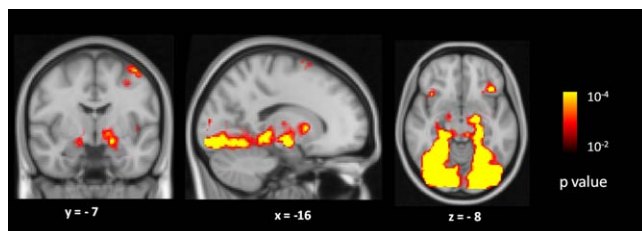


Figure 1.

Clusters in bilateral dorsal amygdalae showing significantly greater signal change between novel high arousal negative images versus fixation (red) at $p < 0.00001$, uncorrected. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

while viewing high arousal negative images versus low arousal images. For this analysis, we used both task-evoked BOLD dorsal amygdala activation (percent signal change in response to high arousal negative novel images versus fixation) and task-free dorsal amygdala connectivity strength as independent variables and the mean difference arousal rating (while viewing high arousal negative images versus low arousal images) as the dependent variable.

To assess the discriminant validity of the a priori hypothesized relationship between the task-free dorsal amygdala connectivity strength within the salience network and feelings of arousal to negative images, we tested whether the strength of task-free connectivity for the medial and ventrolateral amygdala (thought to be involved in affiliative and perceptual social processes, respectively) predicted variance in the intensity of feelings of arousal in response to negative images. For this analysis, we performed a multiple regression analysis using task-free dorsal amygdala, medial amygdala and ventrolateral amygdala connectivity strength (as independent variables and the arousal ratings while viewing negative images as the dependent variable).

Finally, since task-free dorsal amygdala connectivity within the salience network was correlated with feelings of arousal in the hypothesis-driven brain-behavior analysis, we also explored the localization of regions within this network that best predicted feelings of arousal in response to negative images. Using FreeSurfer's implementation of general linear model analysis, we entered arousal ratings while viewing high arousal negative images as a covariate of the dorsal amygdala seed activity into a whole brain regression. The resultant whole-brain map was limited by the dorsal amygdala connectivity mask defined independently in Bickart et al. [2012] and results were considered significant if they met the criteria of $p < 0.01$ with a cluster size constraint of 10 contiguous voxels.

RESULTS

As predicted, there was significantly greater signal change for novel high arousal negative images versus fixa-

tion ($p < 10.5$) in the dorsal subregion of the amygdala (see Fig. 1 and Fig. S1 in Supporting Information). Consistent with prior evidence on the amygdala's role in affective experience, our analyses revealed that increased left dorsal amygdala task-evoked activation in response to high arousal negative novel images was related to heightened feelings of arousal. The results demonstrated that participants with more robust task-evoked dorsal amygdala activation while viewing negative images made higher arousal ratings for those images relative to those individuals with less robust task-evoked dorsal amygdala activation (Fig. 2). This effect was not observed in the right amygdala ($r = 0.16$, $p < 0.43$), consistent with meta-analytic summaries of neuroimaging studies that have indicated a more laterized emotional processing for amygdala with most studies reporting activation in left amygdala [Baas et al., 2004; Fusar-Poli et al., 2009].

As hypothesized, individual differences in the strength of task-free dorsal amygdala connectivity within the salience network predicted feelings of arousal. The dorsal amygdala large-scale connectivity network, binarized at $p < 10.5$ and overlaid on T1 MNI152 1.0 mm template, is shown in Figure 3. The results demonstrated that participants with stronger dorsal amygdala connectivity at rest made higher arousal ratings when they later viewed negative images relative to those individuals with weaker connectivity (Fig. 4). This effect was observed for both unilateral and bilateral dorsal amygdala connectivity ($r = 0.56$, $p < 0.01$). In the remainder of the results, we focus on the left hemisphere. The left dorsal amygdala's level of BOLD activation (high arousal negative novel images vs. fixation) and the task-free dorsal amygdala connectivity strength within the salience network were not related to each other ($r = 0.09$, $p < 0.68$) (see also Fig. S2 in Supporting Information).

Using a multiple linear regression analysis, we found that task-evoked dorsal amygdala activation and task-free dorsal amygdala connectivity within the salience network each independently predicted the intensity of felt arousal, accounting for a total of 45% of its variance. Those individuals that displayed both the greatest task-evoked dorsal amygdala activation and the strongest task-free dorsal amygdala connectivity within the salience network had the most intense feelings of arousal while viewing novel negative images (see Table I and Fig. 5). This effect was significant even when participant's tendency to rate more intense arousal experiences across the images were taken into account. Task-evoked dorsal amygdala activation and task-free dorsal amygdala connectivity were related to mean difference arousal ratings while viewing high arousal negative images versus low arousal images ($r = 0.50$, $p < 0.05$ and $r = 0.59$, $p < 0.01$, respectively) (see also Table SI in Supporting Information).

We next explored the specific regions within the salience network that were driving the relationship between the task-free dorsal amygdala connectivity and feelings of arousal (Fig. 6). This exploratory analysis allowed us to localize the regions within the salience network that best

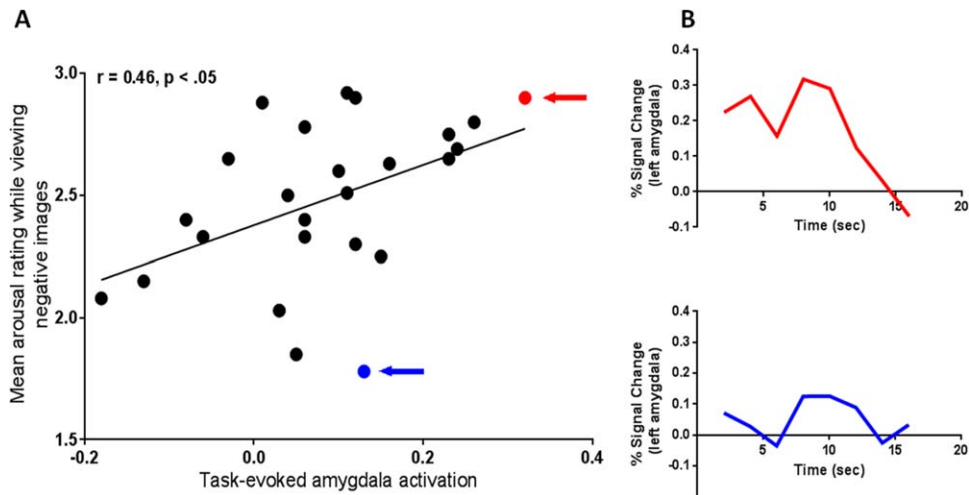


Figure 2.

Task-evoked amygdala activation is related to feelings of arousal. **(A)** More intense feelings of arousal correlated with greater task-evoked amygdala activation, $r = 0.46$, $p < 0.05$. The task-evoked left amygdala activation (percent signal change in response to high arousal negative novel images versus fixation) (x axis) is plotted against mean arousal rating while viewing high arousal negative images (y axis). The subjects with the highest

predicted feelings of arousal in response to negative images. Our findings revealed that connectivity between the dorsal amygdala seed and subcortical regions within the salience network were the best predictors of arousal feelings. Individuals with heightened feelings of arousal had stronger connectivity between the left dorsal amygdala

and the lowest mean arousal rating while viewing high arousal negative images are highlighted with a red and a blue arrow, respectively; **(B)** BOLD time course data for these two individuals is shown for the left amygdala activation from the stimulus onset to 16 s post-stimulus. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

dala and a relatively large cluster in right anterior and ventromedial posterior thalamus. They also had stronger connectivity between the left dorsal amygdala and right anterior hippocampus as well as bilateral posterior hippocampi, lateral putamen, a region near the ventral tegmental area/substantia nigra bilaterally (see Table II). Task-free connectivity between dorsal amygdala and a small cluster in anterior cingulate cortex also predicted feelings of arousal ratings at a more liberal threshold ($p < 0.1$), however (see Fig. S3 in Supporting Information).

A final set of analyses characterized the anatomical and behavioral specificity of the results reported thus far. Task-free connectivity of the medial and ventrolateral amygdala was not related to feelings of arousal while viewing novel negative images ($r = 0.25$, $p < 0.23$ and $r = -0.03$, $p < 0.89$, respectively). Multiple regression analysis that compared the relative contributions of task-free connectivity of the dorsal, medial, and ventrolateral amygdala networks in predicting feelings of arousal showed that task-free connectivity within the dorsal amygdala network was the best predictor of arousal ratings (see Table SII in Supporting Information). Task-evoked amygdala activation and task-free connectivity were not related to arousal ratings to positive images ($r = 0.10$, $p < 0.63$ and $r = -0.15$, $p < 0.48$, respectively).

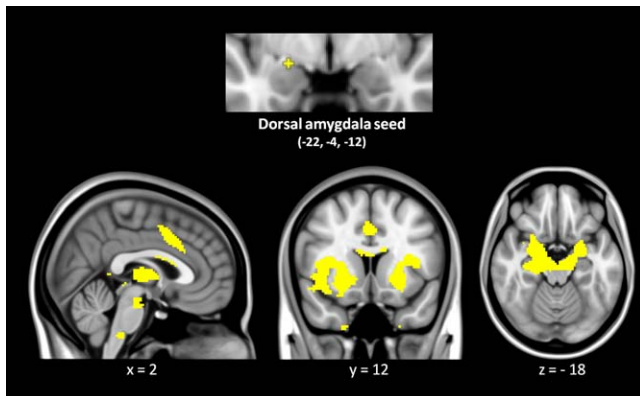


Figure 3.

As previously defined in Bickart et al. [2012], the dorsal amygdala seed anchors the dorsal amygdala large-scale connectivity network (also known as the salience network; see text). The dorsal amygdala map, $z < 0.10$, is overlaid on T1 MNI152 1.0 mm template brain in radiologic convention to demonstrate the cortical and limbic structures within the dorsal amygdala network ($N = 25$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

DISCUSSION

The most novel contribution of this study is the observation that both task-evoked amygdala activation and task-

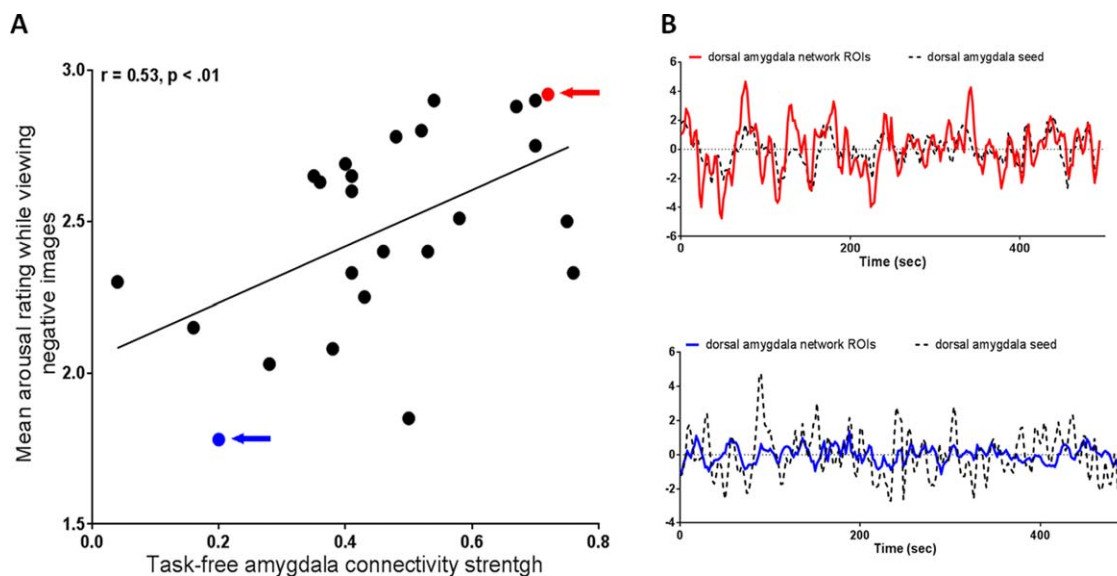


Figure 4.

Task-free dorsal amygdala connectivity within the salience network is related to feelings of arousal. **(A)** More intense feelings of arousal were predicted by the task-free dorsal amygdala connectivity strength within the salience network, $r = 0.53, p < 0.01$. The z-transformed correlation coefficients of left dorsal amygdala connectivity (x axis) are plotted against mean arousal rating while viewing high arousal negative images (y axis). The subjects

with the highest and the lowest level of arousal are highlighted with a red and a blue arrow, respectively; **(B)** BOLD time course data for these two individuals is shown for the left dorsal amygdala seed and the ROIs of dorsal amygdala connectivity within the salience network. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

free amygdala connectivity independently contribute to explaining individual differences in emotional arousal. To our knowledge, the complementarity of these imaging measures has not yet been reported for any brain network or behavior referable to that network. Previous work has shown that task-evoked amygdala activity relates to individual differences in feelings of arousal [Barrett et al., 2007; Colibazzi et al., 2010; Gerdes et al., 2010; Phan et al., 2004; Wilson-Mendenhall et al., 2013] and separately that task-free amygdala connectivity relates to individual differences in arousal [Kim et al., 2010; Seeley et al., 2007]. We extended this work to show that task-evoked amygdala activity and

task-free amygdala connectivity are not redundant measures, but rather that both contribute uniquely to the intensity of reported feelings of arousal. That is, people who feel the emotions evoked by negative pictures most intensely have the greatest amygdala reactivity to the pictures and the strongest task-free amygdala connectivity within the salience network. Our findings indicate that in addition to its reactivity, the amygdala's connectivity to other regions within the salience network plays an independent, additive role in subserving feelings of arousal.

The amygdala functions within a broad network of regions that share both anatomical connectivity and a

TABLE I. Increased amygdala task-evoked activation and task-free connectivity each contributed independently to feelings of arousal while viewing negative images (N = 25)

	Mean arousal rating while viewing negative images		
	B	R ² change	Total R ²
Strength of the dorsal amygdala task-free connectivity within the salience network	0.49 ^a	0.28 ^a	0.45 ^a
Amygdala task-evoked activation	0.42 ^b	0.17 ^b	

Note: This table displays standardized regression coefficients (B) as well as the incremental (R² change) and total variance (total R²) in mean arousal rating predicted by the independent variables entered into a single multiple linear regression model.

^a $p < 0.01$.

^b $p < 0.05$.

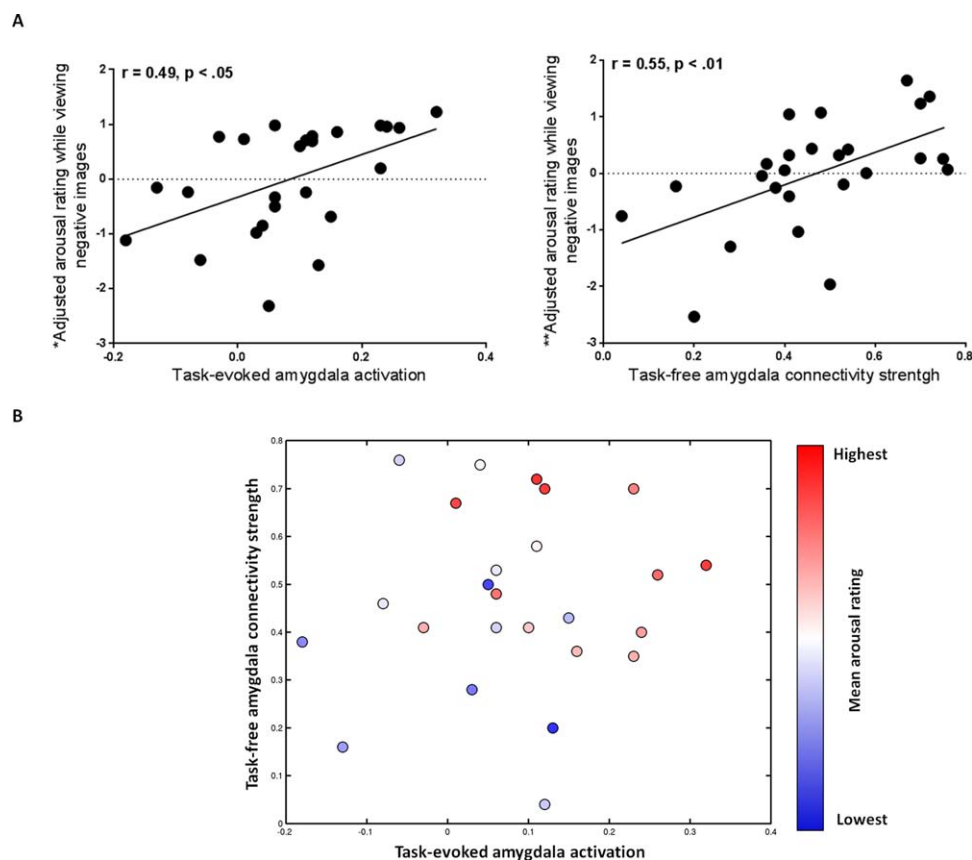


Figure 5.

Greater task-evoked amygdala activation and stronger task-free dorsal amygdala connectivity within the salience network each contributed independently to feelings of arousal. **(A, left graph)** *The y axis displays the residual variance in mean arousal rating after the variance due to task-free dorsal amygdala connectivity within the salience network has been removed; the scatterplot illustrates the relationship between adjusted arousal rating and task-evoked amygdala activation. **(A, right graph)** **The y axis displays the residual variance in mean arousal rating after the variance due to task-evoked amygdala activation has been removed; the scatterplot illustrates the relationship between adjusted arousal rating and task-free dorsal amygdala connectiv-

ity within the salience network. **(B)** Individuals that displayed both increased task-evoked amygdala activation and stronger task-free dorsal amygdala connectivity within the salience network had the highest mean arousal rating while viewing negative images (total variance in mean arousal rating predicted by the independent variables was $R^2 = 0.45$, $p < 0.01$). Individuals with the highest level of mean arousal rating are shown in red, those with a medium level of mean arousal rating are shown in white and those with the lowest level of mean arousal rating are shown in blue. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

common role in detecting and guiding reactions to salient stimuli. Tract-tracing studies in macaques demonstrate that nuclei within the dorsal amygdala and their cellular extensions into the substantia innominata share convergent anatomical connections with the middle cingulate cortex and anterior insula, projecting to interoceptive and pain-related targets in the insula and the rostrally adjacent orbitofrontal cortex, somatosensory operculum, ventrolateral striatum, caudolateral hypothalamus, as well as autonomic and dopaminergic nuclei of the brainstem [An et al., 1998; Carmichael and Price, 1996; Ferry et al., 2000; Fudge et al., 2002; Haber and Calzavara, 2009; Haber and Knutson, 2010; Haber et al.,

2006; Hsu and Price, 2007; Kondo et al., 2003; Kondo et al., 2005; Kunishio and Haber, 1994; McDonald, 1991a,b; Ongur and Price, 2000; Ongur et al., 1998, 2003; Price and Drevets, 2010; Price, 2007; Saleem et al., 2008]. These regions also share a common role in detecting, learning about, and responding to salient, often aversive stimuli [Balleine and O'Doherty, 2010; Craig, 2009; Hayes and Northoff, 2011; Knapska et al., 2007; Murray, 2007; Waraczynski, 2006]. Perhaps the strength of the amygdala's connectivity within the salience network, as measured with task-free functional neuroimaging, reflects how efficiently it communicates the salience of stimuli to the other regions in the network. It is

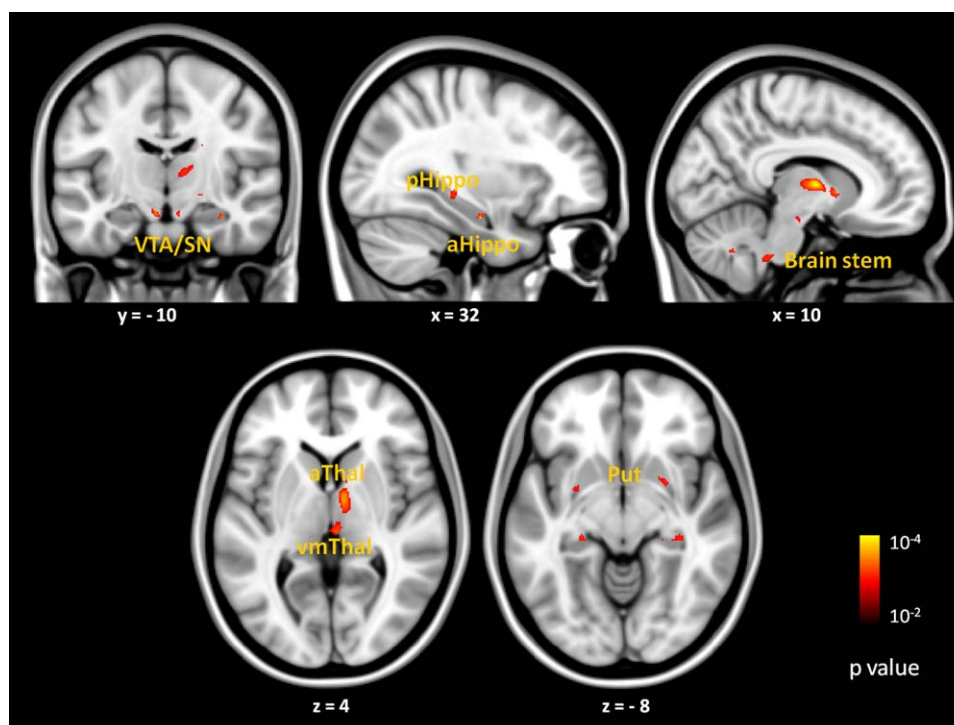


Figure 6.

Exploratory analyses revealed that task-free connectivity between the left dorsal amygdala and subcortical regions within the salience network (dorsal amygdala network previously defined in Bickart et al. [2012]) were the best predictors of feelings of arousal. Brain images show location of voxels where connectivity with dorsal amygdala at rest correlated with mean arousal rating at $p < 0.01$, uncorrected with a cluster size constraint of 10 voxels. Color bars indicate the p values (10^{-2} to

10^{-4}) of correlated voxels, which are overlaid on slices of a T1 MNI152 1.0 mm template brain in radiologic convention. Abbreviations: VTA/SN, ventral tegmental area/substantia nigra; pHippo, posterior hippocampus; aHippo, anterior hippocampus; aThal, anterior thalamus; vmThal, ventromedial thalamus; Put, putamen. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

also possible that the amygdala's connectivity strength at rest contributes to the basal tone of the autonomic nervous system, which biases the system to respond more or less vigorously to salient stimuli.

Whereas prior work has demonstrated a relationship between task-free connectivity and task-evoked activity of regions of the sensorimotor cortex [Fox et al., 2007] or within the frontoparietal network [Mennes et al., 2010, 2011] we found no relationship between the task-free connectivity and task-evoked activation of the amygdala within the salience network. This suggests regional variation in the degree to which task-evoked dynamic responses relate to spontaneous BOLD signal fluctuations. This interpretation would be consistent with recent findings showing that correspondence between task-evoked coactivation patterns and resting-state connectivity patterns is low for limbic subcortical regions but high for association areas that span the frontoparietal cortex [Mennes et al., 2013].

Our findings show that although task-free connectivity and task-evoked activity of the amygdala are independent from each other, both types of brain activity combine to

realize affective experience. Because dynamic stimulus-driven neural responses and basal spontaneous signal fluctuations each provide only one neural correlate of individual differences in behavior, it would seem valuable for future task-related fMRI studies of such differences to also collect resting state data when possible. Although our study employed an affective paradigm and compared the task-free connectivity versus task-evoked activations of regions within the salience network, it is possible that our findings would generalize to other networks.

One limitation of this study is that the present design focused on functional MRI measures alone. Recent studies, however, suggest that variation in the amygdala volume and its anatomical coupling to medial prefrontal cortex also relate to individual differences in negative affect [Holmes et al., 2012; Kim and Whalen, 2009]. Whether structural MRI and diffusion-based tractography measures predict individual differences in feelings of arousal over and above task-evoked activity and task-free connectivity remains an important issue to pursue in subsequent studies. Furthermore, our paradigm included only a state-level

TABLE II. Regions within the dorsal amygdala connectivity within the salience network (defined in Bickart et al. [2012] that correlated with feelings of arousal in an exploratory analysis in our brain-behavior sample (N = 25)

	MNI coordinates of peak voxels		
	x	y	z
Anterior thalamus	10	-6	6
	-6	-10	8
Ventromedial thalamus	6	-22	6
	-6	-22	8
Anterior hippocampus	30	-10	-20
	-30	-8	-22
Posterior hippocampus	30	-30	-8
	-30	-32	-6
Putamen	22	6	-8
	-26	0	-6
Ventral tegmental area/substantia nigra	8	-12	-18
	-8	-12	-16
Brain stem	10	-18	-16
	-4	-16	-16

Note. This table displays MNI coordinates for voxels within the dorsal amygdala connectivity network in which connectivity strength correlated with mean arousal rating at $p < 0.01$, uncorrected with a cluster size constraint of 10 voxels.

measure of affective feelings of arousal. Task-evoked fMRI and task-free functional connectivity MRI, however, reflect state-dependent as well as trait-level sources of variance [Buckner, 2010; Buckner et al., 2013]. Future studies should examine the relative contributions of state and trait on task-evoked activation versus task-free functional connectivity. Additionally, there has been some evidence that attending to subjective experience during the viewing of evocative material increases the activity of the affective circuitry [Taylor et al., 2003]. However, other studies have found no effect of attending to one's subjective experience [McClure et al., 2007]. Future studies should examine the effects of judgments of arousal on task-evoked amygdala responses to emotionally evocative stimuli. Finally, as this study included only healthy young adults, most of whom (16 of 25) were women, further research will be needed to assess whether these findings generalize to more diverse populations.

CONCLUSION

Individual differences in the intensity of feelings of arousal while viewing affectively potent images is associated with two types of fMRI signals in the amygdala, the magnitude of task-evoked amygdala BOLD response and the task-free amygdala connectivity strength within the salience network. We demonstrate that greater task-evoked amygdala activation and stronger task-free amygdala con-

nectivity within the salience network each contribute independently to feelings of arousal to negative images. Importantly, we show that individuals who have both increased task-evoked amygdala activation and stronger task-free amygdala connectivity within the salience network have the most heightened levels of arousal.

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