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Alterations in amygdala functional connectivity reflect early temperament

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ABSTRACT

Behavioral inhibition (BI) is a temperament identified early in life that is associated with increased risk for anxiety disorders. Amygdala hyperresponsivity, found both in behaviorally inhibited and anxious individuals, suggests that amygdala dysfunction may represent a marker of anxiety risk. However, broader amygdala networks have not been examined in individuals with a history of childhood BI. This study uses resting state fMRI to assess amygdala intrinsic functional connectivity (iFC) in 38 healthy young adults (19 with a history of BI, 19 with no history of BI) selected from a longitudinal study. Centromedial, basolateral, and superficial amygdala iFCs were compared between groups and examined in relation to self-report measures of anxiety. Group differences were observed in amygdala iFC with prefrontal cortex, striatum, anterior insula, and cerebellum. Adults characterized with BI in childhood endorsed greater state anxiety prior to entering the scanner, which was associated with several of the group differences. Findings support enduring effects of BI on amygdala circuitry, even in the absence of current psychopathology.

1. Introduction

Behavioral inhibition (BI) is a temperament identified in the first years of life characterized by hyper-vigilance, heightened reactivity to novelty, and social reticence (Fox, Henderson, Marshall, Nichols, & Ghera, 2005; Kagan, Reznick, & Snidman, 1987). This temperament is shown to be stable with time and to increase risk for anxiety disorders (Rosenbaum et al., 1993). The link to anxiety raises questions regarding the degree to which neural correlates are shared across these two phenotypes, subjects with a history of BI and those with clinical anxiety (Lahat, Hong, & Fox, 2011; Perez-Edgar & Fox, 2005). The identification of a shared neural correlate in BI and anxiety may provide a trait marker that ultimately would inform the neurobiology of risk for anxiety. Evidence implicates altered amygdala function as such a marker, demonstrating that amygdala hyperreactivity persists in adolescents and adults with a history of childhood BI even when they do not exhibit clear behavioral manifestations of anxiety (Perez-Edgar et al., 2007; Schwartz, Wright, Shin, Kagan, & Rauch, 2003). To date, the functional integrity of broader amygdala-based networks has not been systematically examined in relation to BI, despite evidence of disruptions in individuals with anxiety disorders (Etkin, Prater, Schatzberg, Menon, & Greicius, 2009; Roy et al., 2013). The present study uses resting-state fMRI to examine the intrinsic functional connectivity of the amygdala in individuals with and without a history of childhood BI in order to identify putative network-level neural markers of anxiety risk.

Recent studies have identified potential biomarkers of clinical anxiety using resting-state fMRI. These studies examined the intrinsic functional connectivity (iFC) of amygdala subdivisions, including the basolateral (BLA), centromedial (CMA) and superficial (SFA) (Etkin et al., 2009; Roy et al., 2013). Compared to healthy peers, adolescents with generalized anxiety disorder (GAD) showed reduced iFC between the CMA and rostral anterior cingulate cortex (rACC), enhanced iFC between the CMA and the insula and enhanced iFC between the SFA and dorsomedial prefrontal cortex (dmPFC) (Roy et al., 2013). The present work extends these findings by taking a similar approach to assess neural markers of temperamental risk for anxiety in young adults with a documented early history of BI.

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Despite the absence of amygdala iFC studies of BI, recent work in related areas can inform study hypotheses. This includes studies in humans on the associations between early-life stress and whole amygdala iFC in adolescence (Burghy et al., 2012), as well as data on amygdala iFC in non-human primate models of BI (Fox et al., 2012; Shackman et al., 2013). Data on task-based amygdala connectivity in BI are particularly relevant. Specifically, in a subset of subjects from the current study, Hardee and colleagues linked early-life BI to perturbed amygdala functional connectivity with anterior insula and dorsolateral prefrontal cortex (dlPFC; Hardee et al., 2013). This study also found that amygdala–insula functional connectivity predicted current internalizing symptoms in the BI group, findings that resembled those in recent report of abnormal CMA–insula iFC in anxious adolescents (Roy et al., 2013). Further, findings of perturbed amygdala–dlPFC connectivity in this study parallel findings from research on monkeys with anxious temperament and children with anxiety disorders (Birn et al., 2014).

The aim of the present study is to examine enduring BI-related alterations in amygdala iFC in a sample of young adults who were initially assessed as infants. Based on work in animal models of BI (Birn et al., 2014; Fox et al., 2012; Shackman et al., 2013) and human studies (Hardee et al., 2013), we predict that adults with a childhood history of BI will show alterations in amygdala iFC with insula and dlPFC. To examine the unique impact of BI on amygdala circuitry, independent of anxious pathology, we only included participants without current or lifetime anxiety diagnoses in our analyses. We propose that BI-related alterations in amygdala iFC similar to those observed in clinical anxiety represent persistent biomarkers of risk for anxiety, while those not observed in anxiety may represent compensatory mechanisms or markers of resilience. Self-reported anxiety levels are examined to further disentangle iFC markers of early childhood BI from those of current anxiety.

2. Methods

The current sample was selected from a larger longitudinal cohort that has been followed since 4 months of age. At that time, 433 participants were assessed for motor and emotional reactivity to novel stimuli. A subset of these infants ($n = 153$), who exhibited either minimal or heightened reactivity, was enrolled in the longitudinal study. Inhibited behavior to novel stimuli was coded at 14 and 24 months and social reticence during standardized social situations with unfamiliar peers was coded at 48 months and 7 years (Fox, Henderson, Rubin, Calkins, & Schmidt, 2001). Parents also completed questionnaires assessing their child's temperament at each of these time points. Behavioral and questionnaire scores were standardized at each time point and used to create a single composite score of behavioral inhibition (Perez-Edgar et al., 2007). Participants with scores in the upper half of the distribution were classified as having behaviorally inhibited temperament (BI) and those with scores in the lower half of the distribution were classified as non-behaviorally inhibited (non-BI).

A total of 60 members of this longitudinal cohort completed the current study when they were between the ages of 18 and 21 years. Six (1 BI, 5 non-BI) were excluded from analyses because they were receiving pharmacological treatment at the time of the scan and six (2 BI, 4 non-BI) were excluded due to a lifetime history of anxiety disorders. An additional ten participants (4 BI, 6 non-BI) were excluded due to excessive movement during the scan. A final sample of 38 subjects was used for the current analyses (19 in BI group, 19 in non-BI group; mean age = 19.5 years). All participants were Caucasian, right-handed, and free from current use of psychotropic medication at the time of the scan. The study was approved by the institutional review boards of the University of Maryland, College Park and the National Institute of Mental Health. Informed consent was obtained from participants prior to participation. The presence of current or lifetime psychiatric disorder was assessed by the Structured Clinical Interview for DSM IV (SCID; First, Spitzer, Gibbon, & Williams, 2002). Anxiety levels were assessed in two ways: (1) the Beck Anxiety Inventory (BAI; Beck, Epstein, Brown, & Steer, 1988) was used as a measure of trait anxiety, and (2) the State subscale of the State Trait Anxiety Inventory (STAI-S; Spielberger, Vagg, Barker, Donham, & Westberry, 1980) was used as a measure of state anxiety at the time of the scan.

2.1. Data acquisition and image processing

Imaging data were acquired on three NIH 3 T scanners with the same acquisition parameters. To control for scanner effects on study results, scanner was entered as a covariate in group analyses as described below. During the resting state (RS-fMRI) scan, participants were instructed to remain still with eyes open while a black screen with a central white crosshair was placed in their field of view. The RS-fMRI sequence consisted of a 6-min acquisition of 180 EPI functional volumes (TR = 2000 ms; TE = 30 ms; flip angle = 90°; FOV = 240 × 192 mm; acquisition voxel size 3 × 3 × 4 mm). T1-weighted anatomical images were obtained for purposes of spatial normalization and localization.

All data were preprocessed at NYU using AFNI (http://afni.nimh.nih.gov/afni/) and FSL (http://www.fmrib.ox.ac.uk/fsl). Preprocessing procedures were identical to those used in previous studies (Di Martino et al., 2009; Roy et al., 2013) and consisted of slice time correction (interleaved acquisition), motion correction, despiking, spatial smoothing (FWHM = 6 mm), mean-based intensity normalization of all volumes by the same factor, temporal band-pass filtering (0.009–0.1 Hz) and linear and quadratic detrending. Visual inspection of images was conducted at each preprocessing step for quality control. RS-fMRI scans with a maximum displacement greater than 3.1 mm were excluded from further analyses. Linear registration of high resolution structural images to the Montreal Neurological Institute MNI152 template with 2 × 2 × 2 mm resolution was carried out using the FSL tool FLIRT, and refined using FNIRT nonlinear registration. The preprocessed data were regressed on nine nuisance covariates, removing variance associated with signals derived from white matter and cerebrospinal fluid, six motion parameters, and the global signal, which is included to control for physiological artifacts. The resultant 4-D residual time series was registered to MNI152 space using linear (FLIRT) and nonlinear (FNIRT) procedures for use in subsequent analyses.

2.2. Time series extraction

Procedures for amygdala region-of-interest (ROI) definition and time series extraction were identical to those used previously (Roy et al., 2009, 2013). Standardized amygdala ROIs were defined based on the Juelich histological atlas implemented in FSL, which defines centromedial (CMA), basolateral (BLA), and superficial (SFA) subdivisions based on stereotaxic, probabilistic maps of cytoarchitectonic boundaries (Amunts et al., 2005). Each ROI included only those voxels that exhibited at least a 50% probability of belonging to their given subdivision and overlapping voxels were delegated to the region with the higher probability. Additionally, due to variation in coverage of the medial temporal lobe across participants, a study-specific mask was derived and used to further refine the amygdala ROIs to insure full coverage across all participants. As a result, the basolateral and total amygdala ROIs were reduced by 464 mm^2 on the left and by 640 mm^2 on the right. This resulted in final volumes for the left and right BLA ROIs of 1376 mm^2 and 1280 mm^2, respectively, and 2496 mm^2 and 2184 mm^2 for the left and right total amygdala, respectively. The sizes of the other amygdala ROIs were the same as used previously (Roy et al., 2009): left and right CMA masks were 176 mm^2 and 224 mm^2, respectively, and the left and right SFA masks were 952 mm^2 and 760 mm^2, respectively. Once ROIs were established, the average timeseries across all voxels of each ROI were extracted for each participant for use in subsequent functional connectivity analyses.

2.3. Voxel-wise examination of group differences in amygdala iFC

For each participant, first-level multiple regression analyses were conducted using FMRIB Improved Linear Model (FILM) in FSL. For each hemisphere a multiple regression GLM was created that included the time series for each of the three subdivisions resulting in individual subject-level maps of all voxels exhibiting positive and negative iFC with each amygdala subdivision, controlling for the others.

Group-level analyses were carried out using a random-effects ordinary least squares model that included two group mean predictors of interest (one for each group), as well as demeaned framewise displacement (an estimate of motion) and scanner as covariates of no interest. Group comparisons were conducted using cluster-level Gaussian Random Field theory for multiple comparison correction, with additional correction for the inclusion of all three amygdala subdivisions ($Z > 2.3$; $p < 0.05/3$, corrected). Further examination of amygdala iFC in the regions exhibiting significant group differences was conducted using SPSS 19.0. Average partial regression coefficients (i.e., iFC) were extracted for the significant cluster(s) for each participant. One-sample t-tests were used to determine whether measures of iFC differed significantly from zero. Correlational analyses were conducted to examine the relation of these iFC measures with BI scores and self-reported anxiety symptoms (BAI and STAI-S) across both groups and within groups. Additionally, a multivariate analysis of covariance (MANCOVA) was conducted to examine whether group differences in self-reported anxiety affected group differences in iFC.

3. Results

As shown in Table 1, no group differences (BI vs. non-BI) were observed for sex, age, IQ, or movement during the resting state scan (mean framewise displacement). No participants had current psychopathology and no group difference emerged in BAI scores. The