You say ‘prefrontal cortex’ and I say ‘anterior cingulate’: meta-analysis of spatial overlap in amygdala-to-prefrontal connectivity and internalizing symptomology

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INTRODUCTION

The past few years have witnessed a paradigm shift in the characterization of neuropsychiatric disorders, away from categorical descriptions towards a dimensional view. 1 This shift is due, in part, to the observations of common behavioral, neurobiological and genetic substrates shared across phenotypically related disorders. This is particularly true among the internalizing disorders (for example, anxiety, depression and posttraumatic stress disorder (PTSD)), which are highly comorbid and have common diagnoses. This is particularly true among the internalizing disorders and genetic substrates shared across phenotypically related parts, to the observations of common behavioral, neurobiological et al. 5 As such, a core emotion psychopathology. 5 A better understanding of the etiopathogenesis of internalizing psychiatric markers (for example, Goodkind et al. 6 ) have prompted the search for potential transdiagnostic neural substrates involved in developmental risk (for example, early adversity and family history), we conducted an activation likelihood estimation meta-analysis of frontoamygdala circuitry. We included all reported amygdala to frontal coordinate locations that fell within a liberal anatomically defined frontal mask. Peak effects across studies were centered in two focal subareas of the ACC: pregenual (pgACC) and subgenual (sgACC). Using publicly available maps and databases of healthy individuals, we found that observed subareas have unique connectivity profiles, patterns of neural co-activation across a range of neuropsychological tasks, and distribution of tasks spanning various behavioral domains within peak regions, also known as ‘functional fingerprints’. These results suggest disruptions in unique amygdala–ACC subcircuits across internalizing, genetic and environmental risk studies. Based on functional characterizations and the studies contributing to each peak, observed amygdala–ACC subcircuits may reflect separate transdiagnostic neural signatures. In particular, they may reflect common neurobiological substrates involved in developmental risk (sgACC), or the broad expression of emotional psychopathology (pgACC) across disease boundaries.

Connections between the amygdala and medial prefrontal cortex (mPFC) are considered critical for the expression and regulation of emotional behavior. Abnormalities in frontoamygdala circuitry are reported across several internalizing conditions and associated risk factors (for example, childhood trauma), which may underlie the strong phenotypic overlap and co-occurrence of internalizing conditions. However, it is unclear if these findings converge on the same localized areas of mPFC or adjacent anterior cingulate cortex (ACC). Examining 46 resting-state functional connectivity magnetic resonance imaging studies of internalizing conditions or risk factors (for example, early adversity and family history), we conducted an activation likelihood estimation meta-analysis of frontoamygdala circuitry. We included all reported amygdala to frontal coordinate locations that fell within a liberal anatomically defined frontal mask. Peak effects across studies were centered in two focal subareas of the ACC: pregenual (pgACC) and subgenual (sgACC). Using publicly available maps and databases of healthy individuals, we found that observed subareas have unique connectivity profiles, patterns of neural co-activation across a range of neuropsychological tasks, and distribution of tasks spanning various behavioral domains within peak regions, also known as ‘functional fingerprints’. These results suggest disruptions in unique amygdala–ACC subcircuits across internalizing, genetic and environmental risk studies. Based on functional characterizations and the studies contributing to each peak, observed amygdala–ACC subcircuits may reflect separate transdiagnostic neural signatures. In particular, they may reflect common neurobiological substrates involved in developmental risk (sgACC), or the broad expression of emotional psychopathology (pgACC) across disease boundaries.

Although the extant literature converges on frontoamygdala circuitry as a core neural substrate altered across internalizing, genetic and environmental risk studies, it is unclear if findings across these studies localize to the same areas of mPFC or adjacent anterior cingulate cortex (ACC). ACC and mPFC are large, heterogeneous regions. Focal subareas within these do not have uniform function, 11,12 cellular composition 13 or position within neuroanatomic circuits. 14,15 ACC/mPFC subregions also have distinct and frequently opposing roles in emotion processing. In general, ventral regions subserves emotion regulation, whereas dorsal regions contribute to the appraisal, expression and facilitation of emotion (see Etkin et al. 16 ). As such, abnormalities in amygdala connectivity with different ACC/mPFC subregions likely have distinct phenotypic consequences.

To test localization of findings across studies, we conducted a coordinate-based meta-analysis of neuroimaging studies reporting disruptions in resting-state FC of the amygdala with frontal regions. We used a data-driven approach to evaluate spatial localization in studies that report significant differences in frontoamygdala FC in patient or at-risk groups. We focused on resting-state FC because it is reproducible and robust to variation exposure to childhood adversity, 10 suggesting that frontoamygdala FC may be a transdiagnostic marker of internalizing psychopathology.

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in experimental parameters. To better understand resulting meta-analytic peak effects, we evaluated their connectivity profiles in healthy individuals using FC mapping and publicly available task activation databases. We also used quantitative functional decoding to identify the distribution of tasks spanning various behavioral domains within each meta-analytic peak, also known as ‘functional fingerprints’. Finally, we assessed the studies contributing to each peak to look for common features (for example, age and diagnosis) that could inform whether the peak was a potential marker of premorbid risk vs expression of symptomology.

**MATERIALS AND METHODS**

**Study selection**

The selection process occurred in multiple stages. First, we searched PubMed (www.pubmed.gov) with the keywords ‘connectivity AND (‘resting-state’ OR rest OR intrinsic) AND amygdala AND (‘fMRI OR ‘functional MRI’ OR ‘functional magnetic’ OR fc-MRI OR fcMRI) AND (PTSD OR Borderline OR internalizing OR ‘behavioral inhibition’ OR stress OR adversity OR abuse OR poverty OR maltreatment OR trauma OR depression OR anxiety OR ‘negative affect’ OR ‘reward sensitivity’ OR anhedonia OR mood OR bipolar or dysthymia OR ‘negative emotionality’ OR neuroticism) AND (‘prefrontal’ OR ‘PPFC’ or cingulate OR ACC OR orbitofrontal)’ for the time frame up to April 2016. This search identified 182 papers. We then refined our search from the 182 identified articles assessing from the title and abstract whether the studies: (1) investigated internalizing conditions (for example, anxiety or affective disorders) or associated risk factors (for example, family history, childhood adversity, trait anxiety and behavioral inhibition); (2) examined resting-state FC of the amygdala (that is, used a seed-based approach); (3) included a group comparison between patients/at-risk individuals and matched healthy control participants, or examined risk factors on a continuum (for example, behavioral inhibition); and (4) reported coordinates in a defined stereotaxic space (Talairach or Montreal Neurological Institute) for the meta-analysis. When multiple patient groups were available, we included results comparing all patients vs healthy controls. Studies were excluded if they (1) used nonhuman animals, (2) used a non-seed-based approach (for example, independent components analysis), (3) seeded regions other than the amygdala, (4) examined externalizing conditions/risk factors (for example, substance use disorder, attention deficit hyperactivity disorder and aggression) or gene polymorphisms, or (5) examined FC following an experimental perturbation (for example, psychosocial stressor, intervention and mood induction). In addition, studies examining postpartum or geriatric depression were excluded, as well as studies with comorbid epilepsy, brain injury or chronic physical condition (for example, end-stage renal disease). Of note, five additional articles were later identified through PubMed and Google Scholar by assessing similar studies that met the initial inclusion/exclusion criteria. After applying outlined exclusion/inclusion criteria (see Figure 1), 55 studies were further assessed for eligibility by reviewing the full text of the article. Nine studies were excluded after full-text review because although these studies fulfilled our initial inclusion criteria, eight studies did not report significant findings in frontal regions and one study did not report coordinate locations (Figure 1; Supplementary Table S1). Thus, 46 studies with a total of 2401 participants (n = 893 in patient/risk group) were ultimately included in the meta-analysis (Table 1). Twelve studies evaluated heritable or temperament risk factors (for example, negative affect and family history), 9 included individuals with major depressive disorder (MDD), 7 with anxiety disorders, 6 with PTSD, 6 environmental risk studies (for example, adversity and stress), 4 with bipolar disorder, 1 with borderline personality disorder, and 1 examined PTSD and environmental risk (early stress) within the same study. Thirty-two studies included adults and 14 included youth ages 18 and under. The majority of included studies (38) contributed more than one foci, and in total, 206 experimental foci were analyzed.

We used a data-driven approach, and included all coordinate locations reported in eligible studies that fell within an anatomically defined frontal mask (Supplementary Figure S1) comprised of the 13 frontal areas defined by the Harvard–Oxford cortical atlas (http://www.cma.mgh.harvard.edu/fsi_atlas.html): frontal operculum cortex, frontal orbital cortex, cingulate gyrus (anterior division), paracingular gyrus, subcallosal cortex, frontal medial cortex, precentral gyrus, inferior frontal gyrus (pars opercularis), inferior frontal gyrus (pars triangularis), middle frontal gyrus, superior frontal gyrus, insular cortex and frontal pole. The mask was dilated by one voxel in all directions. Studies could contribute more than one unique frontal peak.

**ALE meta-analysis**

We used the revised activation likelihood estimation (ALE) algorithm to identify consistent patterns of amygdala resting-state FC changes with frontal regions. This algorithm aims to identify brain areas showing a convergence of reported coordinates across studies, which is higher than expected under a random spatial association. ALE treats reported peak coordinates, or ‘foci’, as centers for three-dimensional Gaussian probability distributions that capture the spatial uncertainty associated with each focus. Width of the probability distribution is weighted based on sample size of the study from which the foci were drawn, such that smaller distributions are used for larger samples and vice versa. Then, for each voxel, probabilities of all foci of a given study are aggregated to produce a modeled activation map. Modeled activation maps are combined to produce voxel-wise ALE scores, which reflect the convergence of results at each location of the brain.
Table 1. Included studies that examined internalizing conditions or risk factors and resting-state functional connectivity between amygdala and frontal regions

| #  | First author      | Year | Journal                  | Participant age (years) | Total number of participants | % Female | Groups or variable of interest                                                                 | Length of scan (min) | Eyes closed or eyes open | n on medications | GSR (global signal regression)? | Scroubling or regressing out affected volumes? | Amygdala seed definition | Whole-brain or ROI analysis | Number of frontal peaks |
|----|-------------------|------|--------------------------|-------------------------|------------------------------|----------|-----------------------------------------------------------------------------------------------|---------------------|--------------------------|---------------------------|----------------------------|---------------------------------|-------------------------|-----------------------------|----------------------|
| 1  | Chai              | 2016 | Biol Psychiatry          | 8-14                    | 43                           | 49       | Children with familial risk of MDD vs HC                                                      | 6.2                 | Eyes open, blank screen                   | Not specified             | No                         | Yes—outlier volumes regressed out       | AAL atlas               | Whole brain                  | 2                     |
| 2  | Zhang             | 2016 | Prog Neuropsychopharmacol | 38-62                   | 66                           | 58       | PTSD vs trauma-exposed controls                                                               | 8                   | Eyes closed                              | Not specified             | Yes                        | Yes                             | AAL atlas               | Whole brain                  | 5                     |
| 3  | Aghajani          | 2016 | Hum Brain Mapp           | 13-17                   | 42                           | 90       | Sexually abused adolescents with PTSD vs controls                                            | 6                   | Eyes closed                              | 3                         | Yes                        | Yes                             | Amunts 2005 (in SPM Anatomy toolbox) | Whole brain                  | 4                     |
| 4  | Kim               | 2016 | Neuropsychiatr Dis Treat  | 12-16                   | 44                           | 32       | Adolescents with MDD and disruptive behaviors vs HC                                            | 12                  | Not specified                             | No                         | No                         | No                              | AAL atlas               | Whole brain                  | 2                     |
| 5  | Barch             | 2015 | Am J Psychiatry          | 7-12                    | 105                          | 41       | Income-to-needs ratio in children                                                             | 6.8                 | Eyes closed                              | Not specified             | Yes                        | Yes                             | Subject-specific seeds derived from FreeSurfer | Whole brain                  | 2                     |
| 6  | Davey             | 2015 | Psychol Med              | 16.5 ± 0.5              | 56                           | 45       | Negative affect in adolescents                                                               | 11.9                | Eyes closed                              | 1 (fluoxetine)            | Yes                        | No                              | ROI BA 25 (via WFU PickAtlas)         | Whole brain                  | 1                     |
| 7  | Liu               | 2015 | Med Sci Monit            | 13-18                   | 46                           | 59       | Adolescents with first-episode GAD vs HC                                                      | 8                   | Eyes closed                              | All med free during 2 weeks before study | Yes                        | No                              | AAL atlas               | Whole brain                  | 3                     |
| 8  | Nicholson         | 2015 | Neuro-psychopharmacology  | PTSD+DS: 37 ± 12.9 PTSD+DS: 37 ± 12.7 HC: 32 ± 11.4 25 ± 2.37 | 89                           | 74       | PTSD + dissociative subtype IDS vs HC                                                        | 6                   | Eyes closed                              | No                         | Current psychotropic use       | Yes—outlier volumes regressed out       | Amunts 2005 (in SPM Anatomy toolbox) | Whole brain                  | 8                     |
| 9  | Rohr              | 2015 | Neuroimage               | 43 ± 53                 | 53                           | 38       | Negative affectivity and task interference (ability to inhibit negative information and negative affect in healthy adults | 7.67 min & 15.33 min (1/2 of sample) | Not specified | Not specified | No                         | No                              | No                              | Harvard–Oxford atlas | Whole brain                  | 2                     |
| 10 | Stoddard          | 2015 | Psychiatry Res           | 9-18.5                  | 53                           | 38       | Youth with BD vs severe mood dysregulation (SMI) vs HC                                        | 6                   | Not specified                             | 42                        | Yes—outlier volumes regressed out       | No                              | Amunts 2005 (in SPM Anatomy toolbox) | Whole brain                  | 1                     |
| 11 | Wang              | 2015 | Behav Brain Res          | MDD: 32.11 ± 11.25 MDD: 32.11 ± 11.25 HC: 33.38 ± 8.83 9-15 | 60                           | 45       | MDD vs HC                                                                                   | 8                   | Not specified                             | Not specified             | Yes                        | Yes—scrubbing in secondary analysis | 6 mm sphere around peak atrophy voxels (~16, ~6 and ~16) | Whole brain                  | 18                    |
| 12 | Thomason          | 2015 | Soc Cogn Affect Neurosci | Trauma-exposed youth vs controls | 42                           | 69       | Trauma-exposed youth vs controls                                                             | 6                   | Eyes closed                              | 4 (3 trauma, 1 comparison) | No                         | Yes—outlier volumes regressed out       | Amunts 2005 (in SPM Anatomy toolbox) | Whole brain                  | 8                     |
| 13 | Arnold Antenaper  | 2014 | Brain Connect            | SAD: 24.7 ± 6.3 HC: 25 ± 7.5 | 34                           | 53       | SAD vs HC                                                                                   | 6.4                 | Eyes open, fixation cross                   | Medication naive           | No                         | No                              | Amunts 2005 (in SPM Anatomy toolbox) | Whole brain                  | 2                     |
| 14 | Baekken           | 2014 | PLoS One                 | 21.7 ± 2.5              | 56                           | 100      | Harm avoidance (personality dimension) in healthy adults                                      | 5                   | Eyes closed                              | None used medications       | No                         | No                              | – 20, – 4, –15 and 22, – 2, 15 (Galler et al) | Whole brain                  | 10                    |
| 15 | Birn              | 2014 | Depress Anxiety          | 22-31                   | 27                           | 0        | Childhood adversity and PTSD symptoms in veterans                                            | 5.5                 | Not specified                             | No                         | Current med use                | Yes—despiking                | 4mm spheres centered on coordinate centers provided by Talairach Daemon | Whole brain                  | 8                     |
| 16 | Blackford         | 2014 | Biol Psychol             | 18-25                   | 40                           | 60       | Social inhibition in young adults (n = 3 met criteria for 1 or more AD)                       | 7                   | Eyes closed                              | No                         | No                         | No                              | Amunts 2005 (in SPM Anatomy toolbox) | Whole brain                  | 12                    |
| 17 | Aghajani          | 2014 | Cogn Affect Behav Neurosci| Trait neuroticism in healthy adults | 40.51 ± 9.45                | 50       | Trait neuroticism in healthy adults                                                        | 7.67                | Eyes closed                              | No                         | No                         | No                              | Harvard–Oxford atlas | Whole brain                  | 3                     |
| 18 | Qin               | 2014 | Biological Psychiatry    | 7-9                     | 76                           | 50       | Anxiety sores in children                                                                   | 8                   | Eyes closed                              | No                         | Current psychotropic use       | Yes—outlier volumes regressed out       | Amunts 2005 (in SPM Anatomy toolbox) | Whole brain                  | 6                     |


| #   | First author Year Journal | Participant age (years) | Total number of participants | Groups or variable of interest | Length of scan (min) | Eyes closed or eyes open | n on medications | GMR (global signal regression)? | Scrubbing or regressing out affected volumes? | Amygdala and definition | Whole-brain or ROI analysis | Number of frontal peaks |
|-----|---------------------------|-------------------------|-------------------------------|------------------------------|---------------------|-------------------------|-------------------|-----------------------------|-----------------------------------------|---------------------|-----------------------------|----------------------|
| 19  | Brown 2014 Neuropsychopharmacology | PTSD: 44 ± 11 Trauma-exposed controls: 40 ± 8.9 | 42 24 | PTSD vs trauma-exposed controls (recent military veterans) | 6.3 | Eyes open, fixation cross | 14 | No | Yes—scrubbing in secondary analysis | Amunts 2005 (in SPM Anatomy toolbox) | Whole brain | 2 |
| 20  | Coombs 2014 PLoS One | Negative affect in healthy adults | 38 29 | Eyes open, blank screen | 6.2 | Eyes open, fixation cross | No lifetime med use | Yes | No | Jeulich Histological atlas | Whole brain | 1 |
| 21  | Fan 2014 Hum Brain Mapp | Early-life stress exposure in adults | 18 0 | | 8 | | Not specified | No | No | AAL atlas and based on meta-analysis of emotion processing (Wager et al., 2012) | Whole brain | 15 |
| 22  | Gollard 2014 PLoS One | Work-related stress in adults | 93 57 | Eyed closed | 8 | All med free during 2 months before study (except contraceptives) | All med free at the time of scan | No | No | AAL atlas based on Talairach Daemon database | Partial brain mask of mPFC, ACC, PCC and insula (AAL atlas) | Whole brain | 5 |
| 23  | Hamb 2014 Biol Mood Anxiety Disord | Pediatric AD (GAD, social phobia and SAD) vs HC | 55 64 | Eyes open, fixation cross | 8 | All med free during 30 days prior to study | | No | Yes—scrubbing in secondary analysis | | Whole brain | 1 |
| 24  | Jacobs 2014 PLoS One | Remitted MDD vs HC | 53 66 | Eyes open | | All med free during 30 days prior to study | | Yes | | | | |
| 25  | Krause-Utz 2014 Psychol Med | Borderline personality disorder (with the history of interpersonal trauma) vs HC | 37 100 | Eyes closed | 6.25 | Free of medication within the past 14 days (28 on fluoxetine) | Yes (repeated analyses without) | No | Yes—scrubbing in secondary analysis | | Whole brain | 2 |
| 26  | Liu 2014 Schizophr Bull | BD (no comorbidities) vs HC | 36 61 | Eyes closed | 18 (bipolar patients) | Yes | No | Amunts 2005 (in SPM Anatomy toolbox) | Prefrontal mask (BA 9-12, 24, 25, 32 and 44-47) | Whole brain | 8 |
| 27  | Pannekoek 2014 J Child Psychol Psychiatry | Youth with MDD vs HC | 52 88 | Eyes closed | | No used medications | Yes | No | Harvard-Oxford Subcortical Structural Probability atlas (in FSL; ± 22, − 6, − 16) | Whole brain | 3 |
| 28  | Ramusubbu 2014 Front Psychiatry | MDD: 36.5 ± 10.41 HC: 32.89 ± 9.97 | 74 59 | Eyes open, fixation cross | 7.67 | All med free at the time of scanning. Fifty-two MDD patients had been previously exposed to antidepressants | No | No | | | | |
| 29  | Ray 2014 Biological Psychiatry | Young adults with childhood history of behavioral inhibition | 38 53 | Eyes closed | | No current psychotropic use | Yes | Yes—despiking | Amunts 2005 (in SPM Anatomy toolbox) | Whole brain | 4 |
| 30  | Singh 2014 Bipolar Disord | Youth with low vs high familial risk of BD | 49 63 | Eyes closed | | No used medications | Yes | Yes—outlier volumes regressed out | Harvard-Oxford atlas | Whole brain | 1 |
| 31  | Zhang 2014 PLoS One | First-episode MDD vs HC | 67 52 | Eyes closed | | Medication naive | Yes | No | AAL atlas | Whole brain | 1 |
| 32  | Anticevic 2013 Biological Psychiatry | Bipolar 1 psychosis | 119 65 | Eyes closed | | | | No | | FreeSurfer-based segmentation | Whole brain | 2 |
| 33  | Carlson 2013 Cortex | Attentional bias to threat | 15 60 | Eyes closed | | | | No | | | | |
| 34  | Heninga 2013 Proc Natl Acad Sci USA | Young adults with maltreatment during childhood | 64 47 | Eyes closed | | | | No | | | | |

*Table 1. (Continued)*
| # | First author | Year | Journal | Participant ages (years) | Total number of participants | % Female | Groups or variable of interest | Length of scan (min) | Eyes closed or eyes open | n on medications | GSR (global signal regression)? | Scrubbing or regressing out affected volumes? | Amygdala seed definition | Whole brain or ROI analysis | Number of frontal peaks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 35 | Prater | 2013 | Depress Anxiety | gSAD: 25.39 ± 5.39 | 37 | 57 | gSAD vs HC | 5 | Eyes open, fixation | 2 | (gSAD patients; SSRIs) | Yes | No | All faces = shapes localized confined within AAL-defined anatomical amygdala Amunts 2005 (in SPM Anatomy toolbox) | Partial brain mask of ACC, mPFC, DLPFC and OFC (AAL atlas) | Whole brain | 2 |
| 36 | Ray | 2013 | J Am Acad Child Adolesc Psychiatry | Youth with GAD vs HC | 35 | 66 | 6 | Eyes open, fixation | 6 | No current or past use of psychotropic medication | Yes | No | Amunts 2005 (in SPM Anatomy toolbox) | Whole brain | 3 |
| 37 | Tahmasian | 2013 | Front Human Neurosci | MDD: 51 ± 15 | 41 | 54 | MDD vs HC | 10 | Eyes closed | 7.6 | Not specified | Yes | No | AAL atlas | Whole brain | 4 |
| 38 | Tang | 2013 | Psychol Med | MDD: 23.6 ± 8.7 | 58 | 53 | MDD vs HC | 6.67 | Eyes closed, fixation | 5 | Not specified | Yes | No | Talairach Deamen and Harvard-Oxford atlas | Whole brain | 3 |
| 39 | Torrisi | 2013 | Bipolar Disord | AD: 32 ± 10 | 108 | 35 | MDD (nonrefractory) vs HC | 108 | Eyes closed | 108 | All med free at the time of scan | Yes | No | AAL atlas | Whole brain | 3 |
| 40 | van der Werff | 2013 | Psychol Med | Adults reporting childhood emotional maltreatment (CEM); before age 16; no physical or sexual abuse vs non-CEM PTSD patients | 88 | 52 | 7.6 | Not specified | Yes | No | AAL atlas | Whole brain | 5 |
| 41 | Sripada | 2012 | J Psychiatry Neurosci | GAD: 27.7 ± 7.2 | 37 | 46 | AD (SAD, PD or both) vs HC | 6 | Eyes open, fixation | 1 | (trazodone as sleep aid) | Yes | No | Amunts 2005 (in SPM Anatomy toolbox) | Whole brain | 3 |
| 42 | Hahn | 2011 | Neuroimage | AD: 32 ± 10 | 29 | 0 | PTSD vs combat-exposed controls | 10 | Eyes open, fixation | 1 | All medications | Yes | No | Amunts 2005 (in SPM Anatomy toolbox) | Whole brain | 3 |
| 43 | Kim | 2011 | Cereb Cortex | AD: 33 ± 10 | 29 | 0 | Anxiety scores in healthy adults | 7 | Eyes open, low-level illumination | 7 | All used medications | Yes | No | Amunts 2005 (in SPM Anatomy toolbox) | Whole brain | 3 |
| 44 | Lui | 2011 | Am J Psychiatry | Nonrefractory MDD: 32 ± 10 | 108 | 35 | MDD (nonrefractory) vs HC | 6.7 | Eyes closed | 108 | All medication free at the time of scan | Yes | No | Amunts 2005 (in SPM Anatomy toolbox) | Whole brain | 3 |
| 45 | Rabinak | 2011 | Front Psychiatry | PTSD: 33 ± 10 | 34 | 0 | PTSD vs combat-exposed controls | 8 | Eyes open, fixation | 8 | All used medications | Yes | No | Amunts 2005 (in SPM Anatomy toolbox) | Whole brain | 3 |
| 46 | Liao | 2010 | PLoS One | SAD: 22.55 ± 4.05 | 43 | 28 | SAD vs HC | 6.83 | Eyes closed | 6.83 | All medication free at the time of scan | Yes | No | AAL atlas | Whole brain | 5 |

Abbreviations: AAL, Automated Anatomical Labeling; ACC, anterior cingulate cortex; AD, anxiety disorder; BD, bipolar disorder; DLPFC, dorsolateral prefrontal cortex; fMRI, functional magnetic resonance imaging; GAD, generalized anxiety disorder; gSAD, generalized social anxiety disorder; HC, healthy controls; med, medication; MDD, major depressive disorder; mPFC, medial prefrontal cortex; OFC, orbitofrontal cortex; PD, panic disorder; PTSD, posttraumatic stress disorder; ROI, region of interest; SSRIs, selective serotonin reuptake inhibitors; SAD, social anxiety disorder.
Significance of convergence was assessed by comparison of ALE scores with a null distribution that includes the same number of peak foci distributed randomly throughout the brain’s gray matter.23 Random-effects inference was applied. Resulting statistical maps show clusters where convergence between foci is greater than would be expected by chance. Statistical maps were thresholded using cluster-level family-wise error correction \( P < 0.05 \) (cluster-forming threshold voxel-level \( P < 0.001 \)). When available, probabilistic cytoarchitectonic maps available through the SPM Anatomy toolbox25 were used to estimate spatial localization of results.

Functional characterization of meta-analytic peaks

To understand the functional significance of identified meta-analytic peaks, and to test whether these represent separate frontoamygdala subcircuits, we performed three complementary analyses in healthy adults to extrapolate what differences in amygdala FC might mean in patient or at-risk groups.

Resting-state FC profiles. First, we derived the pattern of whole-brain resting-state FC for each meta-analytic peak. This analysis was conducted in a sample of 1000 healthy adults via www.Neurosynth.org.26 Results are displayed at a false discovery rate-adjusted threshold of \( P < 0.01 \).

Patterns of task-related co-activation. Next, we evaluated the pattern of task-based co-activation for each peak, using meta-analytic connectivity modeling.27 Spherical (6 mm radii) regions of interest (ROIs) were created for each meta-analytic frontal peak, and the BrainMap database (www.brainmap.org)28 was searched for all functional magnetic resonance imaging (fMRI) and positron emission tomographic (PET) experiments that activated each ROI. We only considered experiments reporting stereotactic coordinates from normal mapping studies in healthy individuals. Thus, pharmacological interventions and group comparisons were excluded. First, three-dimensional peak coordinates from peak areas that activate with each ROI were pooled from retrieved studies. Then, ALE meta-analysis was used to test for spatial convergence in these co-activation peaks, using similar methods as described above. ALE statistical maps were again thresholded using cluster-level family-wise error correction, \( P < 0.05 \) (cluster-forming threshold voxel-level \( P < 0.001 \)).

Behavioral domains associated with activation. To further characterize observed meta-analytic peaks, we tested the distribution of tasks spanning various behavioral domains within peak regions, also known as ‘functional fingerprints’.20 For each ROI, we evaluated the ‘behavioral domain’ metadata from the retrieved experiments in the BrainMap database that elicited activation in that ROI (above). Behavioral domains include cognition, action, perception, emotion and interception, as well as their related subcategories (see http://brainmap.org/scribe for more information on the BrainMap taxonomy). For each domain/subcategory, the number of experiments that reported activation in each ROI was calculated. Domains/subcategories with < 25 corresponding experiments are not shown.

RESULTS

Meta-analysis of frontoamygdala resting-state FC across internalizing conditions and risk factors

The coordinate-based meta-analysis revealed two frontal regions, or ‘clusters’, where amygdala resting-state FC was reliably altered across studies (Figure 2; Supplementary Table S2). Notably, both clusters were centered in the ACC, with limited extension into mPFC. The largest cluster was centered in bilateral pregenual ACC (pgACC), and extended into both anterior dorsal and subgenual ACC (sgACC; 8% probability in s24; see Supplementary Table S2). Hereafter, we refer to this cluster as pgACC. The second cluster was more ventral and centered in right sgACC (72% probability in s24; Supplementary Table S2).

Next, we examined the studies contributing to each cluster (Supplementary Table S2) to look for commonalities across studies. We found that studies contributing to the sgACC cluster consisted predominantly of young people ages 20 and under, with varied environmental (for example, early stress exposure) and temperamental (for example, negative affect and behavioral}

Figure 2. Converging evidence of disrupted amygdala functional connectivity with two separate ACC subregions across 46 internalizing, genetic and environmental risk studies. Results of coordinate-based meta-analysis that included 2401 individuals. ACC, anterior cingulate cortex; ALE, activation likelihood estimation; pgACC, pregenual ACC; RSFC, resting-state functional connectivity; sgACC, subgenual ACC.
Laterality of the amygdala seed region was split across study foci, with 42% reporting effects with right amygdala, 38% with left amygdala and 19% with bilateral amygdala. Laterality was also split under each meta-analytic cluster: 6 of the 12 foci contributing to the pgACC peak reported effects with left amygdala, 5 with bilateral and one with right amygdala. Three of the five foci contributing to the sgACC peak reported effects with bilateral amygdala, one with right and one with left amygdala.

Functional characterization of ACC meta-analytic peaks

Next, we performed functional characterizations of the resulting ACC peaks in healthy individuals to infer what connectivity between amygdala and ACC may mean in patient or at-risk groups. Results may also inform whether the observed ACC peaks represent separate or overlapping brain circuits.

Resting-state FC profiles. We first examined patterns of resting-state FC of each ACC peak in a sample of 1000 healthy individuals. As shown in Figure 3a, whole-brain FC patterns were unique for each peak. In brief, the activity in pgACC was correlated with the activity in precuneus and posterior cingulate cortex, resembling the canonical default mode network (DMN),\textsuperscript{32} as well as amygdala, insula and inferior frontal gyrus, involved in the salience network.\textsuperscript{33} sgACC correlations were observed in local ACC regions, extending into caudate, amygdala and hippocampus, and also in precuneus and posterior cingulate cortex (Supplementary Table S3).

Patterns of task-related neural co-activation. We quantitatively mapped task-based co-activations for each peak using the BrainMap database. Fifty-four and 29 experiments in the BrainMap database reported activations within pgACC and sgACC ROIs, respectively. These studies consisted of 971 and 493 healthy individuals, respectively (see Supplementary Table S5 for metadata from retrieved experiments). Using meta-analytic connectivity modeling, we found distinct patterns of co-activation clusters for each ACC peak (Figure 3b; Supplementary Table S4). In brief, pgACC was associated with co-activation clusters in caudate, posterior cingulate/precuneus, amygdala and parahippocampal gyrus. sgACC was co-activated with caudate and orbitofrontal cortex.

Behavioral domains associated with activation. To outline the functional profiles of observed peaks, we performed a functional decoding analysis based on the BrainMap meta-data. We found that activation in both pgACC and sgACC was associated with the emotion domain and, to a lesser extent, cognition (Figure 4). Relative to sgACC, pgACC activation was more likely to be associated with perception, and language and memory subcategories of cognition.

DISCUSSION

Altered connectivity between amygdala and frontal regions is commonly reported across a range of internalizing, genetic and environmental risk studies. Here we conducted a coordinate-based meta-analysis to test whether findings across studies localize to the same frontal subarea(s). Results converged on two focal subareas of the ACC, centered in pgACC and sgACC. Using FC analyses and publicly available databases of healthy individuals, we discovered that each peak has unique resting-state FC, functional co-activation profiles and ‘functional fingerprints’. These results suggest that observed peaks represent separate frontoamygdala subcircuits. Based on functional characterizations and the studies contributing to each peak, we assert that observed subcircuits reflect distinct transdiagnostic neural signatures. In particular, amygdala–pgACC disruptions were observed broadly in individuals across the internalizing spectrum and may thus reflect general emotional disturbance or specific symptoms that are shared across the internalizing conditions (for example, negative affect\textsuperscript{34}). Altered amygdala–sgACC FC, in contrast, was observed almost exclusively in at-risk youth, implying a potential brain substrate of developmental vulnerability.

The largest meta-analytic cluster was centered in the pgACC, which is involved in automatic forms of emotion regulation, performing a generic negative emotion inhibitory function.
whenever there is a need for suppression of limbic reactivity. Explicit forms of emotion regulation occur by engaging this core circuitry (see Etkin et al.16), which is consistent with this peak’s functional characterization under both cognition and emotion behavioral domains (Figure 4). Here we found that studies across the internalizing spectrum reported abnormalities in amygdala–pgACC circuitry. This raises the possibility that amygdala–pgACC circuitry is broadly involved in emotional psychopathology, or a construct that is shared across the internalizing conditions. For example, prominent models of core affect emphasize that ‘loss’ symptomatology, or a general sense of negative affect or dysphoria (for example, feelings of sadness/withdrawal) is shared across internalizing disorders. Threat symptomology (for example, avoidance and hypervigilance), in contrast, is more specific to the anxiety disorders, and disruptions in positive affect (for example, reward deficits and anhedonia) are more specific to the mood disorders. In line with a general role of amygdala–pgACC circuitry in emotional psychopathology, reduced pgACC gray matter volume is consistently reported in meta-analyses of anxiety as well as affective disorders. Notably, FC and co-activation mapping in Figures 3 and 4 revealed strong connectivity and co-activation of the pgACC with core nodes of the DMN, including precuneus and posterior cingulate cortex. The tight coupling between pgACC and DMN may allow affective disruptions in amygdala–pgACC circuitry to integrate into self-referential processes supported by the DMN, thus propagating negative affect (see Hamilton et al.19). Connectivity was also observed between pgACC and fronto-insular regions, implicated in the salience network. Increased salience network response to negatively valenced stimuli is a consistently reported finding in MDD, suggesting a potential role for pgACC in negative emotion processing. Taken together, abnormalities in core emotion regulation amygdala–pgACC circuitry may underlie the generic negative affect dysregulation observed across internalizing conditions.

Studies contributing to the sgACC cluster consisted of environmental and temperamental risk studies (for example, childhood adversity, negative affect and behavioral inhibition) conducted predominantly in young people (ages 20 and under). Thus, disruptions in amygdala–sgACC circuitry might reflect a state of premorbid risk—a notion supported by prior research. For instance, longitudinal studies demonstrate that dysfunctional response in amygdala corresponds with genetic (that is, family history of depression) and environmental risk (that is, childhood emotional neglect), and that response in sgACC predicts subsequent increases in depressive symptomology during adolescence. Broadly, sgACC is thought to subserve behavioral withdrawal and the promotion of safety behaviors. Thus, early alterations in amygdala–sgACC circuitry may underlie early withdrawal behaviors that could lead to further development of internalizing symptomology. For instance, emergence of emotional psychopathology may depend on later changes in amygdala–pgACC circuitry.

Although the ALE meta-analysis identified significant spatial convergence in two areas of the cingulate cortex, a large portion (~70%) of studies did not contribute to observed meta-analytic peaks. Notably, there was particularly low spatial convergence in MDD. This is consistent with prior ALE meta-analyses in MDD and other psychiatric disorders. For example, one ALE meta-analysis in MDD reported consistent gray matter reductions (relative to healthy controls) in a similar bilateral pgACC region, with 40% of included studies contributing to this peak. In that study, and other ALE studies in psychiatric populations (for example, Chen et al.), as low as 4% of included studies contribute to a single meta-analytic peak. Taken together, these findings suggest significant variability across studies, and particularly within MDD. Convergence within frontoamygdala circuitry might be achieved with the addition of more studies with specific patient subgroups (for example, early age of onset and recurrent). Signal dropout will also contribute to low convergence across studies, as amygdala and ventral frontal regions are highly susceptible to signal loss. Another possibility is that variability reflects significant heterogeneity in network topology among patients. Balsters et al. suggest that conventional methods for generating seed regions may contribute to variable connectivity findings, as these methods do not account for heterogeneous network topology in patient groups.

Our meta-analytic results demonstrate the importance of improved anatomical specificity in reported findings. This point is not unique to the study of internalizing conditions, and there are several examples in the literature illustrating this. There are various means available for improving specificity in reported findings. One resource is cytoarchitectonic maps, including the widely used Brodmann areas and more recent three-dimensional multimodal brain atlases that allow registration of fMRI data into cyto-, myelo- and chemo-architectonic maps. For example, the Eickhoff–Zilles atlas distributed with SPM Anatomy toolbox and the Harvard–Oxford atlas distributed with the FSL software can allow for better understanding of the functional profiles and circuitries in which resulting peak areas are embedded. It is encouraging that results derived from large databases (for example, coordinate-based meta-analysis and meta-analytic connectivity modeling) appear to recapitulate known cytoarchitectonic borders (see Fox et al.25).

Limitations of this work warrant mention. We focused on resting-state FC studies to circumvent variation in behavioral performance and differences in task parameters/paradigms across studies. However, there are still various experimental (for example, eyes open vs eyes closed) and analytic (for example, GSR and motion scrubbing) strategies that differ across FC studies that may have an impact on meta-analytic findings. Indeed, the field still lacks consensus on the best practices for collecting and processing resting-state FC data and, moreover, how these various approaches have an impact on observed findings. We attempted to address this by evaluating studies separately based on the use of GSR, which is known to alter resting-state FC correlations. We also provide key factors that vary between studies (Table 1), and
suggest that there may be other factors contributing to variability across studies (for example, experimental or analytic methods, differences in sample characteristics, disease course and psychological state). Another consideration is that eight additional studies met criteria for inclusion, but did not report significant effects in frontal regions (Supplementary Table S1). Our goal was to examine spatial overlap in studies that do report findings in frontal regions. Future research should test the robustness of these effects using similar methodology. In addition, functional characterizations of observed meta-analytic peaks were conducted in healthy individuals, which allowed us to (1) evaluate whether peaks reflect unique brain areas that are embedded in unique circuits, and (2) infer what behavioral consequences of altered connectivity in these areas might be. A comprehensive developmental and clinical characterization of these circuits across ages and patient populations is warranted. Next, although we focus here on identifying focal subareas of frontal regions, there are also important subregions of the amygdala. Seventeen percent of the experimental foci included in the meta-analysis reported effects of amygdala subregion(s): 17 in basolateral amygdala, 9 in centromedial and 8 in superficial. Further research is needed to understand contributions of amygdala subregion(s) to these findings, and advances in multiband and multiecho neuroimaging will make this all the more accessible.

CONCLUSIONS

The present meta-analysis indicates that findings across intercalizing, genetic and environmental risk studies converge on two focal subareas of ACC. We demonstrate that these ACC subregions have unique patterns of resting-state FC, task-related co-activation and ‘functional fingerprints’, suggesting that they represent distinct frontoamygdala subcircuits. Based on these functional characterizations and the studies contributing to each metaanalytic peak, disruptions in frontoamygdala subcircuits might reflect separate transdiagnostic neural signatures involved in developmental risk (sgACC) or the broad expression of emotional psychopathology (pgACC).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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