Published in final edited form as:

Am J Geriatr Psychiatry. 2020 October ; 28(10): 1089–1101. doi:10.1016/j.jagp.2020.02.014.

Amygdala functional connectivity features in grief: A pilot longitudinal study

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Abstract

Objective.—Acute grief, in an important minority of older adults, can become protracted, intense, and debilitating, leading to the development of complicated grief (CG). However, the neurobiological mechanisms underlying a maladaptive grief response after an attachment loss are unknown. The current study aimed to examine the amygdala brain network features that cross-sectionally explain the symptom variance and longitudinally relate to grief symptom trajectories after an attachment loss.

Methods.—Baseline amygdala functional connectivity (Fc) was assessed using a seed-based resting-state functional MRI method in 35 adults who were within one-year after death of a loved one and 21 healthy comparison (HC) participants. MRI scans were obtained at baseline, and clinical assessments, including the inventory of complicated grief (ICG) were completed at weeks 0, 8, 16, and 26 (endpoint).

Results.—Relative to HC participants, grief participants showed increased amygdala Fc in the posterior default mode (bilateral medial temporal lobes and left precuneus) and thalamus. Amygdala Fc in the default mode and ventral affective regions positively correlated with ICG scores at baseline. Furthermore, increased baseline amygdala functional connections with the dorsal frontal executive control and salience network regions correlated with worsening ICG scores over time. These longitudinal findings persisted after controlling for covariates, including baseline depressive and anxiety symptoms.

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Conflicts of Interest: The authors have no disclosures to report.

Conclusion.—These results provide novel preliminary evidence suggesting amygdala-based brain network measures to cross-sectionally explain symptom variance and longitudinally correlate with grief symptom trajectories in griever. Amygdala brain network function measures may have the potential to serve as biomarkers of CG.

Keywords

amygdala; emotion processing; emotion regulation; resting-state functional MRI; brain network; functional connectivity; bereavement; grief; complicated grief

Introduction

Older adults inevitably lose loved ones, although their responses to bereavement vary. Grief is usually transient, with most griever adapting to attachment loss within 12 months and returning to normal functioning without clinical intervention. However, in an important minority, estimated at about 7–10%, complicated grief (CG) ensues. CG is a unique and recognizable mental health condition characterized by protracted, intense, and debilitating symptoms.^{1, 2} CG can be clearly distinguished from bereavement-related major depression (MDD) in its clinical features^{3, 4} and treatment response,^{5, 6} though these conditions often co-occur in the elderly following bereavement. CG is associated with physical health and cognitive decrements, an increased rate of mental disorders and sleep disturbances, poor quality of life, premature mortality, and increased suicide risk.^{7–12} Despite the magnitude of the problem, little is known regarding the neurobiology underlying the grief symptom variation observed during the first year following bereavement in older adults. Moreover, we cannot distinguish those who will successfully transition to integrated grief from those who are prone to CG trajectories. Identifying functional neuroimaging correlates of a protracted grief symptom course may aid in the early detection of those at risk for CG so that interventions may be targeted to prevent its development. In this preliminary investigation we examined a sample of predominantly older griever within one year following attachment loss, focusing on the amygdala-based brain network – a functional neural circuitry that could serve as a CG biomarker.

An intact amygdala-based functional neuronal system could play an important role in coping with grief, and separation distress, and in eventual acceptance of the loss. Focus is on the *amygdala* because this limbic brain region is implicated in sadness, separation distress, storage of emotional memory, and detecting threats to attachment.^{13–16} A central role for the amygdala is supported by observations that oxytocin, a neurotransmitter that plays an important role in promoting attachment and bonding, attenuates amygdala responses to emotion stimuli.¹⁷ A task-dependent functional MRI (T-fMRI) experiment has demonstrated that an enhanced amygdala reactivity to reminders of the deceased correlates with intense levels of sadness in acute grief.¹⁸ Also, enhanced activation in the default mode, salience, ventral prefrontal, and subcortical limbic and thalamic areas in response to emotional stimuli and to reminders of the deceased are described in acute grief.^{19–21} Emerging evidence suggests that the dorsal prefrontal regulatory regions modulate attentional and emotional aspects of amygdala reactivity in acute grief and may assist in distinguishing grief symptoms of avoidance and intrusive thoughts.¹⁸ Reminders of the deceased also elicit differential

localized activation in these same emotion processing and regulatory brain regions in CG.²² Taken together, these observations have led us to theorize that intrinsic functional decoupling between the amygdala and other nodes involved in emotion processing and regulation will in cross-sectional analysis explain the grief symptom variance observed in older individuals following a loss, and in longitudinal analysis associate with CG symptom trajectories.

We tested this hypothesis by defining amygdala as the seed and using resting-state functional connectivity MRI (R-fMRI), a unique method that measures temporal correlations at rest of spontaneous low-frequency blood oxygenation level-dependent time-series fluctuations between functionally connected but spatially separated brain regions.²³ Amygdala-based R-fMRI studies have been conducted to probe brain network dysfunction in late-life depression (LLD).²⁴ The objectives of this pilot study were to determine the amygdala functional connectivity (Fc) features that distinguish older grief from healthy comparison (HC) participants, to cross-sectionally examine amygdala Fc irregularities that explain the symptom severity variance among predominantly older grief participants, and to use baseline amygdala Fc to longitudinally characterize grief symptom trajectories.

We hypothesized that grief participants, relative to HC individuals, would show increased amygdala Fc in the ventral prefrontal cortices and subcortical limbic and thalamic areas, brain regions that subserve social pain^{25, 26} and emotion processing²⁷ and are germane to grief-related symptoms of separation distress. We also hypothesized that these Fc irregularities would positively correlate with symptom severity in grieverers at baseline. We further posited that worsening grief symptom trajectories over time would be related to increased baseline amygdala functional connections with the dorsal frontal regulatory and salience regions that are implicated in grief symptom dimensions of intrusive thinking and avoidance.

Methods

Study participants

Recruitment—Thirty-eight adults aged 50 years and older who were within 12 months following bereavement were enrolled to a six-month pilot study. The grief participants were recruited via advertisements, and through referrals from grief group and hospice counselors. Twenty-one HC participants also were enrolled; HC participants only completed baseline clinical and neuroimaging visits. All participants provided written informed consent according to our Institutional Review Board-approved protocols.

Inclusion and exclusion criteria—All participants had to be proficient in English and have adequate visual and auditory acuity, a score < 4 on the modified Hachinski Ischemic Scale (HIS), and a score ≥ 24 on the Mini Mental State Exam (MMSE).²⁸ Exclusion criteria included a lifetime history of bipolar or psychotic disorders; alcohol or substance use disorders during the past five years; acute suicidality (assessed using the Scale of Suicidal Ideation [SSI]²⁹ and the third 17-item Hamilton Depression Rating Scale [HDRS]³⁰ item score or judged by a clinician to be at serious suicide risk); a history of neurological illnesses, including seizures, stroke, dementia of any etiology, severe head injury, brain

tumor, etc.; MRI contraindications; and delirium/unstable medical conditions determined using the Cumulative Illness Rating Scale-Geriatric version (CIRS-G)³¹ score of 4 in any category.

Inclusion/exclusion criteria for HC participants were identical to those for grief participants, except for the following differences: No lifetime history of any psychiatric illnesses; no history of death of a spouse/partner or a first-degree relative within two years of enrollment; an HDRS score <7 and/or a 30-item Yesavage Geriatric Depression Scale (GDS) score <8;³² and no psychotropic medications.

Assessment procedures—At *baseline* (week 0 visit), all participants underwent clinical assessments, including a Structured Clinical Interview for DSM-5 Research Version.³³ Sociodemographic characteristics, medical and psychiatric histories, and medication history were obtained, and a neurological examination was performed. All participants also completed self-report questionnaires and a battery of tests. These included the HDRS, GDS, Hamilton Anxiety Scale (HAM-A), CIRS-G, HIS, SSI, MMSE and Mattis Dementia Rating Scale-2.³⁴ All grief participants also completed the Inventory of Complicated Grief (ICG),³⁵ and information related to the relationship of deceased and time since loss (TSL) were documented. ICG is a 19-item, self-report questionnaire that has good to excellent psychometric properties and assesses symptoms of CG.³⁵ ICG has been previously utilized in CG treatment studies,^{5, 6} and the total scores range from 0 to 76; a score ≥30 is highly likely indicative of CG.

Grief participants who met current DSM-5 criteria for depressive, anxiety, or trauma- and stressor-related disorders were not excluded if the onset followed bereavement. Since this was an observational study, grief participants could be on antidepressant medications and/or low doses of benzodiazepines or gabapentin as long as the doses remained stable for at least four weeks prior to baseline clinical and MRI visits.

All grief participants were invited back for the *longitudinal* clinical visits. Follow-up clinical assessments were conducted at weeks 8, 16 and 26 weeks after the baseline (week 0) clinical visit. At each follow-up visit, participants underwent a brief clinical interview with the study physician, and completed a few clinical scales, including the ICG.

Retention and final study sample—Of the 38 enrolled grief participants, three were excluded from all analyses due to either structural MRI motion artifact (n=1) or claustrophobia during MRI (n=2); thus, 35 grief and 21 HC participants completed the cross-sectional study. Three grief participants were lost to follow-up; thus, a total of 32 of 38 grief participants (i.e., ~84%) completed the longitudinal study.

MRI acquisition

An MRI scan was obtained within one month following the week 0 visit, on a GE 3T MR750 scanner (Waukesha, WI) with a standard transmit and receive head coil. A high-order shimming protocol was used on each subject to improve the field homogeneity and reduce image distortion. A whole-brain R-fMRI scan was acquired in sagittal view using a single-shot gradient recalled echo planar imaging (EPI) pulse sequence. The R-fMRI scan

parameters were TR=2000 msec, TE=25 msec, field of view=24×24 cm², matrix size=64×64, voxel size=3.75×3.75×4 mm³, number of slices=36, and a scan time=8 minutes. High-resolution anatomical images were acquired using 3D spoiled gradient-echo sequence. The parameters were: TR=8 msec, TE=3 msec, TI=450 msec, flip angle=12°, matrix size=256×224, voxel size=0.938×0.938×1mm³, continuous 150 axial slices with 1-mm thickness, and scan time=6 minutes.

MRI processing

Structural MRI and R-fMRI preprocessing steps are detailed in the supplemental data file.

Amygdala functional connectivity

The seed-based R-fMRI method was used to examine the whole-brain voxelwise amygdala resting-state Fc. The bilateral amygdala seed region of interest (ROI) was obtained from the Automated Anatomical Labeling template (see Figure S1).³⁶ An *a priori* target mask ROIs was selected using the Wake Forest University PickAtlas software version 3.0.5b (<http://www.fmri.wfubmc.edu/download.htm>).^{37, 38} The target ROI mask included the anterior cingulate, caudate, lentiform nucleus (LN), medial frontal gyrus, superior frontal gyrus (SFG), middle frontal gyrus (MFG), inferior frontal gyrus (IFG), insula, superior and inferior parietal lobules, hippocampus (Hipp), amygdala (Amyg) and parahippocampal gyrus (PHG), posterior cingulate, precuneus (Precu), and thalamus (Thal) (see Figure S2). These ROIs were selected in accordance with previous T-fMRI studies implicating these regions in grief.^{18–20, 39}

Pearson product-moment correlation was used to obtain a whole-brain amygdala Fc map for each participant by cross-correlating between the mean amygdala seed time course and the time courses of all target mask voxels. Next, the correlation coefficient (cc) maps were subjected to Fisher's z-transformation to obtain an approximately normally distributed $z = 0.5 \cdot \ln\{(1+cc)/(1-cc)\}$. Finally, each individual Fc map was smoothed using a 5-mm Gaussian kernel.

Statistical analysis

Demographics (except gender) and clinical characteristics were compared using two-sample t-tests; χ^2 test was used to test for gender differences.

The AFNI program *3dttest++* (student t-test) was used to examine for voxelwise amygdala Fc differences between grief and HC, while controlling for demographics (i.e., age, gender and education), and for voxelwise gray matter concentrations (GMC). We used AFNI program *3dClustSim* to correct for multiple comparisons: two-sided, voxelwise $p < 0.05$, $\alpha < 0.05$, cluster size $> 1056 \text{ mm}^3$ for the *a priori* target, which corresponds to p-value threshold < 0.05 corrected for family-wise error, according to 10,000 iterations in Monte Carlo simulations. We also analyzed the data using a more conservative threshold of voxelwise $p < 0.01$, cluster size $> 376 \text{ mm}^3$.

To determine the cross-sectional relationship between voxelwise amygdala Fc (independent variable) and ICG (dependent variable), while controlling for demographics, GMC, TSL, and HDRS, the following linear regression model was used (two-sided, voxelwise $p < 0.05$, $\alpha < 0.05$, cluster size $> 1056 \text{ mm}^3$, and $p < 0.01$, cluster size $> 376 \text{ mm}^3$ for the *a priori* target mask):

$$ICG(s) = \beta_0(v) + \beta_1(v)Age(s) + \beta_2(v)Sex(s) + \beta_3(v)Edu(s) + \beta_4(v)GMC(s,v) + \beta_5(v)TSL(s) + \beta_6(v)HDRS(s) + \beta_7(v)AmygFc(s,v) + \epsilon(s,v)$$

where AmygFc=voxelwise amygdala Fc, s=subject, and v=voxel. Outliers were detected if the adjusted mean amygdala Fc or the adjusted ICG was > 3 scaled median absolute deviation (MAD) away from the median.

For the longitudinal analysis, the ICG rate of change (ΔICG) was first calculated, using the MATLAB program *regress*:

$$ICG(s,t) = ICG_0(s) + \Delta ICG(s) * t$$

A positive ΔICG means worsening grief symptoms and vice versa.

A linear regression model (two-sided, voxelwise $p < 0.05$, $\alpha < 0.05$, and cluster size $> 1056 \text{ mm}^3$ and a more conservative $p < 0.01$, cluster size $> 376 \text{ mm}^3$ for the *a priori* target mask) examined the relationship between baseline amygdala Fc (independent variable) and ΔICG (dependent variable), while controlling for demographics, GMC, TSL, and baseline HDRS:

$$\Delta ICG(s) = \beta_0(v) + \beta_1(v)Age(s) + \beta_2(v)Sex(s) + \beta_3(v)Edu(s) + \beta_4(v)GMC(s,v) + \beta_5(v)TSL(s) + \beta_6(v)HDRS(s) + \beta_7(v)AmygFc(s,v) + \epsilon(s,v)$$

Participants were considered as outliers if the adjusted mean amygdala Fc or ΔICG was > 3 scaled MAD away from the median.

Exploratory whole cerebrum analyses—Using the whole cerebrum (defined by the 90 cerebral ROIs from the Automated Anatomical Labeling atlas³⁶) as the target mask, we repeated all the above analyses (voxelwise $p < 0.05$, $\alpha < 0.05$, cluster size $> 1392 \text{ mm}^3$). Finally, the cross-sectional and longitudinal regression analyses were repeated while controlling for two additional regressors (i.e., HAM-A and antidepressant/benzodiazepine use status).

Results

Demographic and clinical characteristics

Our sample comprised mostly older adults; however, compared with HC, grief participants were younger, and had higher HDRS, GDS, and HAM-A scores. In the grief group, the mean (SD) ICG score was 27.89 (13.86) and TSL was 163.66 (95.1) days (Table 1). Eighteen of the 35 grief participants had ICG score of ≤ 30 at baseline. Twenty-six (74%) grief participants had lost a spouse/life partner, or child. About half ($n=17$) of the grief

sample met criteria for a bereavement-related psychiatric disorder: depressive disorders alone (MDD: $n=12$; unspecified depressive disorder: $n=1$), unspecified anxiety disorder ($n=1$), co-occurring MDD and unspecified anxiety disorder ($n=2$), and concurrent MDD and post-traumatic stress disorder ($n=1$). Twelve of the 35 grief participants were on antidepressants or benzodiazepines (Table 1). At the week 26 assessment, the mean (SD) ICG score was 20.53 (12.08); 7 of 32 grief participants had an ICG score of ≥ 30 at endpoint.

Cross-sectional findings

A priori target ROI mask: The grief participants, relative to the HC group, showed increased amygdala Fc in the left amygdala/hippocampus/PHG/precuneus/thalamus and the right amygdala/hippocampus/PHG clusters, after controlling for demographics and GMC (Figure 1). No clusters with diminished amygdala Fc were seen.

In grief participants, linear regression revealed positive associations between baseline amygdala Fc and ICG scores in the left amygdala/hippocampus/PHG, and left SFG/orbitofrontal cortex (OFC) clusters, while controlling for covariates (Table 2, Figure 2). Figure 2 illustrates results after an outlier was removed.

A conjunction analysis revealed an overlapping cluster in the left amygdala/PHG between the grief vs. HC group difference and the cross-sectional linear regression results (Figure 3).

We repeated the above analyses using a more conservative $p < 0.01$. In addition to the left amygdala/hippocampus/PHG cluster, increased amygdala Fc in the left posterior cingulate cortex (PCC) and thalamus clusters were seen in the grief group, relative to HC. In the grief group, linear regression revealed positive amygdala Fc–ICG associations in the left amygdala/hippocampus/PHG, right PHG, left SFG/OFC, and left lentiform nucleus clusters. Conjunction analysis revealed similar overlapping cluster in the left amygdala/PHG (Table S1).

Longitudinal findings

A priori target ROI mask: Baseline amygdala Fc positively correlated with ICG rate-of-change in the following clusters, while controlling for covariates, including baseline HDRS: left IFG/MFG/SFG, right SFG and left MFG clusters (Table 2, Figure 4). Figure 4 illustrates results after removing an outlier. To further examine the combined effect size of the longitudinal results, we integrated these results across the three significant clusters. We found that these combined amygdala Fc features explained ~36% of the variance in the ICG-rate-of-change (Figure S3). Using a conservative p threshold < 0.01 , findings remained significant in the left SFG cluster (Table S1).

Whole cerebrum findings

The amygdala Fc differences between grief and HC groups were similar (Figure S4). The cross-sectional relationship of amygdala Fc with ICG scores were also similar (Figure S5). Additionally, negative association between amygdala Fc and baseline ICG score was observed in the left post central gyrus (Figure S5). The longitudinal results were similar to

those discussed above (Figure S6). Additionally, baseline amygdala Fc in the left middle temporal gyrus positively correlated, and in the right cuneus negatively correlated with the ICG rate-of-change. All whole cerebrum findings remained significant after controlling for HAM-A and antidepressant/benzodiazepine status.

Discussion

Our study had three main findings: First, grief participants showed increased amygdala Fc in the posterior default mode regions (i.e., medial temporal lobe [MTL] and precuneus), fusiform gyri and thalamus, relative to nonbereaved HC participants. Second, enhanced amygdala Fc in the ventral affective and default mode regions, and in the basal ganglia was cross-sectionally associated with greater grief symptom severity in bereaved individuals. Finally, increased baseline amygdala Fc with the dorsal executive control and salience regions correlated with worsening CG symptoms in grieverers over the course of six months. Of note, these longitudinal findings remained significant after adjusting baseline depressive and anxiety symptoms.

The observed amygdala Fc irregularities in grieverers are consistent with the extant literature on the neural correlates of grief. T-fMRI experiments have provided early insights into the localized neural activity underlying grief in response to affectively laden stimuli. In individuals who were within one year following the death of a first-degree relative, personalized stimuli such as a photograph of the deceased and grief-related words elicited activation in the fusiform gyri and posterior default mode areas.¹⁹ Acutely grieving women after the loss of an unborn child showed activation in the same default mode areas as well as the thalamus while viewing happy baby faces.²⁰ Neural activation in the precuneus and fusiform gyrus to deceased-related attentional bias is also reported in grieverers.²¹ Collectively, these results indicate that specific amygdala brain network Fc abnormalities underlie grief and may explain the symptom variations seen in acute grief.

We demonstrate that increased amygdala functional connections in the MTL-based default mode regions, OFC, and basal ganglia are associated with higher grief symptom scores at baseline. During the initial months following a loss, grief varies in intensity and is characterized by a wide range of separation distress symptoms. These include yearning and pangs of sadness, ruminative thoughts of the deceased, somatic distress, avoidance behaviors, and difficulty comprehending the painful reality of the death.¹ However, minimal research has been conducted that aims to parse out the brain network correlates of multidimensional symptoms of acute grief. Thus, our preliminary results provide novel data to support hypothesis generation.

An acute grief study reported that amygdala hyperactivity to memories of the deceased correlates with peak intensity of sadness and intrusiveness, but not with yearning.¹⁸ Also, sustained activation in the default mode regions is thought to facilitate ruminative thought processes.^{40, 41} On the other hand, the MTL-based default mode regions and OFC are core nodes of the ventral affective neuronal system and are involved in generating negative emotions, particularly sadness, and somatic symptoms such as sleep and appetite changes.²⁷

Moreover, an increased OFC activity during a grief-related task was reported in those with integrated grief, but a relative inability to recruit this region was seen in CG.³⁹ The OFC, basal ganglia, and ventral striatum are also implicated in hedonic experience; these areas may be linked to yearning for the deceased.⁴² Thus, the enhanced amygdala connectivity with the default mode, ventral affective, and basal ganglia structures observed here may underlie the varying levels of grief-related separation distress symptoms, chiefly sadness, ruminative thinking, somatic distress, and yearning for the deceased.

Enhanced amygdala functional connections with the dorsal frontal executive control and salience regions were associated with maladaptive symptom trajectories. Recent T-fMRI investigations using emotional Stroop paradigms have shed light on the role of executive control and salience brain regions in grief, though the evidence is mixed.^{18, 21, 39} In one study, attentional bias toward deceased-related words was associated with activation in the amygdala, MFG, IFG and the middle temporal gyrus in acute grief. In that study, avoidance correlated with MFG and amygdala deactivation, whereas intrusiveness was associated with amygdala activation.¹⁸ Intrusive thinking about the negative consequences surrounding the loss along with an inability to self-reflect, reappraise, and assimilate new information is hypothesized to result in a maladaptive grief response. Also, it is theorized that in CG, avoidance of reminders of the deceased, which is intended to regulate negative emotions such as sadness and yearning, can be pervasive and impairing.⁴³ In this context, increased levels of intrusive thinking and avoidance behaviors in earlier periods may contribute to the future development of CG. Interestingly, those with higher levels of avoidance grieving paradoxically experience more intrusive thoughts of loss during ongoing monitoring of mind wandering, despite attempts to avoid such thoughts. This contradictory response may be mediated via interactions between a broader cortical network comprising the default mode, cognitive control, and salience regions and the OFC-basal ganglia subnetwork.⁴⁴ Moreover, differential activity in the middle temporal gyrus and the cuneus, additional brain areas seen in our whole cerebrum analyses (Figure S6) have been previously reported in studies of acute grief from romantic rejection²⁶ and after death of an unborn child.²⁰

In LLD, frontal lobe hypoactivation and diminished amygdala Fc with the executive control and salience regions are reported, relative to HC individuals.^{24, 45} Such brain network function differences in LLD are linked to depression severity and cognitive impairments. These observations are consistent with the executive control deficit theory of LLD. In contrast, our longitudinal grief data lead us to hypothesize that bereaved individuals experience a failure to regulate negative emotions due to enhanced amygdala—executive control and amygdala—salience neuronal systems. This could clinically manifest, in a significant minority, as high levels of intrusive thinking and avoidance behaviors, leading to a CG trajectory. Importantly, our results remained significant after accounting for baseline depressive and anxiety symptoms. Moreover, our longitudinal findings, when combined, explained ~36% of the variance in CG symptom rate of change over time. Thus, we propose that these amygdala-based network measures may have the potential to be CG biomarkers.

The upstream mechanisms that cause amygdala network dysfunction in grief are unknown, but several determinants show promise. Bereavement in its early stages could lead to stress-induced activation of the hypothalamus-pituitary-adrenal axis, resulting in neuroendocrine

and physiological dysregulation. Increases in catecholamines and cortisol levels have been previously reported in acute grief.^{46–48} In CG, baseline epinephrine levels may predict psychotherapeutic response.⁴⁹ CG individuals have a flatter diurnal cortisol slope throughout the day relative to those with integrated grief.⁽⁵⁰⁾ Changes in immune functioning are also reported in acute grief.^{46, 51, 52} Bereaved individuals have higher circulatory inflammatory marker levels than their nonbereaved counterparts;⁵³ proinflammatory markers may contribute to abnormal brain network activity in grief.⁵⁴ Risk factors related to the person (e.g., prior mood disorder history, early life trauma or loss, sociocultural influences) and loss (e.g., nature of death, loss of a child) may increase the likelihood of developing CG.

Our study has limitations. First, we had a relatively small sample size; the resulting limited statistical power could have contributed to the disparate cross-sectional and longitudinal brain network-ICG relationship findings. Second, about one-half of the grief subjects met criteria for a current psychiatric disorder; a majority had depressive/anxiety disorders. To clarify whether our amygdala Fc results were specific to grief symptoms, we extended our regression models to adjust for baseline depressive and anxiety symptoms; the findings remained significant. Future studies should use sophisticated multivariate regression models to tease apart the effects of brain network parameters on multidimensional symptoms. Third, even though participants had to be on stable doses before MRI, antidepressants could have modulated brain network function. We therefore repeated the analyses while controlling for antidepressant status. The results did not change (Figures S5 and S6). Fourth, we used a 12-month cutoff to enroll grievers because CG could only be diagnosed, as per the DSM-5 criteria for persistent complex bereavement disorder, if symptoms persist for at least one year after the death. Although we included TSL as a covariate, future work should enroll participants who are within a closer proximity to the loss. Fifth, concerns have been raised regarding inflated false positive rates (FPRs) across existing functional MRI software (including 3dClustsim) when using traditional parametric statistical methods for voxelwise multiple comparison corrections.⁵⁵ Future definitive studies should use more stringent multiple comparison correction methods, such as the mixed model for spatial ACF (for parametric approaches),^{56, 57} or statistical permutation testing (for nonparametric methods),⁵⁵ to estimate FPRs. Finally, studies should examine whether the observed brain network-ICG findings are mediated by different age-related changes, including WM hyperintensity burden.

This pilot study provides novel evidence indicating that amygdala-based brain network features explain the cross-sectional symptom variance and correlate with a CG symptom trajectory following attachment loss. Future research using multimodal imaging approaches and other neurobiological determinants of a grief-induced stress response combined with multidimensional symptom assessments will further enhance our understanding of the biomarkers specific to CG.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments:

This research was supported by the National Institute of Mental Health grant R21 MH109807; Costigan Family Foundation; Froedtert Hospital Foundation; and the National Center for Advancing Translational Sciences, National Institutes of Health, Award Number UL1 TR001436. This research was also completed in part with computational resources and technical support provided by the Research Computing Center at the MCW.

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Highlights

- Do amygdala-based brain network features cross-sectionally explain the symptom variance and longitudinally associate with complicated grief symptom trajectories after an attachment loss?
- Increased amygdala functional connectivity in the posterior default mode, fusiform gyri, and thalamus was observed in grief participants. Increased baseline amygdala functional connections with the ventral affective and default mode areas cross-sectionally correlated with higher grief symptoms, and the executive control and salience network regions correlated with worsening grief symptoms over time.
- Amygdala-based brain network measures may have biomarker potential in complicated grief.

Figure 1.

Amygdala functional connectivity group differences. (A) illustrates brain regions showing significant differences in grief participants relative to the healthy comparison group. Bright color indicates increased functional connectivity. (B) and (C) are histograms indicating individual data distribution for grief (green) and control (yellow) groups, respectively. In the histograms, the whiskers indicate one standard error for the data. The height of the bar indicates the average adjusted m value of the significant cluster.

Abbreviations. R: right; L: left; Thal: thalamus; Precu: precuneus; Amyg: amygdala; Hipp: hippocampus; PHG: parahippocampal gyrus; Fc: functional connectivity; HC: healthy comparison; adj: adjusted for covariates.

Figure 2.

Cross-sectional association between amygdala functional connectivity and inventory of complicated grief scores in grief. (A) illustrates brain regions showing significant results. Relationships between adjusted mean amygdala Fc in the (B) left PHG and (C) left SFG clusters and adjusted ICG scores in grief participants at baseline. The color bar indicates the value of the partial F statistic; a greater partial F value indicates a more significant relationship. R: right; L: left; SFG: superior frontal gyrus; Amyg: amygdala; Hipp: hippocampus; PHG: parahippocampal gyrus; ICG: inventory of complicated grief; Fc: functional connectivity; adj: adjusted for covariates.

Figure 3. Conjunction analysis between the cross-sectional t-test differences (Figure 2A) and linear regression (Figure 3A) data. Overlapped left parahippocampal gyrus/amygdala cluster.

Figure 4.

Linear relationship between baseline amygdala functional connectivity and the inventory of complicated grief score rate-of-change in grief participants. (A) illustrates brain regions showing significant results. Relationships between adjusted mean baseline amygdala Fc in the (B) left IFG/MFG/SFG; (C) right SFG; and (D) left MFG clusters and adjusted ICG rate-of-change in grief participants. The color bar indicates the value of the partial F statistic; a greater partial F value indicates a more significant relationship. R: right; L: left; SFG: superior frontal gyrus; MFG: middle frontal gyrus; IFG: inferior frontal gyrus; ICG: inventory of complicated grief; Fc: functional connectivity; adj: adjusted for covariates.

Table 1.

Baseline demographics and clinical characteristics

	Grief (N = 35)	HC (N = 21)	Statistical Value
Age, yr	66.66±10.01	72.43±7.21	$t=-2.304$; $df=54$; $p=0.025^a$
Gender, F/M	23/12	18/3	$\chi^2(1, n=56) = 2.677$; $p=0.102^b$
Race			$\chi^2(2, n=56) = 0.868$; $p=0.6479^b$
White	32	19	
African American	2	2	
Other	1	0	
Education, yr	15.66±3.21	16.26±2.94	$U=319$; $p=0.418^c$
CIRS-G	6.17±3.48	4.76±2.68	$t=1.593$; $df=54$; $p=0.117^a$
Relationship of Deceased			
Spouse/Partner	19		
Child	7		
Parent	6	NA	NA
Other	3		
Time Since Death, days	163.66±95.10	NA	NA
MMSE	28.49±1.72	28.90±1.34	$t=-0.955$; $df=54$; $p=0.344^a$
MDRS-2 Total	139.94±3.50	140.90±2.10	$t=-1.141$; $df=54$; $p=0.259^a$
HAM-A	7.89±4.84	3.67±4.44	$t=3.256$; $df=54$; $p=0.002^a$
HDRS-17	12.60±7.00	3.11±1.75	$t= 5.636$; $df=51$; $p<0.001^a$
GDS	11.66±8.35	3.33±2.96	$t=4.393$; $df=54$; $p<0.001^a$
ICG	27.89±13.86	NA	NA
Psychotropic Medication Use, n			
No Antidepressant/Benzodiazepine	23	NA	NA
SSRI/SNRI Monotherapy	7		
Combination Antidepressant	3		
Benzodiazepine	2		

Notes. Values reported are mean ± standard deviation. CIRS-G scores are calculated after excluding the psychiatric illness category. HC: nonbereaved healthy comparison; yr: years; F: Female; M: Male; CIRS-G: Cumulative Illness Rating Scale–Geriatric Version; MMSE: Mini-Mental State Exam; MDRS-2: Mattis Dementia Rating Scale–2 raw total scores; HAM-A: Hamilton Anxiety Scale; HDRS: 17-item Hamilton Depression Rating Scale (three HC participants had missing HDRS data); GDS: 30-item Yesavage Geriatric Depression Scale; ICG: Inventory of Complicated Grief; SSRI: selective serotonin reuptake inhibitor; SNRI: serotonin norepinephrine reuptake inhibitor; df: Degree of freedom; NA: not applicable. Computed using

^a2-sample t-test;

^bchi-square test;

^cMann-Whitney U test.

Table 2.

Amygdala functional connectivity results

Description	Regions	Brodmann area	Side	Cluster size (mm ³)	MNI Coordinates			R ²	p-value ¹	df
					X	Y	Z			
<i>Cross-sectional</i> 2-sample t-test: Grief vs. HC	Amyg/Hipp/PHG	20	Left	9392	32	16	-18	NA	0.001 (t=3.463)	54
	Amyg/Hipp/PHG	28	Right	3344	-18	8	-26	NA	0.02 (t=2.334)	54
<i>Cross-sectional</i> Linear regression	Amyg/Hipp/PHG	34	Left	3616	26	2	-18	0.25	< 0.004	32
	SFG	11	Left	1520	16	-64	-16	0.23	< 0.005	32
<i>Cross-sectional</i> Conjunction analysis ²	Amyg/PHG	34	Left	1184	25	9	-25	NA	NA	NA
<i>Longitudinal</i> Linear regression	IFG/MFG/SFG	46	Left	7752	52	-34	24	0.32	< 0.002	29
	SFG	6	Right	1600	-10	-32	60	0.31	< 0.002	29
	MFG	9	Left	1400	52	-22	34	0.32	< 0.002	29

Notes. Results for voxelwise $p < 0.05$, 3dClustsim corrected. MNI: Montreal Neurological Institute; RAI: ; right anterior inferior; X, Y, Z depicts coordinates of peak voxel in the cluster; HC: healthy comparison; df: Degree of freedom; NA: not applicable; Amyg: amygdala; Hipp: hippocampus; PHG: parahippocampal gyrus; SFG: superior frontal gyrus; OFC: orbitofrontal cortex; MFG: middle frontal gyrus; IFG: inferior frontal gyrus.

¹ p-value represents mean amygdala functional connectivity group differences in the significant clusters, and linear relationship between adjusted mean amygdala functional connectivity and adjusted inventory of complicated grief scores in the significant clusters;

² center voxel of the overlapped region.