EVALUATION OF VMPFC-AMYGDALA RESTING STATE FUNCTIONAL CONNECTIVITY AS A TRANSDIAGNOSTIC MODERATOR OF MOMENTARY SADNESS AND RUMINATION

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by

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The undersigned, appointed by the dean of the Graduate School, have examined the thesis entitled

EVALUATION OF VMPFC-AMYGDALA RESTING STATE FUNCTIONAL CONNECTIVITY AS A TRANSDIAGNOSTIC MODERATOR OF MOMENTARY SADNESS AND RUMINATION

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and hereby certify that, in their opinion, it is worthy of acceptance.

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Abstract

Rumination has been identified as a transdiagnostic feature of emotional distress disorders that exacerbates symptoms of anxiety and depression. It is thought that rumination may be due in part to heightened resting state functional connectivity (rs-FC) in the ventromedial prefrontal cortex (vmPFC) and amygdala. Though past fMRI research has linked in-lab self-report measures of emotional distress to vmPFC-amygdala rs-FC, few have examined its relation to symptoms of distress using ecological momentary assessment (EMA). This proposed study utilized EMA to examine vmPFC-amygdala rs-FC as a moderator of momentary rumination and sadness. The sample consisted of 27 women who met criteria for anxiety, depression, and/or borderline personality disorder. Participants completed a 6-minute resting state scan and were administered multiple prompts every day for 14 days. Multilevel models were used to assess the moderating influence of vmPFC-amygdala rsFC on the relationship of momentary- and day-level sadness and rumination. Results showed that though vmPFC-amygdala rsFC did not significantly interact with concurrent and lagged *momentary* rumination to predict *momentary* sadness, it did significantly moderate the relationship between concurrent *day-level* rumination and sadness such that individuals with higher rsFC experienced a stronger relationship between sadness and rumination. This study utilized novel methods to offer vmPFC-amygdala rsFC as a potential influencing factor on the relationship between rumination and sadness people experience in daily life.

Evaluation of vmPFC-Amygdala Resting State Functional Connectivity as a Transdiagnostic Moderator of Momentary Sadness and Rumination

Emotional distress disorders such as anxiety, mood, and borderline personality disorder (BPD), are characterized by increased negative affect and often co-occur with one another (Kotov et al., 2011). It is for this reason that a research focus on specific symptoms and/or characteristics rather than traditional diagnostic categories has been suggested to investigate their underlying mechanisms (Insel, 2010).

One characteristic, rumination, has been identified as a transdiagnostic feature of emotional distress disorders such as depression and anxiety that serves to exacerbate symptoms such as negative affect. Rumination is a process by which individuals repeatedly focus on their symptoms of distress and potential consequences (Nolen-Hoeksema, 2008). Past research has implicated rumination in the development of depressive symptoms at subsequent observations (Broderick & Korteland, 2004). When assessed using a trait-level measure, the Ruminative Response Scale (RRS), it has also been shown to predict onset, number, and duration of major depressive episodes (Robinson & Alloy, 2003).

Trait-level rumination measured through questionnaires has been shown to be an important factor in the severity and maintenance of depressive symptoms, however, a major limitation of trait-level measures is the inability to measure rumination in response to emotional events in daily life. To address this issue, Moberly and Watkins (2008) developed two items representing the definition of rumination to assess ruminative thoughts in the moment through ecological momentary assessment (EMA). One item asked participants to rate the degree to which they were focusing on their feelings, the second prompted them to rate the degree to which they were focused on their problems. Using a sample of 108 community volunteers,

Moberly and Watkins found that the new momentary ruminative self-focus items were able to explain additional variance beyond that of trait rumination measured using the RRS. Importantly, they also found that rumination reported at the current prompt was associated with increased negative affect at the subsequent prompt. The reverse was also true such that negative affect reported concurrently predicted rumination at the following prompt. This study was important in demonstrating the exacerbating influence of rumination on negative affect in real-world, real-time contexts.

A subsequent study that utilized these momentary rumination items found that prompting participants every 80 minutes to focus on ruminative statements (e.g., "think about the way you feel inside," "think about the possible consequences of the way you feel," "think about your current level of energy") for 3 minutes via personal digital assistants (PDAs) decreased positive affect in a community sample (N = 50; Huffziger et al., 2012). These findings suggest that there is a deleterious effect of momentary ruminative self-focus on affect even in individuals who have few depressive symptoms. Importantly, the findings also suggest that rumination is not merely a trait-like process associated with overall symptom severity, but a dynamic, state-like process that may influence the magnitude of negative affect in daily life.

Some have speculated that rumination may be due in part to heightened resting state functional connectivity (rs-FC) between the ventromedial prefrontal cortex (vmPFC) and amygdala (Burghy et al., 2012; Koenigs, 2008). The amygdala has long been associated with negative affect, and the vmPFC is thought to play a role in emotion processing and decision making (Hamilton et al., 2012; Stein et al., 2007; Hiser & Koenigs, 2017). Results from a study by Cooney et al. (2010) suggest that rumination may play a role in the functional connectivity between these two regions. In their study, Cooney et al. compared neural activation in

individuals diagnosed with major depressive disorder (MDD) and healthy controls after undergoing a rumination induction task. They found that individuals in the MDD group displayed significantly higher levels of activation in the amygdala, medial prefrontal cortex, and other brain regions than those in the control condition. Lending additional support, D'Argembeau and colleagues (2005) found that the vmPFC is more active during a self-referential reflective task compared to social and other-person reflection tasks in healthy volunteers. Though not rumination, per se, this study appears to implicate the vmPFC in processes involving a focus on the self. The vmPFC was also found to be implicated in rumination in a meta-analytic study by Hamilton et al. (2015) which found that higher levels of activation in the vmPFC in a sample with MDD predicts increased levels of rumination on depressive symptoms.

Some studies have proposed that the two regions are functionally linked through top-down processing whereby the vmPFC helps to regulate the amygdala (Kim et al., 2011; Urry et al., 2006). One explanation for finding a positive relationship between the two regions in people with emotional distress disorders, is that the vmPFC is unsuccessful in its attempts to regulate the amygdala in persons with higher levels of depressive symptomology. Lending support to this hypothesis, a study by Johnstone et al. (2007) found that activity in the vmPFC and amygdala is positively correlated in individuals with major depressive disorder (MDD) on a task requiring participants to decrease negative affective responses to emotionally valenced pictures, but negatively correlated in a control group.

It is possible that increases in functional connectivity between the vmPFC and amygdala when participants with emotional distress disorders attempt to decrease negative affect may be due to ineffective recruitment of the vmPFC. However, this may not fully explain why some

individuals with emotional distress disorders appear to display this relationship at rest. For example, a study by Burghy et al. (2012) found that vmPFC-amygdala rsFC is associated with higher levels of depression symptoms in female adolescents. Further complicating the hypothesis that heightened vmPFC-amygdala rsFC is due to unsuccessful recruitment of the vmPFC, a study by Koenigs et al. (2008) demonstrated that participants with bilateral vmPFC lesions showed significantly *lower* levels of depression than individuals with lesions in other brain regions. If inadequate recruitment of the vmPFC to regulate the amygdala were solely responsible for preventing the development of an emotional distress disorder, one might expect lesions in this region to yield higher incidence of depression. Instead, findings from Koenigs et al. (2008) may suggest that in some circumstances, recruitment of the vmPFC may be harmful or reflect engagement of maladaptive processes.

Instead, it may be that when persons diagnosed with an emotional distress disorder are at rest, they engage in ruminative self-focus which recruits the vmPFC and elicits activity from the amygdala in the form of increased negative affect. A review discussing the neurobiology of depression by Willner, Scheel-Krüger, & Belzung (2013) suggests that the amygdala may also influence activity in the vmPFC. Therefore, it may also be that input from the amygdala in the form of negative affect may in turn lead to reciprocal activation in the vmPFC and amygdala. This reciprocal activation may eventually lead to the worsening symptoms such as rumination and sadness.

Though previous research has demonstrated relationships between vmPFC-amygdala rsFC and questionnaire measures, little is known about how and whether it relates to symptoms in daily life contexts. To provide further support for increased vmPFC-amygdala rsFC's relationship to symptoms of emotional distress, the current study sought to expand the literature

by examining the moderating effect of vmPFC-amygdala rs-FC on the experience of sadness and rumination measured in daily life.

The present study

Previous studies on vmPFC-amygdala rs-FC have assessed the experience of depressive symptoms and rumination using in-lab self-report measures. However, retrospective assessments of symptoms are subject to participant bias in memory of emotional states (Solhan et al., 2009). To reduce this bias, the present study used EMA to measure symptoms as they happened in participants' daily lives. Additionally, EMA allows for the assessment of individuals in real-world contexts, permitting the observation of participants' levels of sadness and rumination in their natural environments (Stone & Shiffman, 1994). EMA also allows statistical models to disaggregate within-person and between-person processes. As such, the present study was able to account for the relationship between rumination and sadness at different levels of analyses (e.g., momentary, day, person) in individuals who display varying levels of vmPFC-amygdala rs-FC (Curran & Bauer, 2011).

The present study utilized resting-state functional connectivity to examine activation in the vmPFC and amygdala resulting from spontaneous emotional and cognitive processes as opposed to activation induced by task-related stimuli (van den Heuvel & Pol, 2010). Due to the high rate of co-occurrence in the emotional distress disorders (anxiety, mood, and borderline personality disorder [BPD]), the present study sought to expand on previous work by observing momentary- and day-level sadness and rumination across this spectrum of diagnoses rather than in the context of traditional diagnostic categories. The present study utilized data and variables obtained through a collaborative effort by faculty from the University of Missouri- Columbia to

study emotional dysregulation in daily life. The sample purposely included only women as women appear to differentiate from men in their neural activation during emotion-related tasks (Burghy et al. 2012). In line with converging evidence from prior research (i.e., Burghy et al., 2012; Koenigs, 2008; Cooney et al., 2010; D'Argembeau et al., 2005), we predicted that participants with heightened connectivity between the vmPFC and amygdala at rest would report higher levels of rumination and sadness in the moment than those with lower connectivity.

Methods

Participants

The original sample included 32 women in outpatient treatment for emotional distress disorders. Participants were recruited using fliers in a community outpatient clinic and weekly news bulletins through the University of Missouri. Three women in the original sample were left-handed and were excluded from further analyses and two participants were excluded for poor EMA compliance (i.e., < 60%). The resulting sample consisted of 27 right-handed women.

General eligibility for the study required that participants were between the ages of 18 and 45 years old, were not currently pregnant or did not have plans to become pregnant in the month following initial screening, did not have a history of head trauma resulting in deficits in concentration, attention, or mood, were not diagnosed with cystic fibrosis or diabetes by a medical professional, due to their effect on sympathetic nervous system activity, and were not contraindicated for magnetic resonance imaging. Diagnostic eligibility was determined through the Mini-International Neuropsychiatric Interview (M.I.N.I. 7.0) and the Structured Interview for DSM-IV Personality (SIDP-IV; Pfohl, Blum, & Zimmerman, 1997). The diagnostic interviews were completed by trained graduate research assistants. After the initial interview, a second assessor reviewed and rated the video recordings of ten randomly selected diagnostic interviews. The resulting inter-rater reliabilities were excellent for any current anxiety disorder ($\kappa = 1.00$), mood disorder ($\kappa = 0.80$), and BPD ($\kappa = 1.00$). The resulting sample was 7.41% African American (n = 2), 85.2% Caucasian (n = 23), 3.70% Hispanic (n = 1), 3.70% Native American (n = 1) and the mean age was 25.6 years (SD = 6.04 years, Range = 18 – 38 years). Demographic information is presented in Table 1.

55.5% (n = 15) of the present sample met Diagnostic Statistical Manual of Mental Disorders (5th ed.; DSM-5; American Psychiatric Association, 2013) criteria for a mood disorder, 81.5% (n = 22) met criteria for a current anxiety disorder, and 22.2% (n = 6) met diagnostic criteria for borderline personality disorder (BPD). Co-occurrence of diagnoses was high in our sample (see Table 1) as was the proportion of participants taking prescribed psychiatric medications while in the study 92.6% (n = 25).

Procedure

After an initial phone screening process, eligible participants were invited to complete two laboratory visits. In the first, participants completed the diagnostic interview to ensure that participants met criteria for a current mood disorder, anxiety disorder, or BPD according to the DSM-5 (see results above). If participants met criteria, they were deemed eligible and administered self-report surveys during the remainder of the visit. At the second visit, participants completed an fMRI scan that lasted a total of 1 hour and 45 minutes. All participants completed resting state functional scanning lasting approximately 6 minutes. After completing the scan, participants were assigned a study smartphone (Android OS) and received instructions for completing the two-week EMA portion of the study. The EMA phase began on the day of the scan. Participants were paid weekly at two follow-up appointments based on compliance. Participants were able to earn up to $180 each week if they completed more than 80% of the smartphone prompts. If participants completed less than 80% of prompts in a week, they were eligible for compensation up to $160. For every 10% drop in compliance after 80%, participants' pay dropped $35 (i.e., if <70% of prompts were completed they were eligible for $125). After the first week, participants were advised on ways they could improve compliance in their second week of participation. For the 27 participants in this sample, average compliance for random

prompts was 87.5% and 97% for morning prompts. Retention from the first session to the fourth, and final, session was 100%.

Measures

Ambulatory Assessment: For 14 days, participants carried and answered survey prompts on a Motorola Droid MAXX, Android 4.4.4 smartphone. Participants were asked to rate their mood and report on behaviors and daily experiences through surveys administered through a custom-designed app. Sadness and rumination were reported through morning, random, and participant-initiated prompts during the study. Each day, participants were required to complete a *morning prompt* before noon. Participants scheduled each morning prompt for a time they anticipated they would wake the next day (with the caveat that the morning prompt be completed before noon). If the participant did not complete the morning prompt by noon, it was considered "missing." *Six prompts were administered randomly* throughout the day after the participant's morning prompt (or 12:00 pm if missed). Each of these prompts were scheduled at least one hour apart and could not be completed within 30 minutes of another scheduled prompt. Participants were also instructed to *initiate a survey response if they experienced a significant change in mood*.

On average, participants completed 14.89 days of ecological momentary assessment (SD= 0.93, Range = 12 - 17). Participants completed an average of 80.37 prompts while in the study (SD = 13.58, Range = 53 - 101) and an average of 5.39 prompts per day (SD = 0.82, Range = 3.93 – 7.14).

Momentary Sadness. Momentary sadness was assessed using a five-item subscale of the Positive and Negative Affect Schedule-Extended version (PANAS-X; Watson & Clark, 1999).

Participants were asked to rate their experience of the five sadness items in the last 15 minutes on a scale from 1 to 5 (1 = very slightly or not at all, 5 = extremely), M [a] = 1.63, SD [a] = 0.49. Items included the degree to which participants felt "sad," "blue," "downhearted," "lonely," and "alone." Scores on the sadness subscale were calculated by taking the mean of relevant items. Within person and between person reliabilities of the sadness measure were calculated using McDonald's *omega* at the **momentary** (WP = 0.886; BP = 0.901) and **day** (WP = 0.914; BP = 0.907) levels (Geldhof, Preacher, & Zyphur, 2014).

Momentary Rumination. At each morning, random, and participant-initiated prompt, participants were asked to rate their momentary rumination on a scale from 1 (very slightly or not at all) to 5 (extremely; M [a] = 1.92, SD [a] = 0.71) using two items, "How much are you focusing on your feelings right now?" and "How much are you focusing on your problems right now?" Ratings from the two items were averaged to create a momentary rumination variable (Moberly & Watkins, 2008; Huffziger et al., 2012) The reliabilities for rumination at the **momentary** (WP = 0.848; BP = 0.971) and **day** levels (WP = 0.868; BP = 0.974) were high (Geldhof, Preacher, & Zyphur, 2014).

Image Acquisition: Resting state images were acquired using a Siemens Trio 3T scanner with an 8-channel head coil. During the scan, participants were instructed to relax with their eyes open and fixed on a blank screen with a crosshair fixation point, a method consistent with and recommended by previous studies (e.g., Patriat et al., 2013; Smith et al., 2013; Van Dijk et al., 2010). Structural T1 weighted images were obtained using a high-resolution MPRAGE sequence: repetition time (TR) = 1920 ms, echo time (TE) = 2.92 ms, flip angle = 90˚, field of

[a] Prompts collected on the same day from the same participant were averaged together to create a day mean. Day means were then averaged to create the average value of sadness and rumination experienced by a given person while in the study. The sample means and standard deviations reported are derived from these aggregated person means.

view (FOV) = 256 x 256, matrix size = 256 x 256, slice thickness = 1 mm. Functional T2* weighted images were acquired using an echoplanar pulse sequence: 32 contiguous interleaved slices, TR = 2000 ms, TE = 30 ms, flip angle = 90˚, FOV = 256 x 256 mm, matrix size = 64 x 64, slice thickness = 4 mm. Acquisition of images was tilted 30˚ towards the coronal plane from the anterior commisure-posterior commisure (AC-PC) line for the minimization of artifacts and improved scanning (Alexander & Brown, 2010; Deichmann, Gottfried, Hutton, & Turner, 2003).

Data Analysis

Image processing. SPM8 was used to preprocess resting-state data (Wellcome Trust Centre for Neuroimaging, 2009). To normalize structural and functional images to the MNI template, they were first realigned with the AC-PC line, corrected for slice acquisition timing, and coregistered with the T1 structural. Functional images were smoothed with a 6-mm spatial smoothing filter after placing them into the standard MNI template space. 36 realignment parameters including standard parameters, six movement parameters (plus CSF, white, and gray matter) were included to adjust for artifacts captured in the BOLD response. Temporal derivatives of the movement parameters, quadratic terms, and derivatives of quadratic terms were also included as covariates in a confound regression and regressed out from each ROI's time series (Satterthwaite et al., 2019). The data were filtered with a temporal bandpass filter (.01 <f<.08 Hz). Time points exceeding two standard deviations from the participant's mean for motion or intensity parameters were removed (range of time points across participants = 173 to 185 of 187 in time series) according to the scrubbing protocol for temporal censorship.

Regions of Interest (ROIs): Based on a meta-analytic review of emotion regulation research, the vmPFC ROI was defined based on a region found to be active during regulation of negative affect (Diekhof et al., 2011). The MarsBar toolbox of SPM, a program specifically

designed for ROI analyses, was used to develop the amygdala ROIs (Tzourio-Mazoyer et al., 2002). Consistent with previous research, separate analyses were performed for the left and right amygdala (Roy et al., 2009; Kim et al., 2011b). Values from left and right amygdala ROIs were then z-scored and averaged together to create a bihemispheric variable to be used in subsequent analyses (see Figure 1 for locations of ROIs).

Integrating ecological momentary assessment. We conducted multilevel model (MLM) analyses to assess whether momentary rumination significantly interacts with vmPFC-amygdala rs-FC to predict momentary sadness using the PROC MIXED function in SAS. We explored lagged momentary rumination and its interaction with vmPFC-amygdala rs-FC to determine whether there are carry-over effects from levels of rumination reported at one prompt to levels of sadness at the next. Day- and person-level rumination scores were assessed by taking the mean of observations recorded by the same person on the same day (day-level) and the mean of day-level means of rumination for each person (person-level). Day- and person-level measures of rumination were included in the model as covariates to identify the unique effect of rumination in the moment over and above that of day- and person-level rumination. Age, weekday, hour after wake, study day, and momentary fear and hostility subscales of the PANAS-X (for a full list of items see Appendix I; descriptives in Table 2) were included as covariates in the model. We adjusted for the autocorrelation of sadness by including lagged momentary sadness as a day-centered predictor. All predictor variables (with the exception of weekday, hour after wake, and study day) were centered on day-, person-, and sample-level means. We specified random intercepts for (1) participants and (2) days nested within participants. Random intercepts for days were included to account for the likelihood that some days would be characterized by more rumination than others for each participant. In this way, the model accounted for the effect of day

on rumination and sadness. Similarly, random intercepts for participants allowed us to account for individuals who ruminate more than others in the sample, allowing the model to account for the effect of person.

Our measures ask participants to rate the degree to which they are focused on their feelings and problems "right now." Because rumination is conceptualized as a repetitive and persistent process, a single moment may not fully capture its influence on mood. Therefore, it may be informative to assess the influence of the interaction between vmPFC-amygdala rsFC and rumination on sadness aggregated at the day level as well. We explored the interaction between vmPFC-amygdala rs-FC and rumination's effect on sadness at the day-level in a model including day- and person-level rumination and vmPFC-amygdala rs-FC to predict day-level sadness. In the same model, we explored the lagged effects of day-level rumination and vmPFC-amygdala rs-FC on sadness on the following day. We adjusted for the autocorrelation of sadness by including a variable representing day-level sadness centered on person levels of sadness. Age, study day, weekday, and day-level fear and hostility subscales of the PANAS-X were included as covariates. Day-level sadness, fear, and hostility were calculated using the same method described for day-level rumination. As before, all predictor variables included in the day-level model (with the exception of weekday and study day) were centered on person- and sample-level means. We specified random intercepts for person.

Results

Person-level bivariate correlations. Though person-level rumination and sadness were significantly correlated (r = 0.62, p < 0.001), the relationships between person-level sadness and rumination were not significantly correlated with vmPFC-amygdala rsFC (Sadness: $r = 0.15$, $p = 0.45$; Rumination: $r = -0.10$, $p = 0.63$). Due to the neutral-valence wording of the momentary rumination items, we decided to examine the correlation between the positive affect scale of the PANAS-X (Appendix I) and rumination to rule out the possibility that individuals who provided higher ratings of rumination reported adaptive forms of self-focus such as savoring or problem-solving (Bryant, 1989). The relationship was not significant ($r = 0.36$, $p = 0.6$).

Momentary level. In the MLM assessing momentary-level associations between our variables of interest (see Table 2), rumination measured in the same prompt and lagged rumination were significant predictors of sadness in the moment. However, there was not a significant main effect of vmPFC-amygdala rsFC on sadness in the moment. Concurrent momentary rumination did not significantly interact with vmPFC-amygdala rsFC to predict sadness at the same prompt (*Est.* = 0.021, *SE* = 0.018, $p = 0.247$). Further, lagged momentary rumination did not significantly interact with vmPFC-amygdala rsFC to predict sadness at the same prompt (*Est.* = -0.028, *SE* = 0.019, $p = 0.152$).

Day level. In the MLM assessing day-level associations between our variables of interest (see Table 2), rumination measured on the same day and lagged day-level rumination significantly predicted concurrent day sadness. The main effect for vmPFC-amygdala rsFC predicting sadness was insignificant. However, rumination aggregated to the day level significantly interacted with vmPFC-amygdala rsFC to predict overall sadness on the same day

(*Est.* = 0.097, *SE* = 0.027, *p* <.001). The positive interaction implies that the positive relationship between rumination and sadness in the same day is strengthened by heightened vmPFC-amygdala rsFC (see Figure 2). Lagged day-level rumination did not significantly interact with vmPFC-amygdala rsFC to predict overall sadness on the concurrent day (*Est.* = 0.018, *SE* = 0.027, *p* = 0.504).

Discussion

We investigated whether vmPFC-amygdala rsFC connectivity moderated the relationship between rumination and sadness in daily life using a sample of adult women diagnosed with emotional distress disorders. Though the main effect of rumination on sadness in the moment was significant, the main effect of vmPFC-amygdala rsFC on sadness in the moment was not. The finding that vmPFC-amygdala rsFC did not significantly predict sadness in the moment appears generally inconsistent with the findings of Burghy et al. (2012). In addition, the interactions of vmPFC-amygdala rsFC with *concurrent* momentary rumination and *lagged* momentary rumination to predict sadness were not statistically significant. One possible, at least partial, explanation for these null findings is the wording of the items used to capture rumination in the moment. Specifically, the items prompted participants to rate the degree to which they were focusing on their feelings and problems "right now." As rumination is conceptualized as a persistent and repetitive form of thinking, our assessment of the effect of rumination experienced by a participant "right now" as opposed to assessing the effect of rumination experienced "since the last prompt," may not have adequately captured the effect of ruminative self-focus on sadness.

To address this this possibility (of a more pervasive effect of rumination), we conducted an analysis of the interactions of vmPFC-amygdala rsFC with concurrent and lagged *day-level rumination* to predict *day-level sadness*. In this case, we found a significant interaction of vmPFC-amygdala rsFC with concurrent day-level rumination to predict day-level sadness. This suggests that at as vmPFC-amygdala rsFC increases, the relationship between day-level rumination and sadness is strengthened. However, the interaction of *lagged* rumination at the day level and vmPFC-amygdala rsFC did not significantly predict sadness. This suggests that the

relationship between rumination experienced on the preceding day and overall sadness experienced on the current day is not significantly influenced by vmPFC-amygdala rsFC.

The finding that vmPFC-amygdala rsFC moderates the relationship between rumination and sadness on the same day is consistent with the line of research that has shown a link between increased vmPFC-amygdala rsFC and negative affect (Hiser & Koenigs, 2017). The moderating effect of vmPFC-amygdala rsFC on day-level rumination and sadness appears to be consistent with a study finding that heightened vmPFC-amygdala rsFC predicted greater depression symptomatology in a sample of adolescent females (Burghy, 2012). However, it is notable that the main effect of vmPFC-amygdala rsFC on sadness experienced at the day level was still insignificant (apparently discrepant with the findings of Burghy, 2012).

Our finding that vmPFC-amygdala rsFC appears to exacerbate the relationship between day-level rumination and sadness measured through EMA appears to be supported by a study finding increased activity in the amygdala and medial prefrontal cortex in a group with MDD after a rumination induction task relative to healthy controls (Cooney et al., 2010). Though Hamilton et al. (2015) did not examine how the vmPFC's functional connectivity with the amygdala influenced symptoms, our finding also appears consistent with their results suggesting that higher activation in the vmPFC in individuals with MDD predicts higher levels of rumination on depressive symptoms. Our findings also appear consistent with Koenigs et al.'s (2008) study which found significantly *decreased* levels of depression in individuals with vmPFC lesions in that individuals in our sample with emotional distress disorders who display *higher* vmPFC-amygdala rsFC also appear to display a stronger relationship between rumination and sadness in daily life.

On the other hand, this finding may appear contradictory to research that suggests that increased connectivity between the vmPFC and amygdala at rest is adaptive (Johnstone et al., 2007; Urry et al., 2006). However, it may be that increased functional connectivity between the vmPFC and amygdala is only adaptive when the vmPFC is able to successfully regulate the amygdala. Though previous work has suggested that the vmPFC regulates the amygdala through top-down processing, there is also evidence to suggest that the amygdala may influence activation of the vmPFC (Willner, Scheel-Krüger, & Belzung, 2013). This may partially explain our finding that increased resting state functional connectivity appears to exacerbate the relationship between rumination and sadness in daily life. In some circumstances, recruitment of the vmPFC during a maladaptive form of self-reflection, such as rumination, may lead to reciprocal activation between the vmPFC and amygdala. Functional connectivity between these two regions may in turn worsen depressive symptoms such as rumination and sadness.

However, there is also research suggesting that *decreased* functional connectivity between the vmPFC and amygdala appears to be related to group differences between individuals with depression and those without a diagnosis. For example, adolescents with MDD who have never taken psychiatric medications have displayed decreased functional connectivity between the vmPFC and amygdala at baseline relative to controls (Connolly et al., 2017). These mixed findings may be due to in part to a number of factors including sample characteristics (i.e., diagnosis, medication status, age, gender make-up, etc.), analytic approach, and methods. However, it seems likely that rumination and sadness are two complex processes that may not be completely encapsulated or explained by functional connections in the vmPFC and amygdala. The vmPFC alone has been implicated in numerous complex processes such as reward valuation, decision making, social cognition, and emotion processing (Hiser & Koenigs, 2017).

Furthermore, a review by Roy, Shohamy, & Wager (2012) suggests that the vmPFC acts as a sort of "hub" through which many areas relay information to other functional brain systems. It may be that there are other key regions which dictate how functional connectivity between the amygdala and vmPFC affects the expression or severity of psychopathological symptoms such as rumination and sadness.

Limitations and Future Directions

There are limitations with the present study that warrant discussion. First, all but two individuals in the present sample were taking psychiatric medications to alleviate negative affect. It is possible that psychiatric medications may have influenced the findings. For example, our findings are consistent with a study finding heightened functional connectivity between the medial prefrontal cortex and amygdala at rest in a sample of medicated individuals with euthymic bipolar disorder compared to healthy controls (Favre et al., 2014). On the one hand, including individuals who are taking psychiatric medications allows us to increase the generalizability of our findings; however, without a group that is not taking medication, the influence of psychotropic medications on vmPFC-amygdala rsFC in our sample cannot be entirely ruled out. Future studies should investigate the possible influence of psychiatric medications on the relationship between functional connectivity in the vmPFC and amygdala and symptoms such as sadness and rumination.

By using resting state functional connectivity, we are unable to establish temporal precedence between vmPFC-amygdala functional connectivity, rumination, and sadness in daily life. Therefore, we are unable determine whether heightened vmPFC-amygdala rsFC is the cause of increases in rumination and sadness in daily life in the current study. However, past research

indicates that requiring participants to self-reflect or ruminate while in the scanner is associated with heightened activity in the vmPFC and amygdala (D'Argembeau, 2005; Cooney et al., 2010). Future research might seek to disentangle temporal relationships between vmPFC-amydala rsFC, rumination, and sadness by administering relevant tasks while participants are in the scanner.

In addition, the use of resting state functional connectivity as a predictor implies that the connectivity between the vmPFC and amygdala as a static and trait-like process, when it is likely a dynamic process. A review by Bennett & Miller (2010) found modest test-retest reliability of fMRI indices and can be influenced by a number of things including the cognitive state of the participant on the day of the scan. This would appear to suggest that cognitive states, such as a state of ruminative self-focus, may vary between scans and underscores the state-like nature of rumination.

Our sample only included women given the modest sample size from which data were collected and the finding that women and men tend to display differing relationships between vmPFC-amygdala rsFC and depressive symptoms (Burghy et al., 2012). As such, this finding may not generalize to non-female samples. Furthermore, due to the sample size, our findings should be interpreted with caution. In the future, findings from this research study should be replicated in a larger sample size. Future research should also aim to replicate these findings with a longer period of resting state fMRI to ensure their reliability (Birn et al., 2013). In studies combining fMRI and EMA methods, it may also be beneficial to conduct an additional scan at the end of the EMA study procedure to establish the test-retest reliability of vmPFC-amygdala rsFC.

Despite these limitations, to our knowledge, this is one of the first studies to examine the link between the vmPFC-amygdala rsFC and its influence on rumination and sadness measured in daily-life contexts. Though the temporal nature of this relationship is uncertain, our study offers evidence that vmPFC-amygdala rsFC may reflect or influence the relationship between rumination and sadness participants experience in their natural environments. Additionally, the use of EMA allows us to minimize participants' hindsight bias of emotional states. Finally, our study seeks to expand the generalizability of our findings by maintaining a focus on the symptoms of rumination and sadness rather than making group comparisons between diagnostic categories of emotional distress disorders which frequently co-occur with one another.

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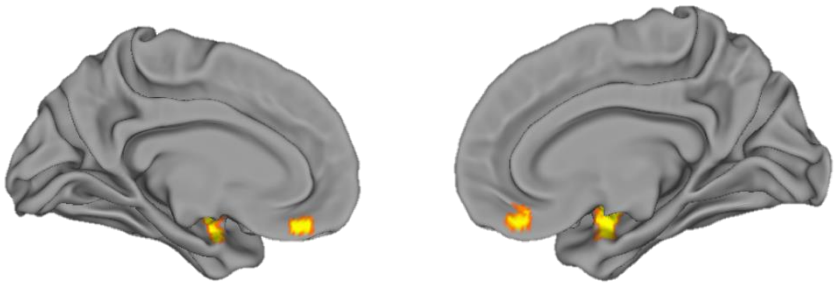

Figure 1. Demonstration of location of vmPFC and amygdala ROIs. The left image represents the locations of ROIs in the left hemisphere. The vmPFC is depicted by the yellow region in the anterior region and the amygdala is represented by the yellow region centrally located. The right image represents location of ROIs in the right hemisphere.

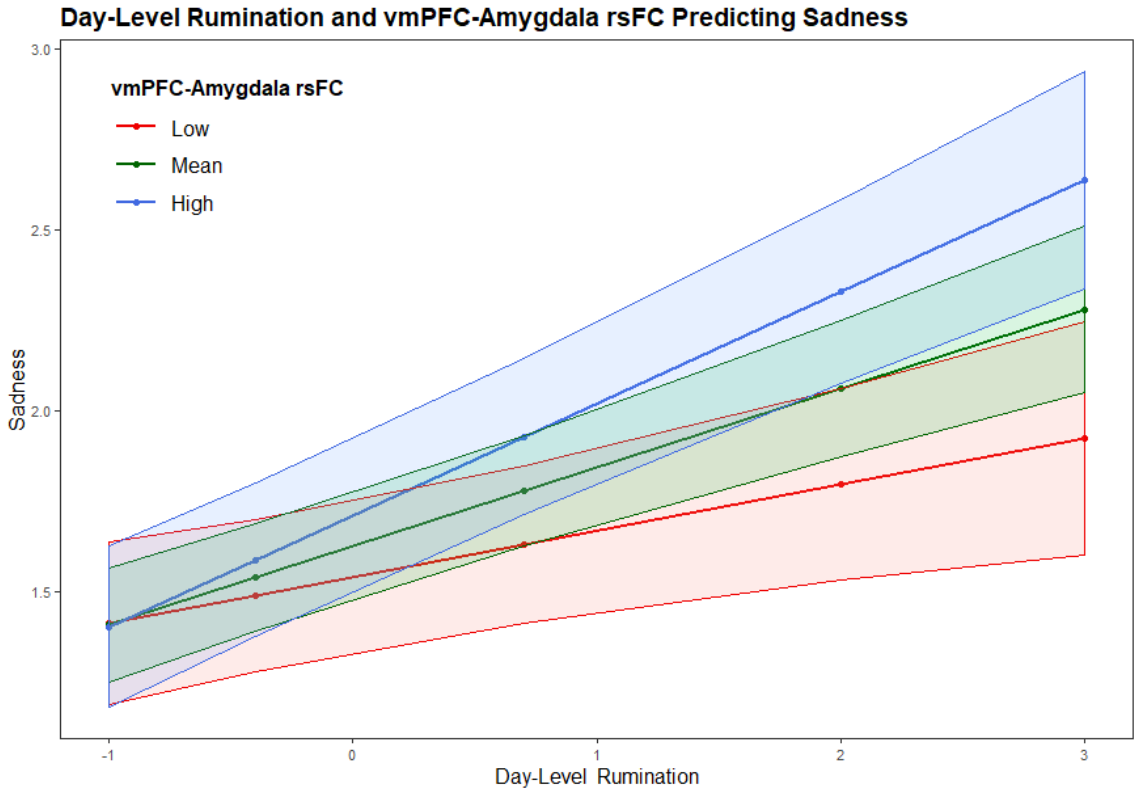

Figure 2. Illustration of the moderating effect of vmPFC-amygdala rsFC on the relationship between same-day rumination and sadness (day level). Figure 2 was plotted using the lme() function of the nlme package and ggplot2 in R Studio (Pinheiro et al., 2020;Wickham, 2016). Day-level rumination was centered on person-level means to disaggregate within and between person-level effects. Day-level sadness was the dependent variable and therefore remained uncentered. Levels of vmPFC-amygdala rsFC correspond to the following: "Low" = 1 SD below the mean, "Mean" = mean level of connectivity in our sample, and "High" = 1 SD above the mean. Shaded regions represent the 95% confidence interval for each regression line.

Table 1. *Demographics*

	N	%
Race/Ethnicity		
Caucasian	23	85.19
African-American	2	7.41
Native American	1	3.70
Hispanic	1	3.70
Marital Status[a]		
Single	17	68
Married	5	20
Cohabitating	3	12
Yearly Household Income[b]		
> 25,00 USD	9	37.50
25,001 USD – 50,000 USD	6	25
50,001 USD – 75,000 USD	3	12.50
75,001 USD – 100,000 USD	3	12.50
>100,000 USD	3	12.50
Number of Children		
0	23	85.19
1	3	11.11
4	1	3.70
Diagnostic Groups		
Mood Disorder Only	3	11.11
Anxiety Disorder Only	9	33.33
Co-Occurring Mood and Anxiety	9	33.33
Co-Occurring BPD and Anxiety	1	3.70
Co-Occurring BPD and Mood	1	3.70
Co-Occurring BPD, Mood, and Anxiety	4	14.81

[a]Two participants did not list their marital status in our sample.
[b]Three participants did not disclose their income in our sample.

Table 2. *Descriptive Statistics for Affect and vmPFC-amygdala rsFC*

	Mean (SD)	Range
Sadness	1.63 (0.49)	1.03 - 3.03
Rumination	1.92 (0.71)	1.02 - 3.72
Fear	1.41 (0.45)	1.00 - 2.58
Hostility	1.33 (0.31)	1.02 - 2.50
vmPFC-amygdala rsFC	0.009 (0.95)	-1.93 - 1.75

Table 3. *Multilevel estimates of rumination, vmPFC-amygdala rsFC, and their interactions predicting sadness*

	Momentary-Level Effects Model [a]			Day-Level Effects Model [a]		
	Estimate	**SE**	***p***	**Estimate**	**SE**	***p***
Momentary-Level						
Rumination[b]	0.24	0.02	**<.0001**	-	-	-
Lagged Rumination[b]	0.05	0.02	**0.014**	-	-	-
Fear[b]	0.16	0.04	**<.0001**	-	-	-
Hostility[b]	0.30	0.04	**<.0001**	-	-	-
Lagged Sadness[b]	-0.10	0.03	**0.001**	-	-	-
Hour After Wake	0.01	0.004	**<0.001**	-	-	-
Momentary Cross-Level Interactions						
Rumination[b] x vmPFC-Amygdala rsFC[d]	0.02	0.02	0.247	-	-	-
Lagged Rumination[b] x vmPFC-Amygdala rsFC[d]	-0.03	0.02	0.153	-	-	-
Day-Level						
Rumination[c]	0.48	0.04	**<.0001**	0.22	0.03	**<0.0001**
Lagged Rumination[c]	-	-	-	0.07	0.03	**0.021**
Fear[c]	-	-	-	0.22	0.06	**<0.001**
Hostility[c]	-	-	-	0.48	0.07	**<0.0001**
Lagged Sadness[c]	-	-	-	0.23	0.04	**<0.0001**
Study Day	0.007	0.005	0.131	0.002	0.004	0.637
Weekend	0.06	0.04	0.124	0.02	0.04	0.571
Day Cross-Level Interactions						
Rumination[c] x vmPFC-Amygdala rsFC[d]	-	-	-	0.10	0.03	**<0.001**
Lagged Rumination[c] x vmPFC-Amygdala rsFC[d]	-	-	-	0.02	0.03	0.504
Person-Level						
Rumination[d]	0.44	0.10	**<0.001**	0.44	0.11	**<0.001**
vmPFC-Amygdala rsFC[d]	0.07	0.08	0.348	0.10	0.08	0.274
Age[d]	-0.02	0.01	0.115	-0.02	0.01	0.10

Note. N = 27. [a]Momentary- and Day-Level models utilized corresponding levels of sadness as the dependent variables (i.e., momentary-level sadness and day-level sadness). [b]Momentary-level variables were centered on day-level means, such that coefficients at this level represent deviations from the average amount of rumination, lagged rumination, lagged sadness, fear, and hostility experienced on a given day. [c]Day-level variables were centered on person-level means, such that coefficients at this level represent deviations from the average amount of rumination, lagged rumination, lagged sadness, fear, and hostility experienced by a given person. [d]Person-level variables were centered on sample-level means, such that coefficients at this level represent deviations from the average amount of rumination and vmPFC-amygdala rsFC displayed by the sample.

Appendix I: Items for PANAS-X

Please indicate the degree to which you felt the following in the past 15 minutes (1 = very slightly or not at all, 5 = extremely).

Negative Affect:

1. Afraid
2. Ashamed
3. Distressed
4. Guilty
5. Hostile
6. Irritable
7. Jittery
8. Nervous
9. Scared
10. Upset
11. Frightened
12. Shaky
13. Angry
14. Scornful
15. Disgusted
16. Loathing
17. Sad
18. Blue
19. Downhearted
20. Alone
21. Lonely

Positive Affect:

1. Active
2. Alert
3. Attentive
4. Determined
5. Enthusiastic
6. Excited
7. Inspired
8. Interested
9. Proud
10. Strong

Fear:

1. Afraid
2. Scared
3. Frightened
4. Nervous
5. Jittery
6. Shaky

Hostility:

1. Angry
2. Hostile
3. Irritable
4. Scornful
5. Disgusted
6. Loathing

Sadness:

1. Sad
2. Blue
3. Downhearted
4. Alone
5. Lonely

Appendix II: Supplemental Results

Table S1. Multilevel estimates of rumination, posterior cingulate cortex-anterior medial prefrontal cortex (PCC/aMPFC) core-amygdala rsFC, and their interactions predicting sadness

	Momentary-Level Effects Model [a]			Day-Level Effects Model [a]		
	Estimate	**SE**	***p***	**Estimate**	**SE**	***p***
Momentary-Level						
Rumination[b]	0.24	0.02	**<0.0001**	-	-	-
Lagged Rumination[b]	0.05	0.02	**0.027**	-	-	-
Fear[b]	0.15	0.04	**0.0001**	-	-	-
Hostility[b]	0.29	0.04	**<0.0001**	-	-	-
Lagged Sadness[b]	-0.10	0.03	**0.002**	-	-	-
Hour After Wake	0.01	0.004	**<0.001**	-	-	-
Momentary Cross-Level Interactions						
Rumination[b] x *PCC/aMPFC core-amygdala rsFC* [d]	-0.06	0.08	0.400	-	-	-
Lagged Rumination[b] x *PCC/aMPFC core-amygdala rsFC* [d]	-0.01	0.08	0.855	-	-	-
Day-Level						
Rumination[c]	0.48	0.04	**<0.0001**	0.24	0.03	**<0.0001**
Lagged Rumination[c]	-	-	-	0.06	0.03	**0.048**
Fear[c]	-	-	-	0.23	0.07	**<0.001**
Hostility[c]	-	-	-	0.51	0.07	**<0.0001**
Lagged Sadness[c]	-	-	-	0.25	0.04	**<0.0001**
Study Day	0.008	0.005	0.125	0.001	0.004	0.691
Weekend	0.05	0.04	0.139	0.03	0.04	0.453
Day Cross-Level Interactions						
Rumination[c] x *PCC/aMPFC core-amygdala rsFC* [d]	-	-	-	0.32	0.12	**0.010**
Lagged Rumination[c] x *PCC/aMPFC core-amygdala rsFC* [d]	-	-	-	0.08	0.13	0.507
Person-Level						
Rumination[d]	0.43	0.10	**<0.001**	0.42	0.11	**0.001**

PCC/aMPFC core-amygdala rsFC [d]	0.02	0.34	0.962	0.02	0.37	0.953
Age[d]	-0.02	0.01	0.087	-0.03	0.01	0.067

Note. N = 27. [a]Momentary- and Day-Level models utilized corresponding levels of sadness as the dependent variables (i.e., momentary-level sadness and day-level sadness). [b]Momentary-level variables were centered on day-level means, such that coefficients at this level represent deviations from the average amount of rumination, lagged rumination, lagged sadness, fear, and hostility experienced on a given day. [c]Day-level variables were centered on person-level means, such that coefficients at this level represent deviations from the average amount of rumination, lagged rumination, lagged sadness, fear, and hostility experienced by a given person. [d]Person-level variables were centered on sample-level means, such that coefficients at this level represent deviations from the average amount of rumination and *PCC/aMPFC core-amygdala rsFC* displayed by the sample.

Table S2. Multilevel estimates of rumination, dorsomedial prefrontal cortex (dMPFC) subsystem-amygdala rsFC, and their interactions predicting sadness*

	Momentary-Level Effects Model [a]			Day-Level Effects Model [a]		
	Estimate	**SE**	***p***	**Estimate**	**SE**	***p***
Momentary-Level						
Rumination[b]	0.23	0.02	**<0.0001**	-	-	-
Lagged Rumination[b]	0.04	0.02	**0.04**	-	-	-
Fear[b]	0.15	0.04	**<0.001**	-	-	-
Hostility[b]	0.30	0.04	**<0.0001**	-	-	-
Lagged Sadness[b]	-0.10	0.03	**0.001**	-	-	-
Hour After Wake	0.01	0.004	**<0.001**	-	-	-
Momentary Cross-Level Interactions						
Rumination[b] x dMPFC subsystem-Amygdala rsFC[d]	-0.19	0.09	**0.025**	-	-	-
Lagged Rumination[b] x dMPFC subsystem-Amygdala rsFC[d]	-0.13	0.09	0.157	-	-	-
Day-Level						
Rumination[c]	0.48	0.04	**<0.0001**	0.22	0.03	**<0.0001**
Lagged Rumination[c]	-	-	-	0.05	0.03	0.156
Fear[c]	-	-	-	0.20	0.06	**0.003**
Hostility[c]	-	-	-	0.51	0.07	**<0.0001**
Lagged Sadness[c]	-	-	-	0.25	0.04	**<0.0001**
Study Day	0.008	0.005	0.125	-0.0007	0.004	0.854
Weekend	0.05	0.04	0.151	0.03	0.04	0.418
Day Cross-Level Interactions						
Rumination[c] x dMPFC subsystem-Amygdala rsFC[d]	-	-	-	-0.29	0.14	**0.037**
Lagged Rumination[c] x dMPFC subsystem-Amygdala rsFC[d]	-	-	-	-0.14	0.14	0.336
Person-Level						
Rumination[d]	0.41	0.11	**<0.001**	0.39	0.11	**0.002**
dMPFC subsystem-Amygdala rsFC[d]	-0.27	0.38	0.493	-0.31	0.41	0.457
Age[d]	-0.02	0.01	0.077	-0.03	0.01	0.057

*Note. N = 27.**The ROIs listed in this table only include voxels of the dMPFC subsystem that overlap the PFC and are anterior to the genu of the corpus callosum. [a]Momentary- and Day-Level models utilized corresponding levels of sadness as the dependent variables (i.e., momentary-level sadness and day-level sadness). [b]Momentary-level

variables were centered on day-level means, such that coefficients at this level represent deviations from the average amount of rumination, lagged rumination, lagged sadness, fear, and hostility experienced on a given day. [c]Day-level variables were centered on person-level means, such that coefficients at this level represent deviations from the average amount of rumination, lagged rumination, lagged sadness, fear, and hostility experienced by a given person. [d]Person-level variables were centered on sample-level means, such that coefficients at this level represent deviations from the average amount of rumination and dMPFC subsystem-Amygdala rsFC[d] displayed by the sample.

Table S3. Multilevel estimates of rumination, dorsomedial prefrontal cortex (dMPFC) subsystem-amygdala rsFC, and their interactions predicting sadness

	Momentary-Level Effects Model [a]			Day-Level Effects Model [a]		
	Estimate	**SE**	***p***	**Estimate**	**SE**	***p***
Momentary-Level						
Rumination[b]	0.23	0.02	**<.0001**	-	-	-
Lagged Rumination[b]	0.05	0.02	**0.028**	-	-	-
Fear[b]	0.15	0.04	**0.0001**	-	-	-
Hostility[b]	0.29	0.04	**<.0001**	-	-	-
Lagged Sadness[b]	-0.10	0.03	**0.001**	-	-	-
Hour After Wake	0.01	0.004	**0.001**	-	-	-
Momentary Cross-Level Interactions						
Rumination[b] x dMPFC subsystem-Amygdala rsFC[d]	-0.24	0.09	**0.008**	-	-	-
Lagged Rumination[b] x dMPFC subsystem-Amygdala rsFC[d]	-0.08	0.09	0.415	-	-	-
Day-Level						
Rumination[c]	0.48	0.04	**<.0001**	0.21	0.03	**<0.0001**
Lagged Rumination[c]	-	-	-	0.05	0.03	0.146
Fear[c]	-	-	-	0.19	0.06	**0.003**
Hostility[c]	-	-	-	0.50	0.07	**<0.0001**
Lagged Sadness[c]	-	-	-	0.25	0.04	**<0.0001**
Study Day	0.01	0.005	0.119	-0.001	0.004	0.821
Weekend	0.05	0.04	0.144	0.03	0.04	0.496
Day Cross-Level Interactions						
Rumination[c] x dMPFC subsystem-Amygdala rsFC[d]	-	-	-	-0.36	0.14	**0.010**
Lagged Rumination[c] x dMPFC subsystem-Amygdala rsFC[d]	-	-	-	-0.13	0.16	0.405
Person-Level						
Rumination[d]	0.39	0.11	**0.001**	0.37	0.11	**0.004**
dMPFC subsystem-Amygdala rsFC[d]	-0.43	0.40	0.289	-0.51	0.43	0.243
Age[d]	-0.02	0.01	0.079	-0.03	0.01	0.057

Note. N = 27. [a]Momentary- and Day-Level models utilized corresponding levels of sadness as the dependent variables (i.e., momentary-level sadness and day-level sadness). [b]Momentary-level variables were centered on day-level means, such that coefficients at this level represent deviations from the average amount of rumination, lagged

rumination, lagged sadness, fear, and hostility experienced on a given day. [c]Day-level variables were centered on person-level means, such that coefficients at this level represent deviations from the average amount of rumination, lagged rumination, lagged sadness, fear, and hostility experienced by a given person. [d]Person-level variables were centered on sample-level means, such that coefficients at this level represent deviations from the average amount of rumination and dMPFC subsystem-Amygdala rsFC[d] displayed by the sample.