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Alcohol Use Disorders and an fMRI Stress Task: A Connectivity Analysis

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ALCOHOL USE DISORDERS AND AN FMRI STRESS TASK: A CONNECTIVITY ANALYSIS

by

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ABSTRACT
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by

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Little research has been conducted on neuronal stress processing in individuals with alcohol dependence (AD). The present study examined neural stress response in AD individuals compared to controls using an fMRI stress task, assessing amygdala activation and its connectivity to the medial prefrontal cortex (mPFC). Further, the study analyzed the impact of hormone levels and subjective stress on frontal-limbic connectivity patterns.

Ten abstinent AD individuals and 11 controls were recruited. Subjects participated in an fMRI stress task. A region of interest (amygdala) analysis was conducted using area-under-the-curve. This activation was then examined in a whole-brain functional connectivity analysis. Follow-up analyses investigated whether brain activation could be predicted by cortisol, ACTH, and subjective stress.

As hypothesized, the present study found increased amygdala activation in the AD group in comparison to controls, as well as decreased bilateral amygdala connectivity with the mPFC, as well as fronto-temporal and cerebellar regions. Hormone levels collected a year prior, but not subjective stress, predicted activation and connectivity.

DEDICATION

They say that those in graduate school do not realize how long they are there, but that those they are closest to do. This is because, while the graduate student's memories all blend together, their support system acutely experiences every stressor, mood swing, victory, and set back with them. For this reason, I dedicate this to those who have supported me throughout the entirety of this project and still love me in spite of it: my immediate family, the Wright family (John, Kathy, Johnny, Stacey, Carl, Leah, Tony, and Chelsie); my Chicago family, the Reveles family (JC, Ruby, CJ, DJ, AJ, and Brian); and my adopted Milwaukee family, the Carter family (Andrew, Erin, Caedmon, and Brian).

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LIST OF ABBREVIATIONS

Alcohol Dependence—AD

Analysis of Functional NeuroImages—AFNI

Area Under the Curve—AUC

Childhood Trauma Questionnaire—CTQ

Corticotropin-releasing factor—CRF

Functional Connectivity—fcMRI

Functional Magnetic Resonance Imaging—fMRI

Medial Prefrontal Cortex—mPFC

Prefrontal Cortex—PFC

Psychophysiological Interaction—PPI

Region of Interest—ROI

Trauma Symptom Checklist—TSC

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“To alcohol: the cause of, and the solution to, all of life’s problems.”

-Homer J. Simpson, *The Simpsons*, Season 8, Episode 18

Introduction

Lifetime prevalence rates of alcohol dependence (AD) range from 5.2% to 12.5% (Kessler, Berglund, Demler, Jin, Merikangas, & Walters, 2005; Hasin, Stinson, Ogburn, & Grant, 2007). Experience of early stressful life events significantly increases the odds-ratio of developing AD (Pilowsky, Keyes, & Hasin, 2009), and recent stress increases alcohol consumption in the short- and long-term (Vlahov et al., 2002; Vlahov et al., 2004). Given this relationship between AD and stress, research examining neuronal response to stress in AD is of great interest.

Neurobiological Theories of Addiction.

Understanding the complexity of AD requires understanding the neurological and biological underpinnings that allow for the initiation and maintenance of addiction. Koob and colleagues describe addiction in three stages: binge and reward, withdrawal and negative affect, and preoccupation and anticipation (Koob & Volkow, 2010). The reward system, specifically the ventral tegmentum and the nucleus accumbens, is implicated in the first stage of this cycle (Koob & Volkow, 2010). With initial alcohol consumption, dopamine levels increase in the nucleus accumbens in rats (Carillo & Gonzalez, 2011). This is hypothesized to be sufficient for reward-based learning through positive reinforcement. Stress may exacerbate stage two, withdrawal and negative affect. Stress increases withdrawal effects through release of corticotropin-releasing factor (CRF) and norepinephrine in the extended amygdala (Koob & Volkow, 2010). CRF is more readily available in extrahypothalamic systems during withdrawal, showing a hyperactivation in the central nucleus of the amygdala (Koob & Volkow, 2010). Injection of CRF1 receptor antagonists directly into the central nucleus of the amygdala (blocking CRF release)

significantly decreases ethanol self-administration in alcohol dependent rats (Funk, O'Dell, Crawford, & Koob, 2006). CRF2 receptors demonstrate an opposite pattern (Funk & Koob, 2007); these combined findings highlight the nuanced factors that influence stress system in alcohol dependence. Subsequent to initial dependence and withdrawal, the third stage, preoccupation and anticipation, is further influenced by stress, with stress often leading to relapse. For example, in rats that have undergone alcohol withdrawal, foot shocks have been shown to reinstate alcohol-seeking behavior (Liu & Weiss, 2003). Importantly, Koob and colleagues hypothesize that addiction leads to an overall allostatic shift, a readjustment of hedonic response as a result of repeated and compulsive drug use and overcompensating by the stress response system (Koob, 2013; Koob & Le Moal, 1997). As physiological adjustments occur, it may be that there are not enough resources available to shut off the stress response effectively. The stress response may also be being sensitized, making it easier to be triggered in response to a stressor.

Expanding upon Koob's model, a biopsychosocial model of addiction specifically addressing stress has been proposed. Garland and colleagues (2011) have suggested that certain schemas are dictated by alcohol use and stress, affecting the cognitive appraisal of threats. A lack of ability to differentiate cognitively or physiologically between stressors leads to an increased reliance on alcohol for its rewarding properties; as allostatic shifts and hedonic-driven actions occur, the ingrained and habitual response to stress through drinking becomes more and more reinforced. Physiological and psychological adjustments (e.g., heightened sensitivity to stress, greater release of cortisol) occur as a result, perpetuating the addiction.

The Stress Response

In response to a stressor, CRF is released, which in turn releases ACTH. ACTH then stimulates the adrenal cortex to release cortisol, with cortisol binding widely throughout the body. Once cortisol levels reach a threshold, cortisol acts within the hypothalamus to stop production of CRF, completing the negative feedback loop. In neural regions, cortisol binds to areas of the PFC, amygdala, and hippocampus (for review, see Dedovic, Duchesne, Andrews, Engert, & Pruessner, 2009). These regions are especially implicated during anticipatory or psychological stressors. For instance, the amygdala regulates glucocorticoid secretion in animal models, partially through activation of the HPA axis (for review, see Herman et al., 2003). In the PFC, decreased activation in the orbitofrontal cortex and anterior cingulate cortex were associated with increased cortisol levels (Pruessner et al., 2008), while increased medial PFC (mPFC) response has been related to decreased cortisol secretion as it may assist in closing the feedback loop (Kern et al., 2008). Other regions of the PFC (e.g., the ventrolateral PFC) as well as the hippocampus are involved in this downregulation of cortisol (Dedovic et al., 2009). Further investigation into the neuroimaging of the stress response is needed to understand these complex relationships. In particular, understanding the functional relationship between the amygdala and mPFC is needed as they are implicated in healthy stress response and AD (Koob & Volkow, 2010; Kern et al., 2008).

Stress, Alcohol, and Neural Function

Neural dysfunction as a result of stress has been noted in healthy samples (Shin & Liberzon, 2010; Ziegler & Herman, 2002) and implicated in leading to AD (Koob & Kreek, 2007), with stress system dysfunction conversely being suggested as a

consequence of AD (Adinoff, Junghanns, Kiefer, & Krishnan-Sarin, 2005). The increased glucocorticoid supply in response to stressful stimuli has been linked to alcohol initiation, risk of dependence, and relapse (for review, see Stephens & Wand, 2012). It should be noted, however, that the function of this increase in glucocorticoid supply is still not well understood; some researchers have hypothesized that it may be a pre-morbid difference in those who abuse alcohol, or it could be in response to the high doses of alcohol consumed as tolerance develops (e.g., King et al., 2006).

Stress and Alcohol Dependence. Chronic stress and trauma history have been linked to increased AD risk (Mills, Teesson, Ross, & Peters, 2006; Pilowsky, Keyes, & Hasin, 2009; Fox, Bergguist, Gu, & Sinha, 2010; Schwandt, Heilig, Hommer, George, & Ramchandani, 2012). Indeed, young adults with an alcohol use disorder were, on average, exposed to eight lifetime traumas (Lloyd & Turner, 2008). This link may be due, in part, to the ability of chronic stress to shift planning into reliance on more habitual processes, reducing judgment abilities (Dias-Ferreira et al., 2009).

Stress has also been implicated in cueing relapse in samples with AD. Initially, Thomas and colleagues (Thomas, Randall, Brady, See, & Drobes, 2011) found that stress induced by the Trier Social Stress Test did not increase craving or alcohol-cue reactivity in non-treatment-seeking individuals with AD; however, in this same sample, a psychosocial stressor did increase alcohol consumption (Thomas, Bacon, Randall, Brady, & See, 2011). Other groups have found a direct link between self-reported stress and relapse. For example, recovering individuals with AD were found to be more likely to relapse when vulnerable (characterized by depression, poor coping skills, low self-efficacy, high alcohol expectancy and low social support) at 3-month and 1 year post-

treatment follow-ups (Brown, Vik, Patterson, Grant, & Schuckit, 1995). Higley and colleagues (2011) also found that increased self-reported stress was related to higher rates of alcohol consumption and decreased time to relapse in treatment-seeking individuals with AD. Similarly, overreaction to stress, alcohol, and neutral cues has been shown to be predictive of relapse in AD and in other substance dependent groups (Seo et al., 2013; for review, see Sinha & Li, 2007). Therefore, at least preliminary evidence suggests that subjective experience of stress following treatment may act as a trigger for relapse.

In individuals with AD, cortisol functioning itself influences each stage of the addiction cycle. This response varies depending on which stage of dependence an individual is in. For example, when actively dependent and drinking individuals with AD tend to have elevated cortisol levels at rest, while having a blunted cortisol response; when abstinent for an extended period of time, cortisol levels and response slowly return to normal (for review, see Stephens & Wand, 2012). This blunted response in dependent individuals has been evidenced in multiple studies (Lovallo, Dickensheets, Myers, Thomas, & Nixon, 2000; Sinha, Fox, Hong, Hansen, Tuit, & Kreek, 2011; Pratt & Davidson, 2009). At the same time, elevated levels of perceived stress also correlate with higher cortisol levels in treatment-seeking AD individuals, and predict shorter time to relapse (Higley, Crane, Spadoni, Quello, Goddell, & Mason, 2011).

Very few studies have linked cortisol levels with neuronal activity. In a study of healthy individuals, cortisol levels were found to influence the functional connectivity between the amygdala and other important structures, such as the hippocampus (Vaisvaser et al., 2010), though these levels change as a person experiences and then recovers from a stressor. Initially, increased cortisol levels were related to increased

amygdala-hippocampal connectivity. After a 2 hour delay post-stressor, they then found that increased cortisol levels were related to decreased connectivity. They hypothesized that this was because of the negative feedback loop, with elevated cortisol levels exhibiting a healthy stress response, eventually shutting down the production of CRF and resulting in less activity in the limbic system. Kern and colleagues (2008) also investigated healthy stress response, finding the PFC, particularly the mPFC, to be key in moderating stress processing and both positive and negatives relationships with cortisol levels depending on regions within the PFC. However, no known studies have examined whether cortisol levels predict neuronal stress processing in AD samples.

Differences in the neuronal stress response occur as a result of AD. Animal models of AD have most frequently been examined to assess stress processing (for review, see Koob, 2013). In humans, however, most studies have assessed emotional processing differences in fronto-limbic regions that also regulate stress. Using the same sample as the present study, our group found abnormal responsiveness to fearful stimuli during an affective processing task in AD (Padula et al., under review). Specifically, results indicated that AD interacted with gender such that females with AD had blunted inferior frontal and superior temporal BOLD response in comparison to same-gendered controls, while males with AD had increased activation in these same areas in comparison to male controls. O'Daly and colleagues (2012) found blunted insula activation in an emotion processing task, as well as decreased connectivity between the amygdala and the bed nucleus of the stria terminalis in abstinent individuals with AD. In cocaine dependent individuals, one fMRI study showed increased activation in cortico-limbic regions in response to a personalized stress script (Potenza et al., 2012), while

another study found the opposite pattern (Sinha et al., 2005). Surprisingly, only one study to date specifically examined the neuronal response to stress in an AD sample. Results showed blunted activity in the ventromedial PFC and anterior cingulate cortex during an idiographic stress script, with hyperactivity during a neutral script (Seo, Lacadie, Tuit, Hong, Constable, & Sinha, 2013).

Stress Habituation. One important area of the stress response that has not often been addressed is the process of habituation to stressful stimuli. Studies have found that individuals with AD typically have both hyperarousal to non-threatening stimuli and blunted stress response to stressful stimuli (Seo et al., 2013), suggesting that individuals with AD may have context inappropriate response or, perhaps, do not properly habituate across stimuli. Research in healthy individuals indicates habituation occurs quickly after a strong initial stress response to emotional stimuli (Wright et al., 2001; Ishai et al., 2004; Britton et al., 2008), while individuals with social anxiety disorder or schizophrenia have been found to have increased initial response and longer habituation times than controls (Sladky et al., 2012; Suslow et al., 2013). As clinical samples may have hyperactive responses to emotional and/or stressful stimuli, research is needed to assess the *pattern* of stress response over time within AD samples.

In sum, conflicting findings across studies and methodologies reveal a complex relationship between stress and AD. Individuals with AD are often characterized as having a blunted cortisol response (Lovallo et al., 2000), yet as Stephens and Wand (2012) point out, specific glucocorticoid supply levels differ depending on what stage in the addiction cycle an individual is in, amongst other factors. In addition, AD individuals have been found to have higher basal levels of cortisol (Thayer et al., 2006; Lovallo et al.,

2000). Increased cortisol levels are needed to initially activate the stress response, reaching a threshold level that then triggers a negative feedback loop (Herman et al., 2003), which is partially moderated by the mPFC (Kern et al., 2008). Social drinkers have increased activation in the amygdala in response to a personalized stress script (Seo et al., 2010), and perceived stress and craving has been predictive of relapse in treatment seeking individuals with AD (Higley et al., 2011). The only known study to investigate functional differences in response to stress found no differences in amygdala response between controls and AD individuals, with blunted response in the vmPFC and ACC in the AD group (Seo et al., 2013). However, the same study found increased activation in the amygdala in AD subjects, amongst other areas, in comparison to a neutral condition. Yet to assess group comparisons, a subset of the AD sample were used and the neutral condition was subtracted from the stressor condition in a whole brain analysis, perhaps reducing the power to see if there was a statistically significant difference. As abstinent AD individuals are often found to have elevated basal cortisol levels and blunted cortisol response, they may have hyperactive amygdalae in response to a stressor as the negative feedback loop of the stress response may be inhibited.

Summary and Aims

Converging lines of evidence link stress processing with alcohol use initiation and AD maintenance. Recent trauma and stressors (Vijayasiri et al., 2012; Vlahov et al., 2004) act as predictors of risk in developing AD, with stress leading to alcohol consumption and/or craving (Thomas, Randall et al., 2011; Thomas, Bacon et al., 2011). Neurobiological shifts then occur as a result of the negative spiral of addiction (Koob & Volkow, 2010). The present study has been proposed to investigate the influences of AD

in stress response. Furthermore, the present study seeks to tease apart the nuances of the stress response, assessing *where* and *how* potential differences in the neuronal stress response are occurring.

Specifically, the present study aims to investigate (1) the effects of AD on initial and sustained amygdala response to a stress task and (2) whether AD impacts amygdala functional connectivity during a stress task. A secondary aim is to assess the influence of brain-behavior relationships using outside measures of stress (baseline stress hormone response collected approximately one year prior to MRI scan and self-reported subjective stress) in brain areas that differed significantly by AD status. Based on previous studies (Potenza et al., 2012; Thomas, Bacon, et al., 2011), it is hypothesized that abstinent AD subjects will have significantly greater activation in the left and right amygdala than controls, both in the initial phase (first minute) of the stress task, as well as in the task overall (sustained activity). Further, it is predicted that the AD group will demonstrate decreased connectivity (O'Daly et al, 2012; Kern et al., 2008) between the left and right amygdala and mPFC during the stress task, compared to controls. For the secondary aim, it is hypothesized that in the AD group increased subjective stress will predict increased amygdala response and reduced amygdala-mPFC connectivity (Herman et al., 2003; Keyes et al., 2012) in regions where AD group differed from controls. It is also hypothesized that increased plasma cortisol and ACTH baseline levels will prospectively predict increased amygdala response and reduced functional connectivity in the amygdala-mPFC (Vaisvaser et al., 2013; Stephens & Wand, 2012).

Methods

The present study analyzed data previously collected as part of a larger imaging genetics study (NCT00226694, PI: Anthenelli; NIMH K23 MH67705, AZ IRUSQUET0456, PI: Nelson). Twenty-one participants (10 AD, 11 controls) were recruited from the original parent study that examined hormonal changes with stress (for details see Anthenelli et al., 2009). Participants answered psychological measures related to stress and trauma and performed an fMRI stress task.

Participants

A total of 21 individuals, aged 23 to 55 years (mean = 40 years old), completed the stress task. Ten abstinent individuals with AD (6 females, 4 males) were recruited from the parent study that examined hormonal changes with stress (NCT00226694, PI: Anthenelli) (Anthenelli et al., 2009). Eleven healthy controls (6 females, 5 males) were recruited from the community as part of a larger study examining stress and brain response in depression; only controls without depression were included for the present study (NIMH K23 MH67705, AZ IRUSQUET0456, PI: Nelson). The Institutional Review Boards at the University of Cincinnati and Cincinnati Veterans Administration Medical Center approved all aspects of the study, and all participants provided written informed consent. Groups were similar in gender distribution [$x^2(1) = 0.06$, $p = 0.80$], age [$t\ (19) = -1.37$, $p = 0.19$], and ethnicity/racial characteristics [$x^2(1) = 0.10$, $p = 0.76$]. Rates of left-handedness significantly differed by group [$x^2(1) = 3.85$, $p = 0.05$], with all of the left-handed participants in the AD group (n = 3). Therefore, handedness was used as a covariate in all analyses.

AD Inclusion Criteria. AD participants met DSM-IV criteria for AD in sustained partial or full remission and were seeking treatment when they enrolled in the parent study (NIAAA R01 AA013307 PI: Anthenelli). At the time of the current study, AD individuals were abstinent from all substances for at least one month prior to the MRI session and continued to meet DSM-IV criteria for AD in sustained partial or full remission. **Control Inclusion Criteria.** Controls had no history of any Axis I or Axis II disorders, including substance use disorders (SUD).

Exclusion Criteria for Both Groups. Exclusion criteria included: Current use of psychotropic medication; lifetime history of serious neurologic injuries or disorders; major medical illness (except hypertension and high cholesterol); known diagnosis of an independent Axis I anxiety, mood or psychotic disorder (or Axis II personality disorder in the control group); use of oral contraceptives; current pregnancy or lactation in women; or MRI contraindications (e.g., metal anywhere in or on the body, greater than 250 lbs., claustrophobia). Recent abstinence from drugs and alcohol were confirmed by drug toxicology (DrugTestStrips.com™12 Panel drug test), cotinine levels (NicAlert) and breathalyzer (FC10 Breath Alcohol Tester® to verify .000 breath alcohol concentration) testing in AD individuals. Although PTSD was exclusionary, sub-clinical symptoms of PTSD and history of trauma were not exclusionary (parent study reported prevalence of trauma history in 80% of AD sample).

Procedure

Eligible participants were asked to come to the local VA Hospital. Participants were consented to this phase of the study, and Timeline Follow-Back data was collected to fill in alcohol and drug use from the parent study's conclusion to the present study.

They were then given urine toxicology, cotinine levels, and breathalyzer tests, as well as pregnancy tests for females. Positive results deemed participants as ineligible, and they were subsequently given $5. If negative, participants were given psychological questionnaires to assess mood and trauma history. Participants then completed the neuroimaging protocol. Consistent with the parent study payment schedules, AD participants were paid $100 for study completion and control participants were paid $75.

Measures

Recent Drug Use. Drug use history was collected using the Time-Line Follow-Back (TLFB; Sobell & Sobell, 1992). Using a calendar to cue special dates and holidays, participants were asked to recount what substances they used since their last study session, as well as when they used substances. Prior to this TLFB data collection, past year substance use history had been collected. Participants reported use of any of the following drugs: ecstasy, marijuana, alcohol, nicotine, sedatives, stimulants, hallucinogens, opioids, and inhalants. Past month abstinence was confirmed.

Stress Hormone Challenge. As part of the parent project, a dexamethasone/ corticotropin releasing hormone (Dex/CRH) hormone challenge was administered at least one year prior to fMRI scanning to assess plasma cortisol and ACTH responsivity. Subjects ingested 1.5mg of Dexamethasone the night before the hormone challenge, then received an injection of ovine CRH (oCRH 1lg/kg) the morning of the challenge. Plasma cortisol and ACTH concentrations were measured following the oCRH injection, from baseline at 15-minute intervals for the first hour then 30-minute intervals for the next four hours (Anthenelli et al., 2009). Baseline ACTH and cortisol levels were used in the present study.

Subjective Stress. Subjective stress was measured through calculating change scores from baseline stress level to post-scan stress level. Participants were asked prior to and post-scan their level of stress on a 100-point scale. Higher levels of self-reported stress indicate higher levels of subjective stress.

Childhood Trauma Questionnaire (*CTQ*). AD participants filled out the CTQ, a 28 item self-report measure (Bernstein & Fink, 1998). Responses are broken into five categories: emotional abuse, physical abuse, sexual abuse, emotional neglect, and physical neglect. Each item was rated on a 5-point Likert-type scale from "Never True" to "Very Often True". Sum scores for each category were calculated with higher scores indicating greater maltreatment.

Trauma Symptom Checklist (TSC). The TSC is a 40-item self-report measure of trauma symptoms experienced within the last two months (Briere & Runtz, 1989). Participants rated the frequency of experiencing various trauma symptoms (e.g., headache, flashbacks, sadness) on a 4-point Likert-type scale, from "Never" to "Often". Scores yield six subscales, with higher subscales indicating the presence of more symptoms.

Coping Styles. Coping style was assessed using the Ways of Coping Questionnaire (Folkman & Lazarus, 1980). Scores were calculated from eight subscales, each containing four to eight questions. Higher scores within a subscale indicated greater use of that method of coping, with each coping subtype functioning independently of other coping styles.

fMRI Data Acquisition

fMRI scans were acquired on a 4 Tesla Varian MRI Scanner using a RF-spoiled FAST 3-D acquisition technique at the University of Cincinnati Center for Imaging Research (CIR). While performing the stress task T2*-weighted gradient-echo echoplanar imaging (EPI) pulse sequence (TR/TE=2000/30ms, FOV=25.6 x 25.6 cm, matrix 64 x 64 pixels, slice-thickness=4 mm, flip angle=75 degrees). Each participant's scan lasted about 60 minutes, with 16 of the minutes being dedicated to the scans for the proposed study. A neuroradiologist assessed each scan for brain abnormalities; no abnormalities were present in the present sample.

fMRI Stress Task

Stress was induced through a variation on the Trier Social Stress Test (Kirschbaum, Pirke, & Hellhammer, 1993). The task includes two math components, a stress-inducing test and a non-stressful control test. The control test, which is not used in the present investigation, was first and included 60 different basic subtraction problems (e.g., 22 – 11 = (a) 11, (b) 15), each being shown for 5 seconds, for a total time of 5 minutes; this task was used to set up the stress component and allow participants to acclimate to the math problems. Participants chose the correct answer from two possible answers using a response box. The second, "stress" task followed the control task. The stress task consisted of 80 subtraction problems that were considerably more difficult than the control problems and that contained three possible answers rather than two (e.g., 31 – 13 = (a) 18, (b) 28, (c) 12). As this task was about to begin, participants saw a video on their video goggles of two "doctors" sitting in the control area of the MRI scanner. Participants were told that these "experimenters," who introduced themselves as doctors,

would be rating and giving feedback on their performance (feedback consisted of six different pre-recorded messages that informed them that they were not performing up to the task, regardless of their actual performance). Participants were also told that they would have between 1 and 5 seconds to answer each question, but would not be told how long was left; if they went over the time, their answer would not count. Finally, participants were told that they had to get enough questions correct for their data to be usable and that if they did not succeed, they would not receive full compensation. The task took approximately 11 minutes. At the completion of the task, participants were debriefed and informed that there were no experimenters rating performance and that the feedback was not based on their performance. Each participant was fully compensated.

fMRI Processing

fMRI data was processed using Analysis of Functional NeuroImages (AFNI; Cox, 1996). Standard pre-processing for each participant was done, including: realigned all images to same orientation, coregistered the anatomical image, removed the first three TRs prior to the machine being ready to scan, tshift (time slice alignments across scans), volreg (register volume), blurred data (5.4 mm), created a brain mask, aligned anatomy, warped the anatomy to standard space (Talairach, TT_N27+tlrc), and created time and curve regressors. Motion parameters were analyzed in 3dToutcount and trials were censored if value was above 0.3. All pre-processing was checked by trained personnel to assess for extensive motion and noise. Greater than 15% of TR removal due to censoring resulted in the participant exclusion; no subjects surpassed this criterion.

Aim 1. Automated left and right amygdala masks were created for each subject, and then nudged to more accurately reflect neuroanatomy (by CP and NW). The average

stress response per ROI over the course of the task was then extracted, representing mean amygdala activity at each TR. To assess initial and sustained amygdala activation, the initial stress response (defined as the first minute of the task) was be measured by area under the curve (AUC). Total AUC over the entire timecourse of the task was then calculated.

Aim 2. For functional connectivity (fcMRI) analysis, linear modeling consistent with psychophysiological interaction (PPI) analysis was used to conduct a voxel-wise regression across the task utilizing the left and right amygdala seed regions (Rogers et al., 2007; Friston et al., 1997). Betas (correlation coefficients) and t-statistics for each individual were saved.

Data Analysis

Demographic data and psychological indices were examined with regressions or chi-square analyses (N = 21) between groups (AD vs. control). No variables differentiated the groups except for handedness, which was included in subsequent analyses as covariates.

Primary Analysis. **Aim 1.** To assess differences in patterns of stress response in the amygdala, mean amygdala activation at each TR was modeled and used to calculate initial (first minute) and total AUC. Multiple regressions were run to examine whether AD group status predicted bilateral amygdala pattern of response.

Aim 2. Group analysis was conducted as follows: average BOLD signal across the time-series in the seed regions (bilateral amygdala) was extracted for each subject and deconvolved as a regressor. We then examined whether AD status predicted voxel-wise functional connectivity between the bilateral amygdala and the whole brain.

To assess neuronal connectivity differences in the amygdala and mPFC between groups on the stress task, 3dttest++ (t-test) were run in AFNI. A Monte Carlo simulation was run to correct for family-wise error (Forman et al., 1995), finding that for an individual voxel threshold of p = .01 and a family-wise error corrected significance of .05, 13 contiguous voxels had to be activated (351 µl). Mean activation for each functional region of interest that significantly differed by group was extracted into SPSS for each participant to confirm relationships are significant after controlling for potential confounding variables. Because of the large number of clusters and to reduce risk of multiple comparisons, a MANOVA was run in both the left and right amygdala. Any brain regions that were significantly different were then followed-up with a regression predicting connectivity with handedness included. The only region that was no longer significant was the increased connectivity in the left cingulate gyrus with the left amygdala.

Secondary Analysis. Multiple regressions whether subjective stress and previously collected plasma cortisol and ACTH levels predicted AUC amygdala response in AD subjects. To assess potential brain-behavior relationships between subjective stress and hormone response with connectivity, a brain mask was created of regions that differed significantly by group. 3dRegAna was run in AFNI, using the above variables (subjective stress, hormone response) as predictors. A Monte Carlo simulation was run to correct for family-wise error (Forman et al., 1995), and data were extracted into SPSS to confirm significance.

Results

Demographics. Groups did not differ significantly on age, education, ethnicity, and gender (see Table 1 for descriptive demographic information). Groups differed on handedness, with all left handed participants in the AD group [$x^2(1) = 3.85$, $p = 0.05$].

Trauma History and Other Symptomatology. Self-reported depressive symptoms in the healthy control group fell within the normal range, with no participants falling out of this range. However, the AD group was on the upper end of normal, with some participants classified within the "moderate depression" range. Self-reports of childhood trauma indicated a wide range of experiences for the AD group, from minimal to extreme history of abuse and/or neglect. Similarly, the AD group reported a wide range of recently experienced trauma symptoms. Looking at the data qualitatively, those who had a more traumatic childhood appear to also have experienced more recent trauma. However, a correlation between these two variables was not significant ($p > .05$).

Table 1. Descriptive Demographic Information

	Alcohol Dependent *%* or *M (SD)*, Range	Healthy Control *%* or *M (SD)*, Range
Age	43.3 (8.3) 30-55	37.6 (10.6) 23-55
% Female	60%	54%
% Caucasian	70%	64%
% Right Handed*	60%	100%
Education (in years)	13.7 (2.1) 10-17	15.4 (1.5) 14-18
BDI Total Score	8.8 (9.7) 0-27	--
HAM-D Total Score	--	0.4 (.8) 0-2
CTQ Total Score	62.5 (22.4) 37-111	--
CTQ-Physical Abuse	9.30 (5.8) 5-25	--
CTQ-Physical Neglect	9.50 (4.1) 5-14	--
CTQ-Sexual Abuse	10.90 (8.0) 5-25	--
CTQ-Emotional Abuse	12.30 (6.5) 5-25	--
CTQ-Emotional Neglect	13.10 (5.4) 5-24	--

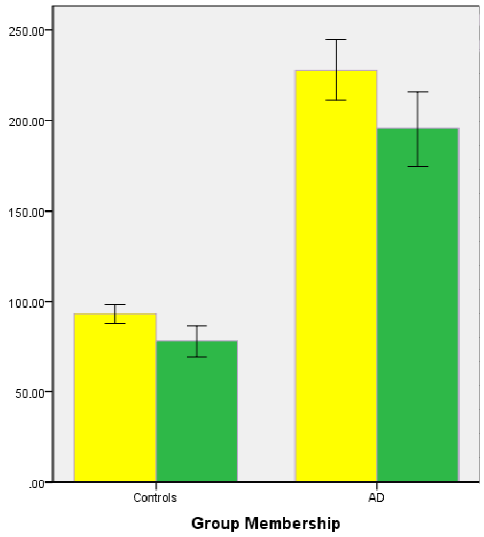
TSC Total Score	18.7 (11.8) 0-42	--
Days Abstinent at fMRI	1628 (2123) 68-4759	--
Days Abstinent at Parent Grant	1326 (2100) 34-5515	--

Notes: * indicates $p < .05$ between groups

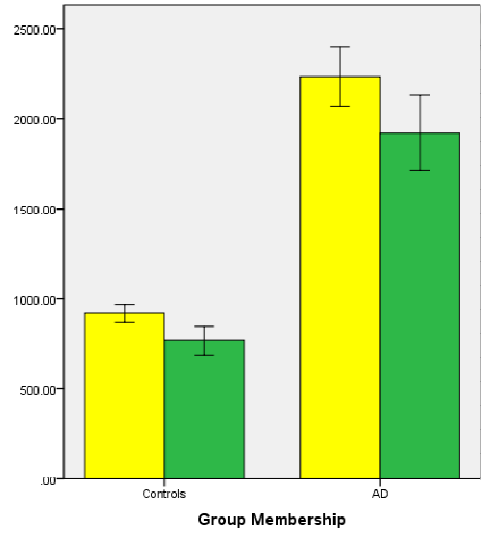
Primary Results

Amygdala Activation. After controlling for handedness, group status predicted initial left and right amygdala activation, with the AD group demonstrating increased activation [Left: $F(1,19) = 7.63$, $p < .001$; Right: $F(1,19) = 4.86$, $p < .001$]. Similarly, the AD group also had significantly greater amygdala left and right activation, which predicted sustained left and right amygdala activation [Left: $F(1,19) = 7.78$, $p < .001$; Right: $F(1,19) = 4.86$, $p < .001$; see Figure 1].

Figure 1. Bar graphs of mean amygdala activation as measured by AUC in (a) initial minute and (b) over the entire timecourse. Controls are on the left; AD individuals are on the right. The yellow bar show left amygdala activation while green show right activation. Results indicate that AD subjects had *significantly greater* amygdala activation over the timecourse.



(a)



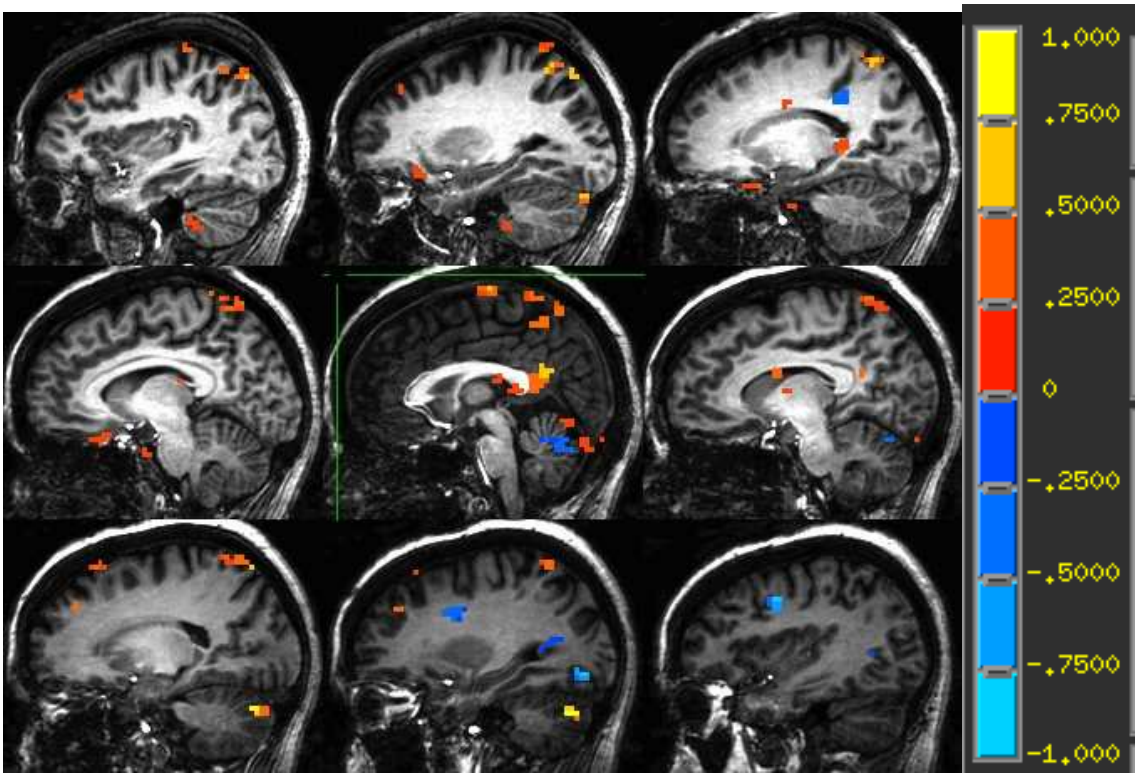
(b)

Amygdala Connectivity. After controlling for family-wise error in AFNI (9 voxels, p = .05), significant differences were found between both left and right amygdala connectivity. Consistent with our hypotheses, decreased connectivity was found between the left and right amygdala and the mPFC. Other differences in activation were also found, with full results for left and right amygdala connectivity listed in Tables 2 and 3, respectively (see Figure 2). Results do not appear to be lateralized.

To control for handedness and confirm findings, significant clusters were exported into SPSS for a MANOVA analysis. If any region differed significantly by handedness, it was then confirmed in a regression analysis. In the left amygdala, the MANOVA revealed differences in a left cingulate gyrus region, which had shown increased connectivity [$F(1,16) = 18.18$, $p < .01$]; a second left cingulate gyrus region with decreased connectivity marginally approached significance [$F(1,16) = 3.08$, $p < .10$]. Multiple regressions were then run on the two clusters significantly different by handedness. In the cluster that demonstrated increased connectivity, handedness still

predicted connectivity (beta = -.67, p = .002; see Figure 3). In the cluster that demonstrated decreased connectivity, handedness did *not* significantly predict connectivity (beta = .05, p = .75). There were no other changes in results.

Figure 2. Left amygdala (top) and right amygdala (bottom) connectivity patterns. Yellow indicates healthy controls have greater connectivity than AD; blue indicates AD has greater connectivity than controls.



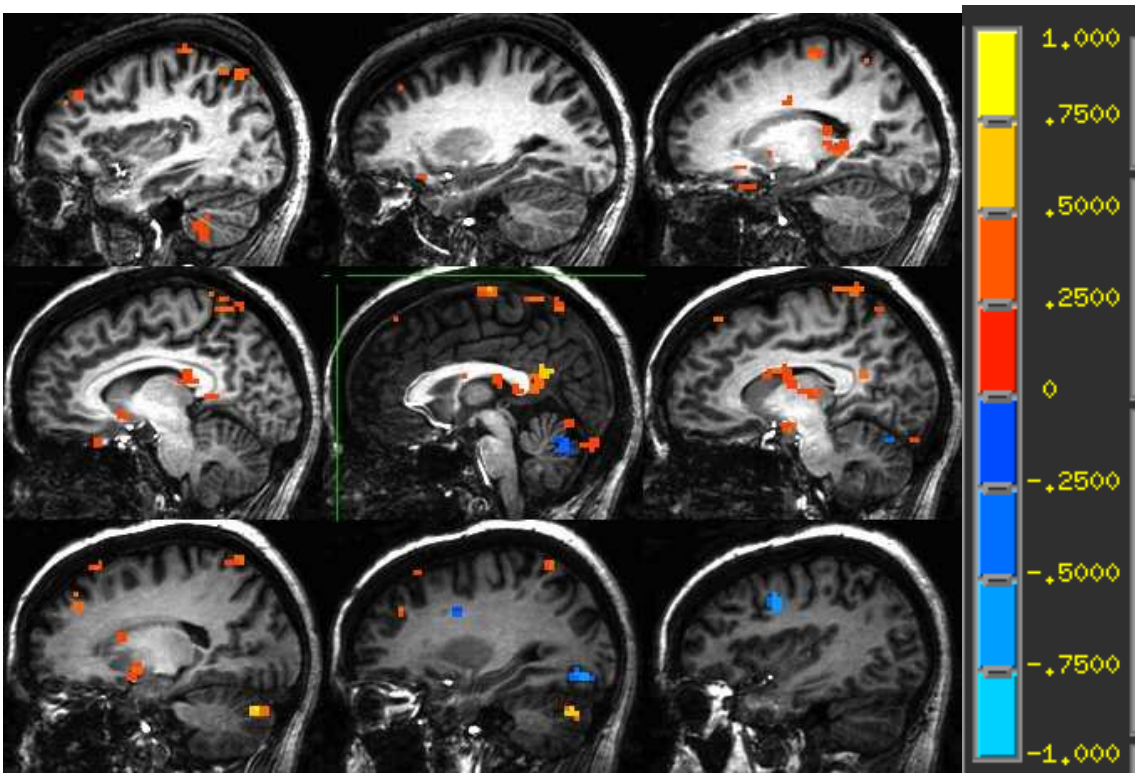


Table 2. Left Amygdala Connectivity. Most clusters revealed significantly greater connectivity in the control group than the AD group; * denotes clusters that revealed greater connectivity in the AD group than the control group.

Region	Talairach Coordinates			Volume (voxels)
	CM x	CM y	CM z	
Right Declive*	-3.6	62.3	-23.6	43
Right Cerebellar Tonsil	-23.7	69.5	-29.1	38
Left Cerebellar Tonsil	31.5	34.6	-43.3	22
Right Declive	-4.9	79.2	-19.5	20
Right Culmen	-5.5	62.7	-5.8	19
Left Uncus	11.8	7.3	-28.6	15
Left Uvula	25.3	74.5	-25.5	13

Right Caudate	-8	2.2	20	26
Left Precuneus	1.4	45.1	47.4	20
Right Posterior Cingulate	-2.9	47.8	19.2	40
Right Posterior Cingulate*	-28.5	55.9	11.2	26
Left Cingulate Gyrus	12	5.9	27.8	21
Left Parahippocampal Gyrus	16	38.4	6.2	18
Left Posterior Cingulate	1.7	27.7	17.2	14
Right Superior Parietal Lobule	6	56.3	58.3	237
Left Inferior Parietal Lobule	40.8	43.2	54	14
Right Precentral Gyrus*	-33.4	-0.2	33.3	42
Left Inferior Temporal Gyrus	50.6	49.2	-22.9	28
Right Superior Temporal Gyrus	-53	-5.7	-2.6	27
Right Lingual Gyrus*	-30.9	73.5	-6.9	19
Right Middle Temporal Gyrus	-63.4	43.5	-10.2	14
Right Inferior Temporal Gyrus	-57.7	59.2	-3.7	14
Left Inferior Frontal Gyrus	10.8	-25.4	-19.2	20
Right Superior Frontal Gyrus	-23	-24.5	55.1	18
Left Middle Frontal Gyrus	30.6	-35.8	37.2	16
Left Postcentral Gyrus	36.6	29.5	62.4	16
Left Inferior Frontal Gyrus	20	-14.8	-17.9	14
Left Inferior Frontal Gyrus	24.9	-22.8	-11.6	14
Right Superior Frontal Gyrus	-23.6	-37.3	33.6	13

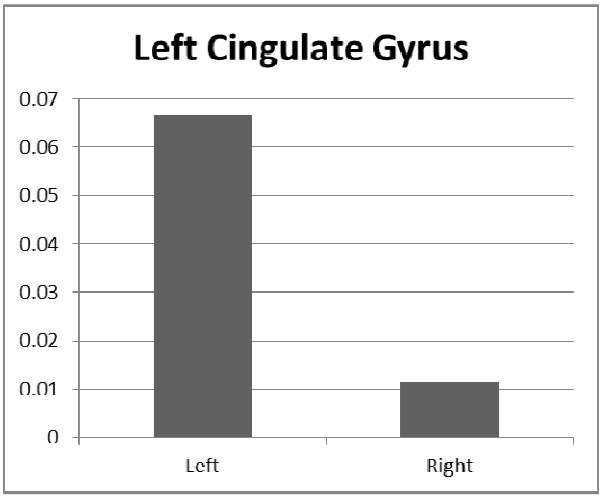
Right Medial Frontal Gyrus	-2	17.4	70.7	13

Table 3. Right Amygdala Connectivity. Most clusters revealed significantly greater connectivity in the control group than the AD group; * denotes clusters that revealed greater connectivity in the AD group than the control group.

Region	Talairach Coordinates			Volume (voxels)
	CM x	CM y	CM z	
Right Pyramis	-23.4	69.1	-29.3	40
Right Declive*	-3.7	63.6	-24	39
Right Declive	-4	79.9	-20.3	23
Right Culmen	-5.8	62.4	-5.4	22
Left Cerebellar Tonsil	33.2	37.8	-4.4	21
Right Lentiform Nucleus	-17.1	1.9	-6	35
Right Claustraum	-20.5	-7.8	16	16
Right Caudate	-8.9	6.9	17.8	62
Left Caudate	12.7	-12.5	-1.6	40
Left Parahippocampal Gyrus	14.5	36.5	6.2	27
Left Cingulate Gyrus	12.8	6.9	30.5	19
Right Posterior Cingulate	-0.6	42.9	19.2	81
Right Lingual Gyrus*	-31.4	73	-6.9	28
Right Precuneus	-7.6	55.3	60	17
Right Superior Parietal Lobule	-23.5	58.1	59.2	32
Left Superior Parietal Lobule	9.2	53.6	59.8	24

Left Superior Parietal Lobule	31.9	58.2	50.7	20
Right Middle Temporal Gyrus	-63.3	42.9	-10	13
Left Postcentral Gyrus	1.5	47.6	65.7	38
Left Postcentral Gyrus	36.3	29.4	61.8	21
Right Postcentral Gyrus	-9.8	42.5	70.6	14
Right Precentral Gyrus*	-35	-0.2	34.8	26
Left Middle Frontal Gyrus	30.4	-39.7	35.1	23
Right Medial Frontal Gyrus	-2.5	17.9	71	14
Left Inferior Frontal Gyrus	16.8	-20.8	-17.7	43
Right Superior Frontal Gyrus	-18.4	-28.7	54.8	27
Right Superior Frontal Gyrus	-22.8	-37.1	35.1	23

Figure 3. Average connectivity between left cingulate gyrus and left amygdala by handedness.

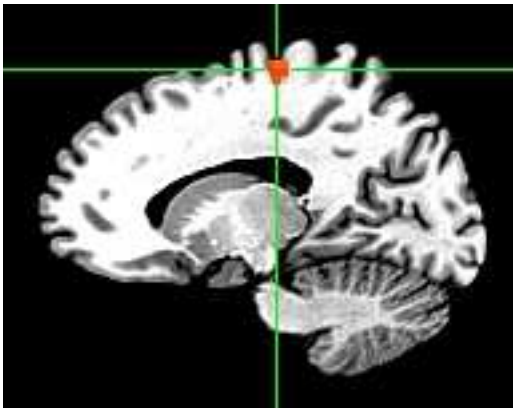


Secondary Results

Subjective Stress. Within the AD group, changes in subjective stress from pre- to post-scan did not predict initial left or right amygdala activation ($p > .05$), nor did it predict sustained left or right amygdala activation ($p > .05$). Similarly, subjective stress largely did not predict connectivity patterns. One notable exception was in the left amygdala, with increased subjective stress predicting increased activation with the right middle frontal gyrus (9 voxels, $p < .05$).

Stress Hormones. Increased baseline cortisol level predicted initial [$F (2,7) = 2.85$, $p = .025$] and sustained [$F (2,7) = 2.63$, $p = .034$] activation in the left, but not right, amygdala. Baseline ACTH did not predict amygdala activation patterns. In assessing amygdala connectivity, baseline cortisol predicted increased connectivity between the right amygdala and the right superior parietal lobule (15 voxels, $p < .05$) and baseline ACTH predicted increased connectivity between the right amygdala and the left medial frontal gyrus (11 voxels, $p < .05$).

Figure 4. (a) Higher baseline cortisol predicted increased right amygdala and right superior parietal lobule connectivity (15 voxels, $p<.05$); (b) Higher baseline ACTH predicted increased right amygdala and left medial frontal gyrus connectivity (11 voxels, $p < .05$).

(a)  (b) 

Discussion

The present study sought to investigate neuronal differences in stress processing in AD individuals. Using the amygdala as the primary region of interest, we found that AD individuals had both increased initial and sustained activation in the bilateral amygdala during the stress task. We then examined connectivity differences using the left and right amygdala as seed regions. In general, we found decreased connectivity between the bilateral amygdala and prefrontal, temporal, parietal, and cerebellar structures. Finally, in assessing subjective stress response and hormone response, we found that subjective stress experienced during the MRI scan significantly predicted increased amygdala-right mPFC connectivity. Increased baseline cortisol collected a year prior to scan predicted increased amygdala-parietal connectivity while increased baseline ACTH levels prospectively predicted increased amygdala-parietal and frontal connectivity.

More specifically, the first aim of the present study was to assess the effects of AD on initial and sustained amygdala response to a stress task. The AD group exhibited more amygdala reactivity to the stress fMRI task. As in other clinical samples (Sladky et al., 2012), the AD group had increased initial response to the stress task. However, unlike

other clinical samples (Sladky et al., 2012; Suslow et al., 2013), the present sample of AD individuals maintained significantly higher amygdala activation than the controls throughout the timecourse of the task. These findings are consistent with previous studies on amygdala activation in response to chronic stress. In adolescent and adult rats, repeated stressors have been found to cause hyperactivity in the basolateral amygdala (Padival, Quinette, & Rosenkranz, 2013; Hetzel & Rosenkranz, 2014). Similarly, in humans, repeated, chronic stressors can increase amygdala responsivity (Dannlowski et al., 2012; Ganzel et al., 2012). Interestingly, in combat veterans, those with PTSD had greater amygdala response to fearful tasks than combat veterans without PTSD and healthy controls. Yet, when looking at shapes, both veterans with and without PTSD showed hyperactive amygdala patterns (Simmons et al., 2011), showing increased experience of stressful life events may lead to long-term functional changes in the amygdala. In contrast to these findings of hyperactivity, when using an emotional faces task in this same sample, our group did not find any differences in amygdala activation between individuals with AD and healthy controls (Padula et al., under review), nor did the only other known fMRI study of stress in individuals with AD (Seo et al., 2013). However, neither of these studies examined amygdala response as a region of interest over time, which may be a more sensitive measure of amygdala reactivity to stress. Despite this contrast with other studies of AD samples, the present study fits within the broader stress literature. This hyperactivation of the amygdala may explain some key differences in how individuals with AD respond to stress, as increased perceived stress can be predictive of relapse (Brown et al., 1995; Higley et al., 2011) and stress has long be hypothesized to be involved in each stage of the addiction cycle (Koob & Volkow,

2010). As the present study did not have anatomical specificity, future studies should target subregions of the amgydala. In particular, the basolateral amygdala has been found to be key to the development and maintenance of AD (Koob & Volkow, 2010) and should be further investigated.

The second aim was to determine whether AD impacts amygdala functional connectivity during a stress task. With the left amygdala as a seed region, we found 31 significant clusters in frontal, parietal, temporal, and cerebellar regions. With the right, we found 28 significant clusters in these same primary regions. Specifically, individuals with AD demonstrated *decreased* connectivity in the majority of clusters. Within the PFC, we found a range of connectivity differences in superior, inferior, middle, medial, and precentral regions. Further, we found that decreased baseline cortisol levels within the AD sample predicted decreased connectivity between the amygdala and the mPFC, as well as the parietal lobe. Though most studies have looked at emotional processing in response to pictures versus creating a stressful environment, our results of reduced frontolimbic connectivity are largely consistent with both the stress and emotion literature. In a study of healthy controls that used a cold pressor task as a stressor, the authors found reduced resting-state functional connectivity between the amygdala and orbitofrontal cortex (OFC) and vmPFC in response to stress, although this occurred irrespective of cortisol release (Clewett, Schoeke, & Mather, 2013). Another study on healthy controls conducted by Veer et al. (2011) found negative connectivity between the amygdala and the medial PFC, although cortisol levels did not predict brain response. However, both studies did not assess amygdala activation outside of functional connectivity, amongst other differences (e.g., the stressor tasks occurred outside the

scanner, and Clewett and colleagues used a physiological pain stressor), and these studies only included healthy controls.

Our fronto-limbic results may reflect underlying structural differences. Networks of structural pathways connect to the amygdala, such as with the parahippocampal gyrus and orbitofrontal cortex (Stein et al., 2005). There are also many direct neuronal projections from the prefrontal cortex to the amygdala, such as in the cingulum white matter tract with the orbitofrontal and dorsolateral PFC (McDonald, 1998; Catani et al., 2012). Bidirectional projections from both the orbitofrontal cortex and mPFC to the central and basolateral amygdala tend to be excitatory (Ghashghaei & Barbas, 2002; Barbas, Saha, Rempel-Clower, & Ghashghaei, 2003), and inhibiting this connection has been related to inappropriate affective regulation and stress response. For example, disrupting the connection between the amygdala and mPFC in mice resulted in ineffective and passive coping techniques, indicating an inability to process stress (Andolina et al., 2013). Repeated stress can also increase dendritic growth in the amygdala and decrease dendritic length in the mPFC (McEwen, 2007), perhaps resulting in reduced connectivity and abnormal stress response. Chronic alcohol use is associated with decreased gray and white matter volume, particularly in the PFC (Pfefferbaum et al., 1997). Few studies have assess the role of the dlPFC and its connections to the amygdala in emotion processing (e.g, Zhong et al., 2013), with no known studies on stress processing, and therefore greater understanding of the function of the dlPFC in such stress regulation is needed. The dlPFC may indirectly influence emotion and stress processing and has limited connections to limbic regions (for review, see Price & Drevets, 2010) and therefore its mediating role should be assessed. Overall, our findings are

consistent with previous studies regarding evidence of connectivity differences in abnormal stress response and suggest both functional and structural differences that future studies should assess. It should also be noted that we found connectivity differences in many areas within the PFC outside of the hypothesized role of the mPFC, suggesting broad differences in higher order cognitive functioning and stress processing in AD individuals in comparison to controls.

Though few studies have assessed the role of the temporal lobe in response to a stressor (Qin et al., 2012; Cousijn, Rijpkema et al., 2012), our findings suggest a relationship between amygdala connectivity and regions within the temporal lobe in stress processing. Perhaps one reason for the AD group's negative connectivity in the present task may be due to the anterior temporal lobe's function in social cognitions (for review, see Olson, McCoy, Klobusicky, & Ross, 2013). In the AD group, the anterior temporal lobe, especially parts of the superior temporal lobe, exhibited decreased connectivity with the amygdala, which may have led to a decreased ability to deal with a social stressors such as the one used in the present task. Others have studied the functional response to stress of healthy individuals during a working memory task embedded within stressful films (Qin et al., 2012) as well as in a similar stress paradigm as in the present study (Pruessner et al., 2008), finding the temporal lobe showed significant deactivation. In the stressful film task, a separate analysis found that temporal lobe deactivation accompanied by deactivation of the amygdala would be hypothesized to increase cognitive functioning (Cousijn, Rijpkema et al., 2012). However, in the present study, we found increased amygdala activation in conjunction with decreased connectivity to temporal regions, suggesting a potential difficulty in social cognitive

processing. The broader AD literature has also found deficits in white matter integrity within the temporal lobe (Monnig et al., 2013; Durazzo et al., 2014), though Durazzo and colleagues found recovery in white matter, but not gray matter, with abstinence. As our sample had a relatively long period of abstinence (mean = 1628 days), there may have been some recovery; however, other studies have shown improvements for up to five years with sustained abstinence (see Rosenbloom & Pfefferbaum, 2008). In addition, the uncinate fasciculus white matter tract connects the temporal pole to the amygdala and then the orbito-frontal cortex, allowing for bidirectional transport of information (Fortin, Aubin-Lemay, Bore, Girard, Houde, Whittingstall, & Descoteaux, 2012; Von Der Heide et al., 2013), and suggesting structural connectivity deficits may further exacerbate stress processing deficits. Therefore there may be preliminary evidence to suggest that the temporal lobe does have a role in stress processing in individuals with AD, particularly in social stress. Future studies are needed to further determine mechanisms of the temporal cortex in processing stressors, both within individuals with AD and in healthy individuals.

Amygdala functional connectivity with the parietal cortex was also abnormal in the AD group, suggesting the parietal cortex may also have a role in stress response, or alternatively may be differentially activated in response to mathematical problems. In 3-5 week abstinence AD males, a painful anticipatory stressor resulted in decreased activation of the bilateral parietal cortex, amongst other areas (Yang et al., 2013). Decreased white matter integrity (Monnig et al., 2013) and gray matter volume (Fein, Shimotsu, Chu, & Barakos, 2009) within the parietal cortex in remitted AD individuals in comparison to healthy controls has also been found. In non-AD samples, decreased white matter fractional anisotropy (FA; a measure of white matter integrity) were found in the

parietal lobe, right prefrontal lobe, basal ganglia, and right parahippocampus, in those who had recently experienced a severe earthquake in comparison to individuals who had not (Chen et al., 2013), indicating differences in not only function but structure within the parietal lobe in response to stress. However, the parietal lobe has also been known to be a hub for mathematical cognition (Park, Park, & Polk, 2013; Andres et al., 2011) as well as for working memory and attention (Soto, Rotshtein, & Kanai, 2014), and therefore our results may be influenced by the arithmetic task used to induce stress. Even so, our results highlight different parietal areas than the most often implicated math-processing regions (in math processing, areas activated often include the horizontal segment of the intraparietal sulcus and the posterior superior parietal lobule, while we found activation in the left inferior and bilateral superior parietal lobule). Further research is needed to clarify potential underlying mechanisms within the parietal lobe that may uniquely contribute to either math or stress processing. In particular, a stress task with no math component may result in no findings within the parietal lobe.

Recently, the cerebellum has been hypothesized to have a greater role in stress and emotion processing than once thought (for review, see Stoodley & Schamahmann, 2010; Baumann & Mattingley, 2012). In particular, the vermis has been proposed as the "limbic cerebellum" (Stoodley & Schamahmann, 2010), fitting with our findings regarding altered function of limbic regions *including* regions within the vermis such as the culmen and uvula. In healthy individuals, mental stressors have been shown to result in increased regional cerebral blood flow (rCBF) in the vermis in positron emission tomography scans, while a control task showed increased amygdala activity (Critchley et al., 2000). Therefore in healthy individuals increased vermis activity in a stressful

condition with increased amygdala activity *only* in the neutral condition may be better for stress processing, which is not the pattern displayed within the current AD sample. These cerebellar functional differences may also be due to structural changes, as alcohol exposure in animals (Phillips, 1990), adolescents (Lisdahl et al., 2013), and adults with AD, even after 7.5 months of abstinence (Durazzo et al., 2014), has been linked with cerebellar atrophy. As much of this cerebellum-specific research is preliminary, more research is needed to assess the influence of cerebellum structure and function on alcohol-related outcomes and the underlying mechanisms that drive cerebellar stress processing.

In sum, the functional connectivity patterns indicate both an alternate and exacerbated pattern of stress processing. By alternate pattern, we mean that the AD group exhibited decreased connectivity in regions not typically thought to be involved in healthy stress response (e.g., regions within the temporal and parietal lobe), perhaps indicating that these regions are important for stress processing or amygdala regulation. These results may suggest that these secondary stress response regions are underutilized in AD individuals, inhibiting their ability to appropriately handle stressors. In addition, individuals with AD utilize expected stress processing regions and patterns (e.g., negative connectivity between the amygdala and the mPFC), but exacerbate this above and beyond the response exhibited by healthy controls. This abnormal stress response may then influence an individual with AD to turn to the hedonic properties of alcohol to handle the stress they are under, rather than use healthier stress processing networks, as Garland and colleagues (2011) have suggested. Alternatively, as alcohol use has known structural consequences (see Rosenbloom & Pfefferbaum, 2008) and these structural difference

may lead to a reorganization of neural systems (see Crews et al., 2005), structural deficits in gray and white matter may lead to aberrant stress processing.

In the secondary analyses, baseline cortisol collected a year prior to the neuroimaging session significantly predicted initial and sustained amygdala activation. Additionally, both baseline cortisol and ACTH predicted increased connectivity patterns. Consistent with van Stergeren and colleagues (2007), we found evidence of increased amygdala activation being related to increased baseline cortisol level, though our study has found this relationship in AD individuals rather than healthy controls. Higher cortisol levels collected a year prior in AD individuals were also predictive of greater amygdala connectivity, though this was not assessed in comparison to controls. As our initial data collection of basal hormone levels was a year prior to the scan date, these brain-behavior relationships may suggest a long-term change in hormonal response that is predictive of neuronal function. Even if hormone response may return to near-normal with sustained abstinence, as others have suggested (Stephens & Wand, 2012), the underlying neuronal function may not. Therefore it may be that, even with prolonged abstinence, years of chronic alcohol exposure can damage both neuronal and hormonal functioning. Alternatively, individuals may be predisposed to certain hormonal function that also makes them more likely to develop AD, or even previous traumatic events may result in abnormal hormone function and then influence the onset of AD. As it stands now, the increased connectivity exhibited in relation to higher cortisol levels may be indicative of better utilization of stress processing networks. Future research should assess neuronal stress response and its relation to cortisol in AD and healthy samples, and see if this

connectivity pattern then predicts treatment outcomes or can be predicted by other predictors of AD (e.g., history of trauma).

Stress is an emotional response most often linked to negative affect, and studies examining stress response often refer to overall emotion processing (e.g., Andolina et al., 2013; Clewett et al., 2013), as well as assessing the effects of stress on emotion processing (e.g., Golkar et al., 2014). Therefore it may be helpful to assess the present findings in light of emotion processing and negative affect literature. Though it is suggested that stronger amygdala and mPFC connectivity is better for emotion processing and anxiety reduction in emotional tasks (for review, see Kim et al., 2011), the present findings suggest that individuals with AD are not able to effectively activate this circuit and are therefore unable to mediate the stressors they experience. Notably, Kim and colleagues also suggest that increased mPFC and decreased amygdala response is evident in those who have successfully processed emotions. Greater activation in the lateral and medial prefrontal cortices, amongst other areas, and decreased amygdala activation have been found to be related to reappraisal of negative stimuli (Oschner et al., 2002). If this relationship were flipped, then, it may reflect poorer appraisal of emotional stimuli and, perhaps, a deficit in stress and emotional processing, as exhibited in this study of AD individuals.

The present sample of individuals with AD appears to fit the typical experience and characterization of the general population of individuals with AD. High rates of co-morbid diagnoses are common in AD, as well as sub-threshold diagnoses and trauma histories, (Kessler et al., 2005; Pilowsky, Keyes, & Hasin, 2009), indicating that having sub-threshold PTSD symptoms as well as high rates of childhood trauma is fitting for this

population. As also indicated in the present sample, there is a wide range of experiences within those with AD—from co-occurring diagnoses to minimal or no symptoms of other psychiatric disorders (e.g., depression)—making it appear to be highly generalizable to the general population. However, this does raise questions regarding the driving factors of the observed stress response differences. Perhaps the experience of childhood or recent trauma has led to the development of altered stress response or even develop AD. Unfortunately as the present study did not assess trauma history in the controls, the current study is unable to adequately answer these questions and future studies should be aimed at assessing the longitudinal pattern of varying degrees of trauma on stress response and AD. Yet as the present findings fit more typical community samples, including through the inclusion of varying degrees of traumatic history, the findings regarding altered stress response in AD individuals remain important and novel.

Implications of Stress Processing and Emotion Dysregulation in AD

As the present findings suggest stress processing abnormalities, treatments specific to stress and emotion regulation would seem beneficial. In particular, mindfulness has been found to be an efficacious treatment in improving stress regulation capacity (Lutz et al., 2013). For example, a simple, brief mindfulness intervention in a healthy sample resulted in reduced amygdala activation as well as reduced PFC response, suggesting that less brain power was needed to process emotions (Lutz et al., 2013). Similarly, individual differences in mindfulness disposition predicted down-regulation of emotion in response to negative stimuli through increased dorsalmedial PFC activation and inversely related amygdala response (Modinos, Ormel, & Aleman, 2010). The brain areas highlighted in this mindfulness literature map on to several of the clusters found in

the present study. Indeed, in theorizing the biopsychosocial model of alcohol dependence, Garland and colleagues (2011) hypothesized mindfulness would be a useful treatment, as it decreases automatic thoughts through increased awareness of triggers that may relate to alcohol craving and relapse, creates new schemas related to alcohol and stress, and teaches an individual how to refocus attention away from alcohol stimuli, rather than attempting to use thought suppression.

Our results also suggest amygdala activation as a potentially unique and simple biomarker for AD. A relatively brief fMRI scan (perhaps as brief as one minute) during a stressful task and then mapping out the timecourse of amygdala activation may produce a robust biomarker for patients. Correlating the pattern of left amygdala activation with basal cortisol levels may further strengthen the utility of amygdala activation as a biomarker. Other outcomes may then be predicted from this observed pattern, such as potentially predicting relapse and response to stressors. Additional research into the utility and outcomes of the amygdala activation as a biomarker are needed.

Limitations

Several limitations to the present study should be noted. This was a pilot study with a small sample size; therefore, although findings were significant and very robust in the case of amygdala activation, they will need to be replicated in a larger sample to ensure generalization. Our sample included left-handed individuals, which may have introduced extra variance into our between-group differences due to lateralization effects (Vingerhoets, Acke, Alderweireldt, Nys, Vandemaele, & Achten, 2012; Willems, Peelen, & Hagoort, 2011). However, it should be noted that differences are predominately in language and fine motor function (for review, see Gutwinski, Loscher, Mahler, Kalbitzer,

Heinz, & Bermpohl, 2011) and that right-vs-left handed individuals may have minimal lateralization differences (Serrien & Sovijarvi-Spape, 2013). Our sample also included psychiatric co-morbidities in the AD group, including those with nicotine dependence, other substance use disorders in remission, and anti-social personality disorder. Even so, alcohol dependence was the primary diagnosis and the reason for enrollment in the study and these conditions are commonly comorbid in AD treatment samples. Gender is often an important moderator of emotion processing and brain connectivity (Tomasi & Volkow, 2012), but, due to our limited sample size, we did not assess the potential moderator effects of gender. We suggest future studies directly test whether these findings are consistent across both genders. All secondary analyses were conducted in only the AD group. Future research is needed to assess if the relationships found in the present study are unique to individuals with AD or if other samples have similar results. Finally, as the stress-inducing task was based on math subtraction problems, there is a possibility that the observed differences were due to mathematic and working memory abilities, though it should be noted that subjective stress levels did increase during the task, the amygdala was engaged, and stress hormones significantly predicted connectivity patterns.

Conclusions and Future Directions

The present study demonstrated increased amygdala activation in response to a stress task in individuals with AD, as well as decreased connectivity between the amygdala and brain areas important for stress and emotion processing. Further, findings suggest a long-lasting change in hormone and stress response in individuals with AD, as baseline hormone levels collected a year prior to the scan prospectively predicted bilateral amygdala response and right amygdala functional connectivity. Future studies

with larger samples of both men and women are needed to assess the functional response to stress in AD subjects. Further, as structure may predict function (Greicius, Supekar, Menon, & Dougherty, 2008), understanding the underlying microstructure of neural networks could further elucidate differences in AD stress response. Longitudinal studies would be beneficial to assess whether the stress response dysregulation causes or is a result of alcohol dependence. Interventions aimed at treating stress dysregulation in AD may be especially efficacious, such as through mindfulness training. Investigations should assess the potential of amygdala activation patterns as a robust biomarker. Finally, as this stress response dysfunction appears to be long lasting, prevention should be aimed at adolescents and young adults prior to the development of AD. In particular, research developing early interventions to improve stress and emotional regulation may be beneficial.

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