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14. ABSTRACT
Hazardous use of alcohol negatively impacts the treatment of PTSD. However, the mechanisms that underlie the association between PTSD and hazardous alcohol use in veterans are poorly understood. The current research takes a multi-level approach to study the psychological, behavioral, cognitive and neural relationships between PTSD and alcohol use. Progress to date has been focused on building the infrastructure to conduct neural imaging by training of personnel, setting up recruitment of veteran populations, and conducting test runs to ensure the recently purchased fMRI equipment and experimental procedures were suitable to test the study hypotheses with veteran participants. The ability to now conduct functional brain imaging at the University of South Dakota represents the first and only opportunity in the State of South Dakota to perform such research, and complements the strong emphasis and infrastructure already in place to conduct psychological, behavioral and cognitive assessment of South Dakota's underserved rural veteran population. Preliminary data collected in the current reporting period suggests that emotional stimuli used in the fMRI tasks elicit arousal responses and expected neural activity in non-veteran and veteran participants, thus validating the paradigms for testing the behavioral and neural association between PTSD and hazardous alcohol use. Research in the next funding period will build on recruitment efforts to increase subject numbers, thus allowing full analysis of the psychological, behavioral, cognitive and neural data to identify behavioral and neural predictors of poor psychological outcomes related to PTSD and hazardous alcohol use in South Dakota veterans. Overall, the current funding has built research infrastructure and tools to allow comprehensive studies regarding mental health and neurological issues specific to veterans in rural areas.

15. SUBJECT TERMS
Posttraumatic stress disorder; Alcohol; Neurobiology of anxiety disorders; Limbic System; Emotional Regulation.

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Introduction:
Posttraumatic stress disorder (PTSD) is a signature impairment of US soldiers arising from Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (OEF) (Kennedy et al., 2007; Gaylord et al., 2008; Hoge et al., 2008). Furthermore, 25-35% of returning OEF/OIF veterans report some alcohol misuse (i.e., using more than intended) (Hoge et al., 2004), and approximately 15% of veterans screen positive for both PTSD and hazardous drinking (Jacobson et al., 2008; McDevitt-Murphy et al., 2010). The association between PTSD and alcohol use has clear therapeutic implications given that impairment associated with PTSD may be compounded by alcohol use (Dao-Castellana et al., 1998; Gilman and Homer, 2008). Furthermore, patients with PTSD and comorbid substance use disorder are least likely to benefit from treatment and more likely to relapse when compared to patients who have substance use disorder alone (Ouimette, et al., 2007; Schafer and Najavits, 2007). Therefore, it is important to study the behavioral and neural relationships between PTSD and alcohol use.

Objective 1) *To test a model linking PTSD symptoms and alcohol-related problems in a population of recently returned soldiers.*
Associations between PTSD symptoms and alcohol use disorder likely represent complex reciprocal relationships. However, longitudinal and experimental research indicates that PTSD symptomatology increases risk for alcohol use disorders. This objective will focus on enhancing understanding of the potential mechanisms by which PTSD may increase risk for alcohol use disorder. Recently returned veterans will be assessed for PTSD symptoms and other mental health issues, alcohol use and dependence issues, and factors related to stress coping and behavioral disinhibition. Specifically, we will test a model linking PTSD symptoms and alcohol use disorder via behavioral disinhibition and affective lability. Behavioral disinhibition is posited to link PTSD symptoms and alcohol-related negative consequences (e.g., interpersonal aggression), whereas affect lability is posited to link PTSD and symptoms of alcohol dependence. Secondary analyses will examine whether hyperarousal and reexperiencing symptoms represent core symptoms underlying the link between PTSD and alcohol use disorder.

Objective 2) *To test a neural circuitry model linking trauma, alcohol consumption, and PTSD symptoms.*
This objective will focus on enhancing understanding of potential neural mechanisms by which traumatic experience and excessive alcohol use may contribute to PTSD symptoms in recently returned veterans. We will test a model linking trauma and alcohol consumption with PTSD symptoms via altered functioning in the anterior cingulate cortex and amygdala. The non-recursive model will incorporate bi-directional effects linking alcohol consumption, neural functioning, and PTSD symptoms. This will be achieved by conducting an fMRI study to examine activity of the amygdala during a masked emotional faces paradigm and to examine the activity of anterior cingulate cortex during the emotional counting Stroop task. The central hypothesis of this objective predicts that trauma and heavy alcohol use negatively impact the activity of and balance between ACC and amygdala function, thus increasing dysfunction associated with PTSD.

A further purpose of this funding was to enhance the capacity of USD to perform fMRI research at USD's health care partner, Avera Sacred Heart Hospital. Therefore, the technical objectives of this project are:

1) To substantially increase the ability of The University of South Dakota and its health care partners to research the neural bases of psychological health impairment, diagnosis and treatment by initiating fMRI research.
2) To conduct a short-term initial feasibility study to bring together the components of recruitment, screening, imaging, testing, and data analysis; all critical for subsequent fMRI longitudinal studies.

Body:
<u>Objectives and Milestones:</u>
The scientific objectives for this research project were:

1) To test a model linking PTSD symptoms and alcohol-related problems in a population of recently returned soldiers.

2) To test a neural circuitry model linking trauma, alcohol consumption, and PTSD symptoms.

Given a year no-cost extension and the delays incurred in the first year of the award, the originally approved Milestone timeline was no longer relevant. Table 1 and Figure 1 below show the updated timeline for each task, which were presented at the Product Line Review meeting on June 12th 2012 and included in the June 2012 quarterly report.

Table 1: Project Objectives, Milestones and Timeline

Tasks/Milestones	Start Date	Duration (days)	End Date
Objective 1: Testing a model linking PTSD symptoms and alcohol-use.			
Task 1: Obtain IRB approval.	9/24/2010	447	12/15/2011
Task 2: Hiring of personnel.	10/1/2010	304	8/1/2011
Task 3: Advertising & recruitment.	12/15/2011	502	4/30/2013
Task 4: Interview participants.	1/1/2012	486	5/1/2013
Task 5: Analysis/statistical modeling of assessment data.	4/30/2012	426	6/30/2013
Task 6: Manuscript/s for assessment data prepared for publication.	5/30/2013	116	9/23/2013
Objective 2: Testing a neurocircuitry model linking trauma, alcohol consumption and PTSD symptoms.			
Task 7: Purchase, set-up & test infrastructure for fMRI study.	9/24/2010	495	2/1/2012
Task 8: Conduct fMRI study.	2/1/2012	469	5/15/2013
Task 9: Analysis of images.	4/30/2012	426	6/30/2013
Task 10: Statistical analysis/modeling of fMRI & behavioral data.	7/1/2013	45	8/15/2013
Task 11: Manuscript/s for fMRI study prepared for publication.	5/30/2013	116	9/23/2013

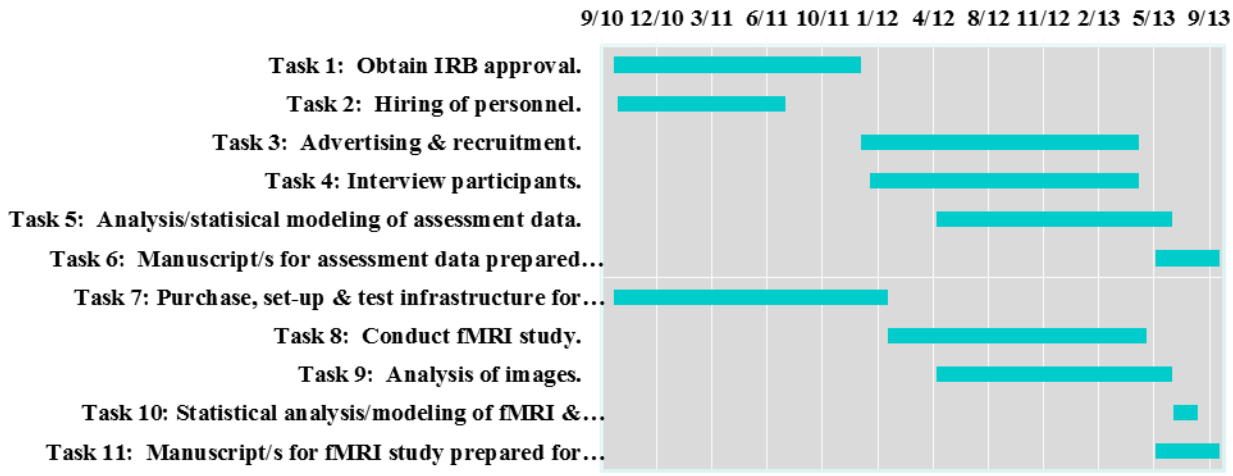


Figure 1: Gnatt chart illustrating the timeline for the various tasks listed in Table 1.

As outlined by Table 1 and Figure 1, the major objectives for the second year of the award included work towards:

- Objective 1 Task 1: Obtain IRB Approval
- Objective 1 Task 3: Advertising and Recruitment
- Objective 1 Task 4: Interview Participants
- Objective 1 Task 5: Analysis/Statistical Modeling of Assessment Data
- Objective 2 Task 7: Infrastructure for fMRI
- Objective 2 Task 8: Conduct fMRI Study
- Objective 2 Task 9: Analysis of Images

Work Towards Objective 1 Task 1: Obtain IRB Approval:

Detailed information regarding the delays and progression of IRB approval over the previous reporting periods was supplied in the 2011 annual report. At that stage (September 2011), the University of South Dakota IRB had approved our human subjects protocol in June 2011, but the results of the review by the DoD Office of

Research Protection (ORP) had not been made available to the PI. In October 2011, reviews from the DoD ORP were sent to the PI, and minor revisions required. The revisions were made, and sent to the University of South Dakota (USD) IRB and to the DoD ORP. The final version of the protocol was approved by the USD IRB on December 12th 2011, and by the DoD ORP on December 14th 2011. Therefore, this task has been completed.

Work Towards Objective 1 Task 3: Advertising and Recruitment:
The investigators generated several forms of advertising materials targeting veterans, including fliers, letters, email scripts, and newspaper advertisements. These were approved in their final form by the DoD ORP and USD IRB in mid-December 2011, and these materials were emailed or mailed to Iraq/Afghanistan veterans in South Dakota directly, in addition to fliers being posted on notice boards on University campuses, National Guard offices and local clinics. Fliers were also distributed during meetings of the USD veterans association. Furthermore, newspaper advertisements in daily and weekly newspapers within the southeast corner of South Dakota and adjacent Nebraska towns were placed. These efforts resulted in the first participants being scheduled for the study in the first week of January 2012. To date, we have recruited 46 veterans who met the selection criteria for phase I of the study (psychological and alcohol use assessment). Of these, 22 participants met the MRI screening criteria and agreed to participate in phase II of the study (fMRI and emotional suppression tasks). See Table 3 for more details on participant recruitment. Work towards this task will continue in year three of this award to ensure sufficient participant numbers (at least 20 per group) for appropriate analysis of the data.

Work Towards Objective 1 Task 4: Interview Participants:
The psychological assessment interview represents phase I of the study. Participants were invited to an interview session with the one of the Clinical Psychology investigators in the location of the participant's choice (Sioux Falls, Yankton or Vermillion). The participants filled in on-line validated assessment tools probing combat exposure, social support, PTSD symptoms, other mental health and health symptoms, alcohol use and misuse, stress coping and personality traits. They then underwent a clinical interview to confirm mental health status and cognitive status. Finally, screening for MRI contraindications was performed to assist in determining eligibility for phase II of the study. Each interview session was 2-3 hours in length. Forty-six participants were interviewed for phase I of the study. Work towards this task will continue in year three of this award.

Work Towards Objective 1 Task 5: Analysis/Statistical Modeling of Assessment Data:
Clinical interview data is being collated by the Clinical Psychologist investigators, and at this stage there are not enough participants to begin the statistical modeling of this data. However, individual subject information is analyzed on a weekly basis as it relates to MRI compatibility, PTSD symptoms and alcohol use, and is made available by a confidential online process (PsychData) to the Neuroscience investigators of this project, so that they can quickly determine whether the participant could be invited to participate in phase II of this study (Objective 2 Task 8). Participants that do not have contraindications to MRI and that fall into one of the following groups qualify for phase II of the study: 1) low/no alcohol use and low/no PTSD symptoms; 2) low/no alcohol use and PTSD symptoms; 3) high alcohol use and low/no PTSD symptoms; 4) high alcohol use and high PTSD symptoms (Table 2). Table 3 shows the number of participants in each group to date.

Table 2: Criteria Used for Allocating Participants to PTSD and Alcohol Use Groups

	Low-Moderate Alcohol Use	**Hazardous Alcohol Use**
No PTSD	< score of 29 on PCL < 7/14 drinks/week (women/men) < score of 8 on AUDIT	< score of 29 on PCL ≥ 7/14 drinks/week (women/men) ≥ score of 8 on AUDIT
PTSD	≥ score of 29 on PCL < 7/14 drinks/week (women/men) < score of 8 on AUDIT	≥ score of 29 on PCL ≥ 7/14 drinks/week (women/men) ≥ score of 8 on AUDIT

PCL = PTSD checklist, is used clinically to diagnose PTSD, with a score of 29 or more indicating PTSD symptoms.

The number of drinks (shown for both men and women) is based on the National Institute for Alcohol Abuse and Alcoholism (NIAAA) guidelines for problem drinking within a week, over the last 30 days.
AUDIT = Alcohol Use Disorders Identification Test, a score of 8 or more is used to diagnose alcohol use disorder (questions relate to problems associated with alcohol use in the last year).

Table 3: Number of Participants Tested in Phase I (Psychological Assessment) and Phase II (fMRI) within each Experimental Group

	Low-Moderate Alcohol Use	**Hazardous Alcohol Use**
No PTSD	Phase I = 6/ Phase II = 4	Phase I = 8/ Phase II = 4
PTSD	Phase I = 15/ Phase II = 6	Phase I = 15/ Phase II = 8

Phase I total = 46 participants, Phase II total = 22 participants.

Work Towards Objective 2 Task 7: Infrastructure for fMRI:
As detailed in the first annual report for this award, the 1.5 T MRI unit at Avera Sacred Heart Hospital was replaced with a 3 T scanner, and outfitted for research (e.g. presenting stimuli, recording subject responses) during the second half of Year 1. The new scanner (Siemens 3T Open Bore Magnetom Skyra) and the Esys fMRI system (stimulus presentation-response system with E-Prime stimulus generation software) required optimization during the current reporting period, prior to being used to image veteran populations. The PI and study personnel received training on the use of the fMRI equipment and software in September and October 2011, and obtained IRB approval to conduct test experiments with student subjects. During December 2011-Feberurary 2012, the PI and co-investigator Dr. Vince Magnotta (Director of MR Research Facility at the University of Iowa) conducted studies with test subjects recruited from USD student groups. We optimized the parameters for basic visual and motor tasks, and for the emotional tasks that were to be used in the scanner with the veteran populations. One problem did arise with the equipment when presenting the emotional tasks which involved the rate at which stimuli needed to be presented was not being supported by the Esys stimulus system. This was solved by the investigators and Esys technical staff with modifications to the stimulus design and mode of presentation. Also in this current reporting year, a DICOM server with XNAT interface was set up between Avera Sacred Heart Hospital and USD, with password protected access being given to Dr. Magnotta so that he can download and process images. This system was tested with the data collected from the student participants in the fMRI optimization studies, and was a very successful means by which to transfer images to Dr. Magnotta at the University of Iowa. The fMRI infrastructure for this study was fully functional by March 2012, allowing the fMRI phase of the current study with veteran subjects to begin. Therefore, this task is complete.

Work Towards Objective 2 Task 8: Conduct fMRI Study:
Following psychological assessment as outlined in Objective 1 Task 4 above, qualifying veteran participants were invited to Avera Sacred Heart Hospital in Yankton for the fMRI component of the study. Participants were re-screened for MRI compatibility, tested for blood-alcohol levels, and then within the scanner, passively viewed masked emotional faces and performed an emotional stroop counting task (a task of emotional suppression – see Figure 3 for more details regarding each task). After the tasks in the scanner were complete, participants then filled out a self-assessment manikin to rate their arousal levels upon viewing the images that comprised the two tasks in the scanner (Figure 2). Although low participant numbers at this stage preclude any statistical analysis of the arousal rating data, preliminary data suggests that participants rate fearful faces higher than neutral and happy faces, and rate combat and negative words higher than neutral words. This suggests that the negatively-valanced emotive stimuli used in the fMRI-based tasks appear to elicit a greater state of arousal in participants. The entire phase II study is approximately 2 hours long, with participants in the scanner for 45 minutes to 1 hour. Twenty-two veteran participants have been scanned in phase II of the study to date. Work towards this task will continue in year three of this award to increase participant numbers to a total of 20 participants each group.

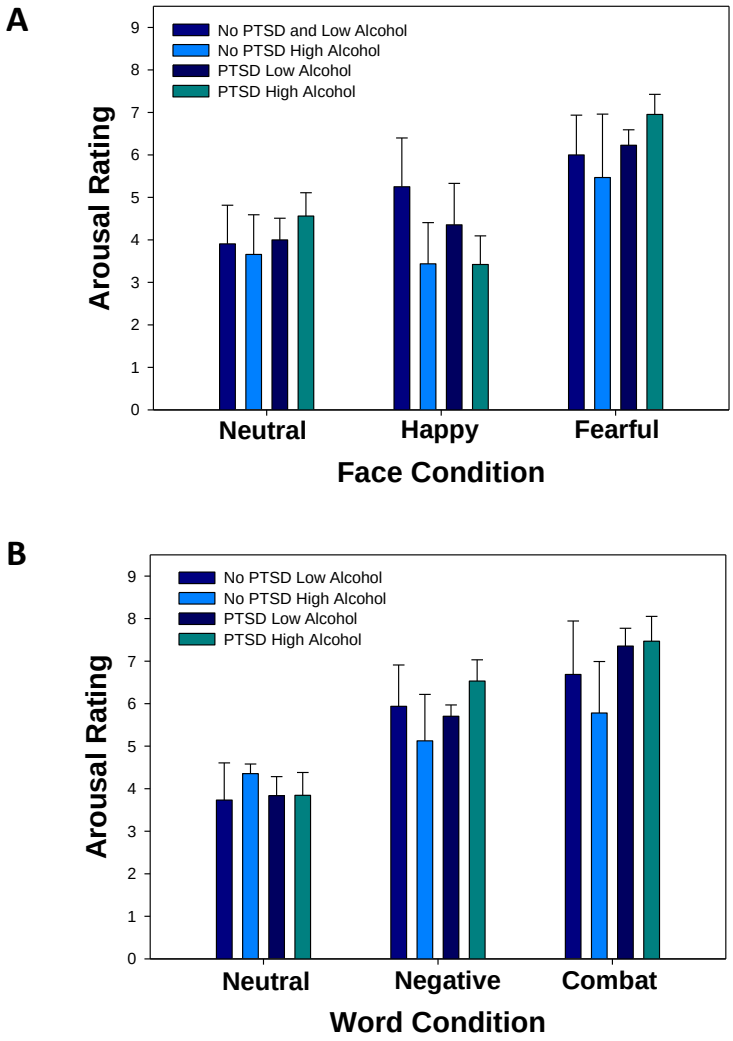
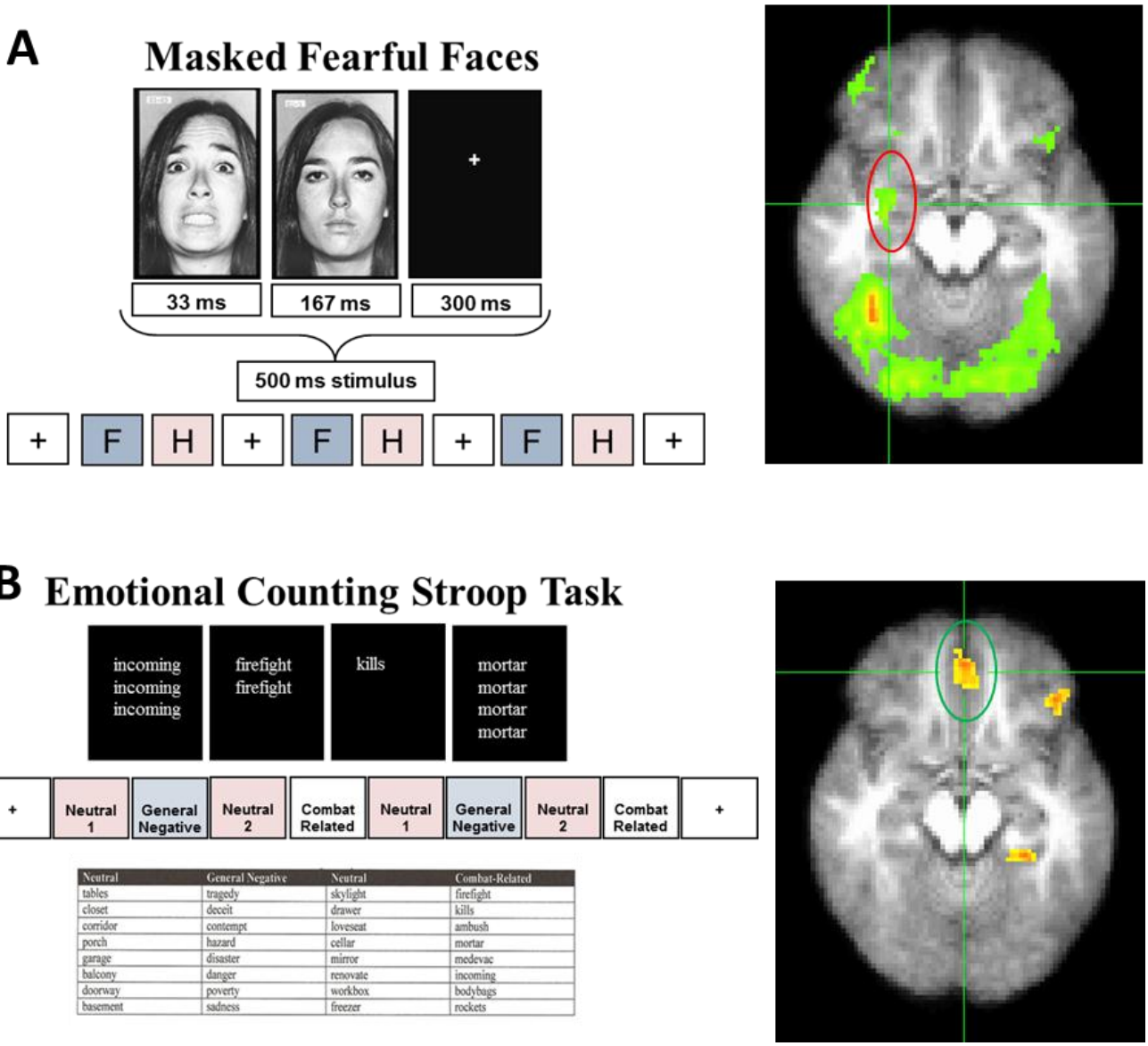


*Figure 2: Very preliminary arousal ratings for stimuli used in fMRI tasks (4-8 participants per group, see Table 3). Following the scan, participants were asked to rate their arousal level (scale 1-9 with 9 being high arousal, 1 being very calm) using the self-assessment mannikin (SAM). Participants were presented with each stimulus used in the **A)** masked faces paradigm (fearful, neutral, or happy face) and in the **B)** emotional stroop task (neutral, negative or combat word) for 3 sec each, and given 8 sec to rate each stimulus. See Figure 3 for more details regarding the stimuli. While very preliminary, there is a trend for participants to rate fearful faces higher than neutral and happy faces, and there is also a trend for participants to rate combat and negative words higher than neutral words.*

Work Towards Objective 2 Task 9: Analysis of Images:

Preliminary fMRI analysis of the first 12 participants from phase II was performed by Dr. Magnotta in June 2012 to determine whether the stimuli and tasks performed in the scanner elicited the expected neural activity. The data from the first 12 participants analyzed was sufficient to determine activation in the regions of interest (amygdala and anterior cingulate cortex) with the planned contrasts that will be employed when performing between-group analyses (Figure 3). Specifically, masked presentation of fearful faces elicited increased activation within the amygdala (Figure 3A) as has been observed in other studies using Vietnam veterans and non-combat participants (e.g., Shin et al., 2005). Furthermore, robust activation of the anterior cingulate cortex was observed during the combat-word condition of the emotional counting stroop task (Figure 3B), as has been observed by previous studies with Vietnam veterans (using Vietnam war-related words; e.g. Shin et al., 2001). Therefore, the paradigms and stimuli used in the current study were validated by this preliminary analysis.



Neutral	General Negative	Neutral	Combat-Related
tables	tragedy	skylight	firefight
closet	deceit	drawer	kills
corridor	contempt	loveseat	ambush
porch	hazard	cellar	mortar
garage	disaster	mirror	medevac
balcony	danger	renovate	incoming
doorway	poverty	workbox	bodybags
basement	sadness	freezer	rockets

Figure 3: Preliminary analysis (n = 12) of fMRI data collected during the masked faces paradigm and the emotional counting stroop task. ***A)*** *Masked Faces Paradigm: Used to elicit processing of fearful information in the brain. Each emotional face (happy or fearful) is backward masked by the neutral expression, as illustrated by the composite stimulus above (500 ms total). Stimuli are derived from the Ekman-Friesen stimulus set, and comprise 8 individuals (5 male, 3 female). Each fearful or happy block comprises 56 presentations of masked fearful or masked happy faces respectively. There are three blocks of each within the 10 block run. Two runs are conducted, with the presentation of fearful and happy blocks counterbalanced between runs. Significant activation in the region of interest – the amygdala (circled in red) was observed in fearful vs. fixation conditions. With an increase in subject number, we expect to see more robust activation of the amygdala.* ***B)*** *Emotional Counting Stroop Task: Used to assess suppression of emotional information. Each word (in the table above) is presented 1-4 times on the screen as illustrated above, and the subjects are required to count the number of words and report that number via button press. Words from neutral, negative and combat conditions are matched for length, number of syllables and frequency of use in American-English. Each word condition block comprises 20 word presentations (each 1450 msec duration with 50 msec between each), and each block is presented twice within the run. Two runs are conducted with the presentation of neutral, negative and combat blocks counterbalanced between runs. Robust significant bilateral activation of the region of interest, the rostral anterior cingulate cortex, was observed (circled in green) in combat vs. negative word conditions.*

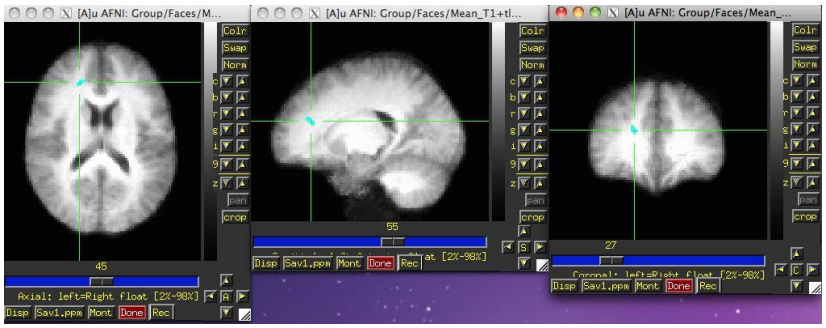
In September 2012, Dr. Magnotta performed a preliminary group-based analysis of the fMRI data collected from twenty participants scanned in phase II of the study. Since there were not sufficient subject numbers to analyze the data across all four groups (see Table 3), within-group analyses were performed for each of the task conditions for no-PTSD groups, the PTSD with no hazardous alcohol use group, and the PTSD with hazardous alcohol use group. Analyses were conducted with a threshold of 0.01 and a cluster size of 50 voxels. The results are summarized in Tables 4-5. While very preliminary due to low subject numbers, the vast majority of regions identified within each group during the masked faces paradigm (Table 4) are regions known to be activated during the processing of emotional faces or images. For example, changes in activation in the lingual gyrus, superior temporal gyrus, anterior and posterior cingulate, cuneus, fusiform gyrus, and parahippocampal gyrus are associated with processing of positively-valanced images while changes in activation of the middle frontal gyrus, parahippocampal gyrus, precuneus, cingulate, superior temporal gyrus, middle temporal gyrus, fusiform gyrus are associated with processing of negatively-valanced images (Kensinger and Schacter, 2006). Furthermore, changes in activity of the middle frontal gyrus, parahippocampal gyrus, fusiform gyrus and middle temporal gyrus are specifically associated with emotional face processing (Shin et al., 2005; Sabatinelli et al. 2011). Similarly, the majority of regions identify within each group during the emotional counting stroop task (Table 5) are consistent with those known to be activated during the processing of negatively-valanced words. It is known that changes in activity within the middle frontal gyrus, inferior temporal lobule, superior temporal gyrus, middle temporal gyrus, precuneus, and cingulate are associated with processing of negative words (Kensinger and Schacter, 2006). Furthermore, combat-related words are specifically associated with regional changes in the middle frontal gyrus, precentral gyrus, anterior cingulate, precuneus, insula, superior temporal gyrus, middle temporal gyrus, lingual gyrus and claustrum (Shin et al., 2001). Therefore, the preliminary within-group analysis of our fMRI data for both paradigms suggests activation in expected regions of the brain associated with processing of emotional content.

Table 4: Summary of Regions in which Changes in BOLD Signal was Observed during the Masked Faces Paradigm.

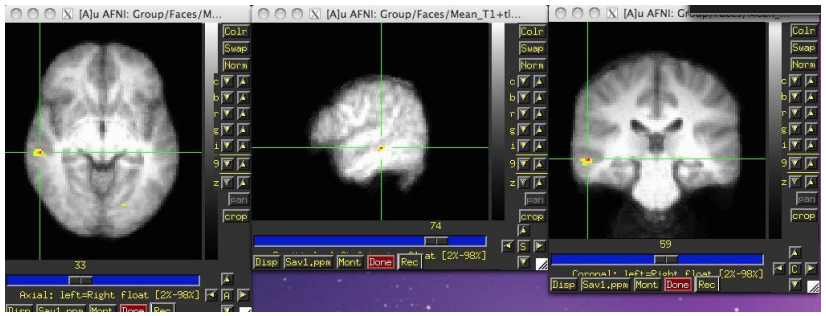
Group	Task Condition	Region
No PTSD	Happy Faces	Left Lingual Gyrus Right Parahippocampal Gyrus Left Parahippocampal Gyrus Right Superior Temporal Gyrus Right Insula Right Cingulate Gyrus Right Cuneus
PTSD	Happy Faces	Right Lingual Gyrus Left Lingual Gyrus Left Fusiform Gyrus Right Inferior Frontal Gyrus
PTSD with Hazardous Alcohol Use	Happy Faces	Left Lingual Gyrus Right Caudate Tail Left Caudate Tail Left Parahippocampal Gyrus Right Superior Temporal Gyrus Right Insula Right Cingulate Gyrus Right Cuneus
No PTSD	Fearful Faces	Right Parahippocampal Gyrus Left Parahippocampal Gyrus Left Precuneus Right Precuneus Right Cuneus Right Insula Right Precentral Gyrus Right Postcentral Gyrus Right Cingulate Gyrus Right Superior Temporal Gyrus Left Posterior Cingulate Gyrus
PTSD	Fearful Faces	Right Fusiform Gyrus

		Left Fusiform Gyrus Right Middle Frontal Gyrus Left Precentral Gyrus
PTSD with Hazardous Alcohol Use	Fearful Faces	Right Parahippocampal Gyrus Left Parahippocampal Gyrus Right Insula Right Cuneus Right Precuneus Left Precuneus Right Precentral Gyrus Right Postcentral Gyrus Right Cingulate Gyrus Left Posterior Cingulate Gyrus Right Superior Temporal Gyrus
No PTSD	Fearful – Happy Faces	Left Lingual Gyrus
PTSD	Fearful – Happy Faces	None
PTSD with Hazardous Alcohol Use	Fearful – Happy Faces	Right Middle Temporal Gyrus Right Anterior Cingulate Gyrus

The summary presented in Table 4 also suggest activity of many of the same regions in participants within no-PTSD and PTSD groups within the happy faces or fearful faces conditions, as would be expected with similar regions processing emotional content across participants. However, the fearful-happy contrast analysis (Table 4; Figure 4) suggests differential activation of regions in those participants reporting hazardous alcohol use and PTSD when compared to PTSD alone, specifically related to emotional processing (e.g. middle temporal gyrus and anterior cingulate). Like the masked faces paradigm, the presentation of negatively-valanced words in the emotional counting stroop task (Table 5) elicited activity in many of the same brain regions across PTSD and no-PTSD groups. However, some notable differences were observed – for example, combat words elicited changes in regions involved in top-down control of emotional/attentional processing (such as the claustrum and anterior cingulate gyrus) in the PTSD with hazardous alcohol use group only (Table 5, Figure 5). Increasing subject numbers in each group over the next year will determine whether these findings are confirmed.



Anterior Cingulate Gyrus



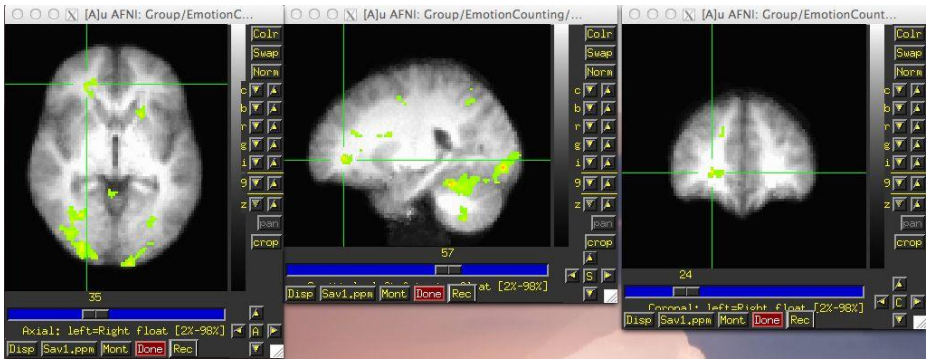
Middle Temporal Gyrus

Figure 4: Preliminary analysis (n = 8) of fMRI data collected during the fearful faces condition in the masked faces paradigm from participants in the PTSD with hazardous alcohol use group. These participants show

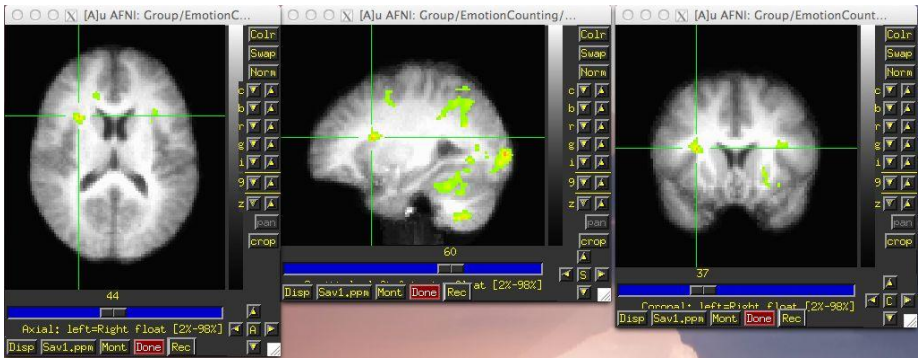
reduced activation of the anterior cingulate and increased activation of the middle temporal gyrus when viewing masked fearful faces.

Table 5: Summary of Regions in which Changes in BOLD Signal was Observed during the Emotional Counting Stroop Task.

Group	Task Condition	Region
No PTSD	General Negative Words	Left Lingual Gyrus Left Posterior Cingulate Gyrus Left Inferior Parietal Lobule Left Inferior Parietal Lobule Right Middle Temporal Gyrus Left Middle Temporal Gyrus Left Precentral Gyrus Right Insula Right Middle Frontal Gyrus
PTSD	General Negative Words	Right Lingual Gyrus Left Lingual Gyrus Right Precentral Gyrus Left Postcentral Gyrus Right Fusiform Gyrus
PTSD with Hazardous Alcohol Use	General Negative Words	Left Lingual Gyrus Left Posterior Cingulate Gyrus Right Inferior Parietal Lobule Left Inferior Parietal Lobule Right Middle Temporal Gyrus Left Middle Temporal Gyrus Left Precentral Gyrus Left Postcentral Gyrus Right Insula Right Middle Frontal Gyrus
No PTSD	Combat Words	Left Precuneus Left Precentral Gyrus Left Insula Left Superior Temporal Gyrus Right Cingulate Gyrus
PTSD	Combat Words	Left Middle Frontal Gyrus
PTSD with Hazardous Alcohol Use	Combat Words	Right Anterior Cingulate Gyrus Right Cingulate Gyrus Left Cingulate Gyrus Left Lingual Gyrus Left Precentral Gyrus Left Postcentral Gyrus Left Insula Right Middle Temporal Gyrus Right Middle Frontal Gyrus Left Inferior Temporal Lobule Right Claustrum



Anterior Cingulate Gyrus



Claustrum

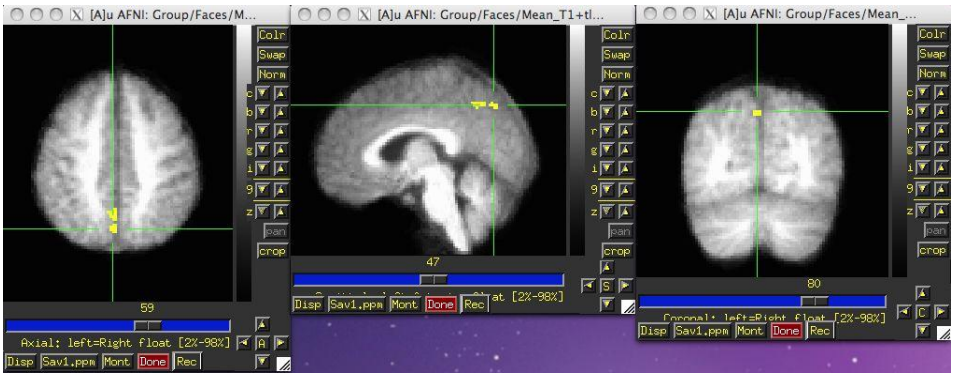
Figure 5: Preliminary analysis (n = 8) of fMRI data collected during the combat word condition in the emotional counting stroop task from participants in the PTSD with hazardous alcohol use group. These participants show increased activation of the anterior cingulate and claustrum when viewing combat-related words.

Furthermore, subject numbers allowed contrasts between-groups for PTSD vs. No PTSD and PTSD vs. PTSD with hazardous alcohol use for each of the task conditions. These contrasts were performed with a threshold of 0.01 and 20 voxel clusters and are thus quite preliminary. The results are summarized in Tables 6-7 and illustrated by Figures 6-8. Of relevance to the planned comparisons when the full data set is available, we observed regional differences in precuneus and cuneus areas as well as the middle temporal gyrus when PTSD groups were contrasted with the PTSD and hazardous alcohol group in the fearful-happy faces contrast condition of the masked faces paradigm (Table 6, Figure 6). Furthermore, changes in activity of the postcentral gyrus and middle temporal gyrus were noted when PTSD groups were contrasted with no-PTSD groups, and in the precentral gyrus when PTSD groups were contrasted with the PTSD and hazardous alcohol group in the combat word condition of the emotional counting stroop task (Table 7, Figures 7-8). Changes in these regions within PTSD populations during the combat word condition of the counting emotional stroop task have been noted previously (Shin et al., 2001), with the current data adding the variable of hazardous alcohol use. Work towards this task will continue in year three of this award, with greater subject numbers allowing full within-group and between-group contrasts for each task condition in the future.

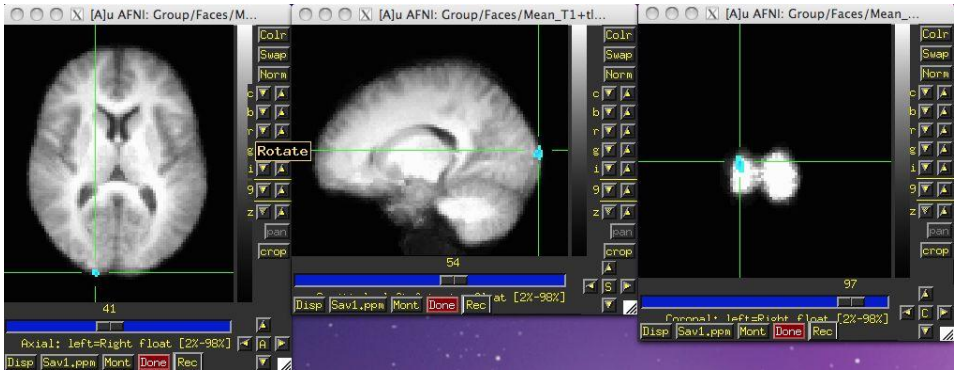
Table 6: Differences in BOLD Signal Between Experimental Groups during the Masked Faces Paradigm.

Group Contrast	Task Condition	Region
PTSD - No PTSD	Happy Faces	Right Insula Right Cingulate Gyrus Left Posterior Cingulate
PTSD – PTSD & Hazardous Alcohol	Happy Faces	Right Putamen Right Cingulate Gyrus Left Posterior Cingulate
PTSD – No PTSD	Fearful Faces	Right Postcentral Gyrus Right Cingulate Gyrus
PTSD – PTSD & Hazardous Alcohol	Fearful Faces	Right Precentral Gyrus

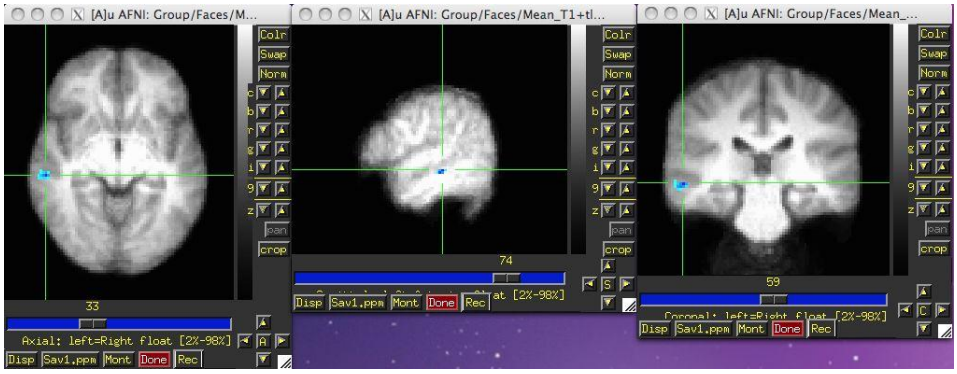
		Right Postcentral Gyrus Right Cingulate Gyrus Left Cingulate Gyrus Right Anterior Cingulate Right Insula Right Inferior Frontal Gyrus Right Precuneus Left Precuneus
PTSD - No PTSD	Fearful – Happy Faces	Right Middle Temporal Gyrus
PTSD – PTSD & Hazardous Alcohol	Fearful – Happy Faces	Right Precuneus Right Cuneus Left Cuneus Right Middle Temporal Gyrus



Precuneus



Cuneus

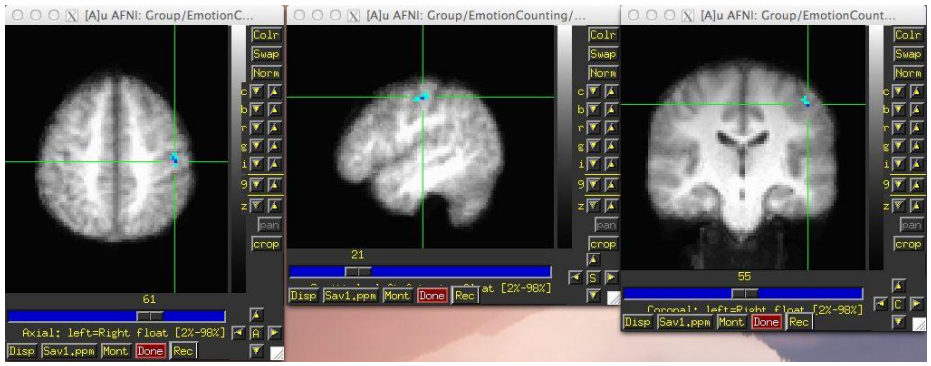


Middle Temporal Gyrus

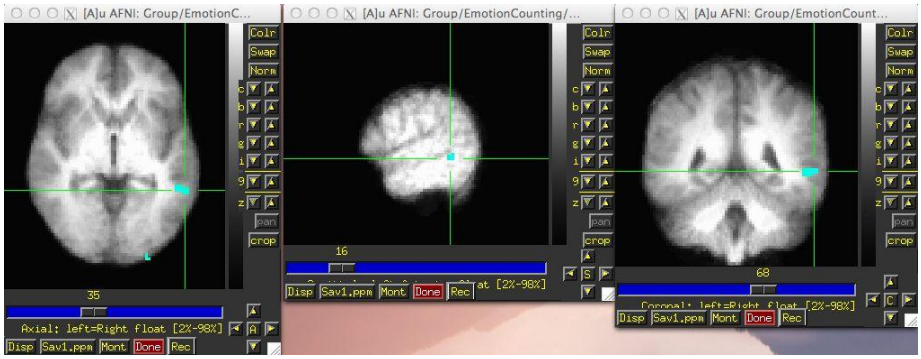
Figure 6: Preliminary analysis (n = 12) of fMRI data collected during the masked faces paradigm from participants in the PTSD vs. PTSD with hazardous alcohol use groups. Data represents fearful vs. happy faces conditions. Participants with PTSD show increased activation of the precuneus and reduced activation of the cuneus and middle temporal gyrus as compared to participants with PTSD and hazardous alcohol use when activity elicited by masked fearful faces is contrasted with masked happy faces.

Table 7: Differences in BOLD Signal Between Experimental Groups during the Emotional Counting Stroop Task.

Group Contrast	Task Condition	Region
PTSD - No PTSD	General Negative Words	Right Precentral Gyrus
PTSD – PTSD & Hazardous Alcohol	General Negative Words	Right Precentral Gyrus
PTSD – No PTSD	Combat Words	Left Postcentral Gyrus Left Middle Temporal Gyrus
PTSD – PTSD & Hazardous Alcohol	Combat Words	Right Precentral Gyrus

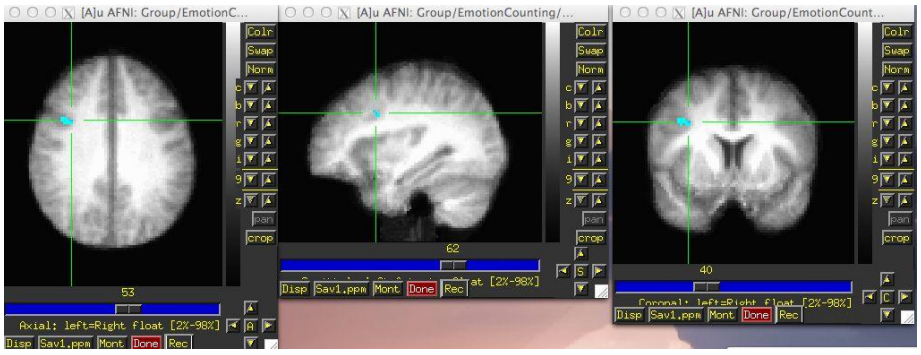


Postcentral Gyrus



Middle Temporal Gyrus

Figure 7: Preliminary analysis (n = 20) of fMRI data collected during the combat word condition in the emotional counting stroop task from participants in the PTSD vs. no-PTSD groups. Participants with PTSD show decreased activation of the postcentral gyrus and middle temporal gyrus as compared to participants without PTSD when viewing combat-related words.



Precentral Gyrus

Figure 8: Preliminary analysis (n = 12) of fMRI data collected during the combat word condition in the emotional counting stroop task from participants in the PTSD vs. PTSD with hazardous alcohol use groups. Participants with PTSD show decreased activation of the precentral gyrus as compared to participants with PTSD and hazardous alcohol use when viewing combat-related words.

Key Research Accomplishments:

In relation to the objectives and tasks in Table 1, key accomplishments for Year 2 were:

- Obtaining IRB approval and setting up the infrastructure for fMRI research with veterans – the first of its kind in South Dakota.
- Recruitment of mid-west Iraq/Afghanistan war veterans to participate in a study assessing the relationship between alcohol use, PTSD symptoms and neural processing of emotions.
- Preliminary analysis validating that the emotional stimuli used in the fMRI tasks elicit arousal responses and expected neural activity in non-veteran and veteran participants.

Reportable Outcomes:

- Review Article in Press (see Appendix):
  Forster GL, Novick AM, Scholl JL, Watt MJ. (2012). The role of the amgydala in anxiety disorders. In B. Ferry (Ed.), *Amygdala*. Intech (in press).

- Conference Presentation of Preliminary Data:
  Olson, D., Hansen, J., Baugh, L., Gaher, R., Simons, J, Magnotta, V and Forster, G. (2012). Neural Imaging: The Effects of Alcohol Use on Emotional Processing in Posttraumatic Stress Disorder (PTSD). Presented at the *University of South Dakota Health Affairs Research Symposium,* Vermillion, SD, April 2012.

- Conference Presentation of Preliminary Data:
  Olson, D., Hansen, J., Baugh, L., Gaher, R., Simons, J., Magnotta, V. and Forster, G. (2012). Neural Imaging: The Effects of Alcohol Use on Emotional Processing in Posttraumatic Stress Disorder (PTSD). Presented at the *Sanford School of Medicine Research Symposium*, Vermillion SD, May 2012.

Conclusions:

Hazardous use of alcohol negatively impacts the treatment of PTSD. However, the mechanisms that underlie the association between PTSD and hazardous alcohol use in veterans are poorly understood. The current research takes a multi-level approach to study the psychological, behavioral, cognitive and neural relationships between PTSD and alcohol use. Progress to date has been focused on building the infrastructure to conduct neural imaging by training of personnel, setting up recruitment of veteran populations, and conducting test runs to ensure the recently purchased fMRI equipment and experimental procedures were suitable to test the study hypotheses with veteran participants. The ability to now conduct functional brain imaging at the University of South Dakota represents the first and only opportunity in the State of South Dakota to perform such research, and complements the strong emphasis and infrastructure already in place to conduct psychological, behavioral and cognitive assessment of South Dakota's underserved rural veteran population. Preliminary data collected in the current reporting period suggests that emotional stimuli used in the fMRI tasks elicit arousal responses and expected neural activity in non-veteran and veteran participants, thus validating the paradigms for testing the behavioral and neural association between PTSD and hazardous alcohol use. Research in the next funding period will build on recruitment efforts to increase subject numbers, thus allowing full analysis of the psychological, behavioral, cognitive and neural data to identify behavioral and neural predictors of poor psychological outcomes related to PTSD and hazardous alcohol use in South Dakota veterans. Overall, the current funding has built research infrastructure and tools to allow comprehensive studies regarding mental health and neurological issues specific to veterans in rural areas.

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Appendix:

Forster GL, Novick AM, Scholl JL, Watt MJ. (2012). The role of the amgydala in anxiety disorders. In B. Ferry (Ed.), *Amygdala*. Intech (in press).

The Role of the Amygdala in Anxiety Disorders

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Additional information is available at the end of the chapter

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1. Introduction

1.1. Defining anxiety and fear

Anxiety is a term often used to encompass feelings of apprehension, dread, unease or similarly unpleasant emotions. Trait anxiety defines the affect of an organism over time and across situations, whereas state anxiety is the response or adaptation to a given situation [1]. Anxiety can be differentiated from fear, both biologically and behaviorally [see 1 for an extensive review]. Converging theories and evidence from clinical psychology and comparative neuroscience suggest that fear can be considered a negatively-valenced emotion that is brief, focused on the present, occurs in situations of specific threat, and aids in avoidance or escape [1,2]. Anxiety, on the other hand, is a negatively-valenced emotion that is characterized by sustained hyperarousal in response to uncertainty, is thus future-focused, and aids in defensive approach or risk assessment [1,2]. Both anxiety and fear are emotions experienced by all individuals and can serve to be adaptive in shaping decisions and behaviors related to survival of an organism [1,3]. However, when excessive, or pathological, or triggered inappropriately, fear and anxiety form the basis of a variety of anxiety disorders [3,4,5; Table 1]. As illustrated by Table 1, some anxiety disorders such as generalized anxiety disorder (GAD) or obsessive-compulsive disorder (OCD) are characterized by excessive anxiety as defined above [1]. However, other anxiety disorders are characterized, at least in part, by excessive and inappropriate fear, such as posttraumatic stress disorder (PTSD), specific phobias and social anxiety disorder [1,3; Table 1]. Thus, it is important to understand the neurobiology of both anxiety and fear to obtain a comprehensive picture of the physiological basis of anxiety disorders.

1.2. Anxiety disorders

One in three people will develop one of the anxiety disorders outlined by Table 1 within their life-time, with the life-time prevalence at least two times more likely for women [5,6].

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Furthermore, individuals may present with one or more comorbid anxiety disorders, and anxiety disorders are highly likely to be comorbid with other psychiatric illnesses, such as major depressive disorder, psychosis, mania, and substance abuse disorder [4-6]. Several non-psychiatric disorders are also associated with anxiety disorders, and these include hyperthyroidism, Cushing's disease and mitral value prolapse [4,5]. Thus, anxiety disorders are one of the most prevalent psychiatric disorders, posing great personal, economic, and societal burdens [4-6].

Generalized Anxiety Disorder (GAD)
Excessive worry occurring more days than not over at least a 6 month period, accompanied by restlessness, fatigue, sleep disturbances, muscle tension or irritability.
Posttraumatic Stress Disorder (PTSD)
Characterized by a history of trauma and symptoms related to avoidance, re-experiencing, and physiological hyperarousal in the face of triggering cue.
Obsessive-Compulsive Disorder (OCD)
Compulsions (repeated actions) produced to reduce anxiety associated with obsessions (unwanted, intrusive thoughts).
Panic Disorder
Characterized by panic attacks; a period of intense fear or discomfort accompanied by a variety of physiological symptoms (e.g. sweating, trembling, chest pains, tachycardia).
Agoraphobia
Fear and avoidance of situations from which escape would be difficult in the event of having panic-like symptoms.
Specific Phobia
Excessive or unreasonable fear in anticipation or in response to a specific object or situation.
Social Anxiety Disorder (Social Phobia)
Excessive/unreasonable fear and avoidance of social situations (including performances) in which the person is exposed to unfamiliar people or possible scrutiny by others.

Table 1. Major Classes of Anxiety Disorders [4,5,7]

1.3. Goals of the current review

The neurobiological bases of anxiety and fear appear to be very similar across species [1], thus complementary findings from both animal models (most often rodents) and human studies can contribute to theories of the neurobiological basis of anxiety disorders. State fear within animal models is most often studied by measures of freezing and fear-potentiated startle, both acquired via classical conditioning of rodents [1,8]. State anxiety, on the other hand, is most often studied using apparatus such as an open field, elevated plus maze, or light-dark box, which all take advantage of the rodent's preference for familiar, dark, and/or enclosed areas [1,9]. Notably, these paradigms do not rely on the processes underlying classical conditioning, although McNaughton and Corr [2] caution against defining fear verses anxiety as conditioned versus unconditioned responses. While trait fear is not well-

defined by animal studies [1], trait anxiety is often examined in animal models by the use of selective breeding, resulting in high- and low-anxiety strains and lines of rodents [for example, see 1, 10]. However, one can argue that experimental manipulations (such as early-life stress or amphetamine withdrawal) that drive a group of animals towards greater fear- and anxiety-like phenotypes also examine the underlying basis of trait fear or anxiety [e.g. 11, 12]. As noted by Sylver et al [1] clinical studies most often examine trait anxiety, whereas experiments involving animal models most often focus on state anxiety and fear, and then relate these findings to concepts associated with trait anxiety. Regardless, both human and animal studies suggest an important role for the amygdala, and subregions within, in mediating fear and anxiety, and in the manifestation of anxiety disorders (Sections 2 and 3). Therefore, the goals of this review are to first evaluate and integrate classical and recent findings from human studies and relevant animal models that reveal the specific role the amygdala plays in fear and anxiety, and then to elucidate how anxiolytic drugs may affect the amygdala function to ameliorate heightened fear and/or anxiety. This is important, given that traditional drug and cognitive behavioral therapy (CBT) are effective in reducing symptoms of the various anxiety disorders for many individuals, but often do not provide long-term relief, and relapse is a common post-treatment outcome [as reviewed by 3]. Therefore, the final goal of the current review is to identify future potential therapeutic targets for the treatment of anxiety disorders.

2. Human imaging studies: Amygdala hyperfunction and anxiety disorders

2.1. Amygdala reactivity and anxiogenic or fearful stimuli

Human imaging studies that explore the neurobiological bases of anxiety or fear processing typically use functional magnetic resonance imaging (fMRI) or positron emission tomography (PET) as measures of neural activity or cerebral blood flow. Imaging experiments that are designed to study neural reactivity to fearful stimuli utilize either conditioned fear paradigms similar to those used in animal models, or involve the presentation of unconditioned stimuli such as fearful faces [1]. It has become clear that masked stimuli can elicit conditioned and unconditioned fear responses from human subjects, suggesting unconscious, implicit processing of these cues [as reviewed by 1]. Similarly, increased activity of the amygdala is observed in response to both conditioned and unconditioned fearful stimuli, independent of whether the subject is aware of the stimulus [1,13-16].

Comparable studies that have examined neural correlates of anxiety in healthy controls are limited. One of the reasons for this is that many studies use fearful stimuli, such as the fearful faces or conditioned fear paradigms [1], blurring the distinction between fear and anxiety. Therefore, conclusions regarding neural bases of anxiety are better drawn from studies that include trait anxiety as a variable while utilizing fearful stimuli, or those fewer studies in which an anxiogenic situation is created within the experimental design. Like for studies of fear processing, the majority of these studies show a relationship between trait anxiety and greater amygdala reactivity [as reviewed by 17]. For example, a study of healthy

subjects found that reactivity of the amygdala was positively correlated with anticipatory anxiety, and when the anticipated event was imminent, amygdala activation positively correlated with the degree of trait anxiety [18]. Furthermore, college students who scored in the upper 15th percentile for trait anxiety show greater amygdala reactivity to emotional faces as compared to students who scored in the normative range, suggesting that anxiety-prone individuals have greater amygdala reactivity [19]. A similar hyperactivity of the amygdala in high trait anxiety participants is noted when a masked emotional faces or unattended faces paradigm are used [20,21], suggesting the individual does not need to be aware of the stimulus to exhibit heightened amygdala activity. Interestingly, Etkin et al., [21] differentiate between different subregions of the amygdala (see Section 3.1 for more details on amygdala subregions), with the basolateral amygdala activated during masked presentations of emotional faces while the dorsal/central amygdala was activated during unmasked presentations. Thus, there may be subregion specificity within the amygdala when processing unconscious versus conscious emotionally-valenced stimuli.

When gender has been examined as a factor in populations of healthy subjects, higher trait anxiety is associated with greater amygdala responses to unattended fearful faces in female but not male participants [22]. A further factor potentially mediating the relationship between trait anxiety and amygdala reactivity appears to be perceived social support. To illustrate, Hyde et al. [17] show a positive correlation between the degree of trait anxiety and amygdala reactivity to fearful faces in subjects that report below-average social support, but not in those who report above average support. Related, it is also thought that the degree of social anxiety rather than trait anxiety may be more closely related to amygdala reactivity to emotional faces [23]. These factors, and other similar considerations, may explain why some, but not all, studies show a positive correlation between trait anxiety and amygdala reactivity in non-patient populations [18-21,23].

2.2. Amygdala reactivity in anxiety disorders

Hyperactivity of the amygdala in response to negatively-valenced stimuli also appears to be a common finding from a variety of clinical anxiety populations [16]. For example, individuals suffering from social anxiety disorder show heightened amygdala responses to both social and non-social highly emotive stimuli as compared to healthy control groups, with the degree of social anxiety positively correlated with amygdala reactivity [24-27]. Furthermore, activation of the amygdala by non-social stimuli has been correlated with trait anxiety in social anxiety disorder, leading to the conclusion that social anxiety disorder is characterized by a more general dysfunction in emotional processing in addition to altered processing of social stimuli and situations [26]. Importantly, reduced symptoms in a public speaking situation following either CBT or antidepressant treatment was associated with reduced amygdala reactivity [24], further suggesting a tight link between symptomology and amygdala reactivity in social anxiety disorder.

Like social anxiety disorder, a commonly replicated finding from various PTSD populations is hyperactivity of the amygdala in response to masked fearful faces or trauma-related

stimuli [3,28,29]. This manifests as higher amygdala reactivity as compared to non-PTSD groups and/or a positive correlation between severity of PTSD symptoms and amygdala reactivity [28,30-33]. Furthermore, in a group of unmedicated acute PTSD subjects (1 month post trauma), the degree of PTSD symptoms also positively correlated with activity of the amygdala in response to masked fearful faces [34]. Thus, amygdala hyperactivity observed in chronic PTSD appears early in the disorder. However, it should be noted that in these same individuals, the degree of PTSD symptoms negatively correlated with activity in the amygdala in response to unmasked fearful faces [34]. This suggests amygdala hypoactivity in response to consciously-processed fearful stimuli in the early stages of PTSD, further implying a dissociation in amygdala activity in response to consciously-processed versus unconsciously-processed fearful stimuli. Interestingly, activity of the amygdala in response to fearful stimuli might not only be characteristic of PTSD, but might predict treatment outcome. Bryant et al [33] show that individuals diagnosed with PTSD that do not respond to CBT (8 one weekly sessions) show significantly greater pre-treatment amygdala activation in response to masked fearful faces as compared to those PTSD subjects who did respond to CBT, as defined by a 50% or more reduction in scores on the Clinician-Administered PTSD Scale (CAPS). Therefore, hyper-function of the amygdala might provide a useful tool for future selections of treatment options for PTSD.

Similar to PTSD and social anxiety disorder, amygdala hyperactivity as a result of highly emotional stimuli presentation or symptom provocation has been observed in specific phobia, panic disorder, and OCD [35-38]. Given the prevalence of GAD, it is surprising that few studies have assessed amygdala reactivity in GAD participants. Somewhat more surprising is that of those studies that have determined amygdala activity in response to emotive stimuli in adult GAD populations, a lack of amygdala hyperactivity has been observed [27,39,40]. This stands in contrast to findings from pediatric GAD, where hyperactivity of the amygdala is apparent in response to emotional stimuli and positively correlated with symptom severity [41,42]. However, recent findings examining amygdala function within paradigms that elicit anticipatory anxiety or emotional conflict have implicated a role for amygdala hyper-reactivity in adult GAD populations. For example, Nitschke et al. [43] report greater anticipatory amygdala activation in response to both emotional and neutral images in adult GAD subjects. Furthermore, Etkin et al [44] found that adult participants with GAD exhibited poor performance on a task that involved emotional conflict (incongruent visual emotional stimuli), accompanied by a failure of the frontal cortex to exert negative top-down control of amygdala activity (see Section 3.1 for more on top-down control of the amygdala). Therefore, amygdala hypofunction in adult GAD might be better revealed by imaging studies that create anxiogenic or conflict situations, rather than the standard presentation of fearful stimuli. While this conclusion requires direct testing, the findings that anxiogenic but not fearful stimuli reveal hypofunction of the amygdala in GAD, whereas fearful stimuli consistently elicit amygdala hyper-reactivity in other anxiety disorders (such as social anxiety disorder, PTSD and also pediatric GAD), suggests a neural dichotomy between GAD and other anxiety disorders on the anxiety to fear continuum.

In summary, there appears to be reasonable overlap across various experimental paradigms and study populations to conclude that the amygdala is reactive to fearful stimuli and anxiogenic situations, and exhibits hyper-function to emotive stimuli, anxiogenic situations and/or symptom provocation in anxiety disorders. However, which neurotransmitters and subregions of the amygdala mediate these responses if often better answered by animal studies, where spatial and neurochemical resolution is greatly improved over human imaging studies.

3. Amygdala subregions, connectivity, neurotransmission and fear/anxiety

3.1. The role of amygdala subregions in mediating fear and anxiety

As discussed above, hyper-function of the amygdala appears to be a key component of human anxiety disorders. However, the contribution of particular amygdalar subregions in the development and maintenance of this hyperactive state in humans is still being established. Only very recently have refinements in the acquisition and analysis of fMRI data allowed subregion function to be segregated effectively during emotional tasks such as avoidance learning [45] and facial expression recognition [21,46]. Similarly, effective structural identification of human amygdalar subregions and assessment of their functional connectivity using imaging techniques is still fairly new [for example, see 47-51]. Therefore, most of our understanding of causal neurochemical pathways in amygdalar circuitry related to fear and anxiety has derived from extensive studies using rodent and non-human primate models [for example, see 9,52-58].

Anatomical arrangement of the mammalian amygdala appears to have been evolutionarily conserved, with particular subregions being connected to homologous brain structures across species [as reviewed by 59]. The lateral (LA) nucleus of the amygdala is reciprocally connected with the auditory, somatosensory and visual sensory association centers in the temporal and insular cortices [59], and in rats also receives further auditory information via projections from the posterior thalamus [59,60]. The medial amygdala (MeA) is reciprocally connected with the accessory olfactory bulb and many hypothalamic and preoptic nuclei [59,61], creating a locus for assimilation of olfactory stimuli and information regarding internal hormonal state [62,63]. Information summated within the LA and MeA is then conveyed to the adjacent basal (B) and accessory basal (AB) nuclei [64], which also receive projections from the CA1 and subiculum areas of the ventral hippocampus [65-67]. The B/AB nuclei send excitatory and inhibitory projections back to the LA and MeA [64,68], creating a localized circuit that may assist in fine-tuning the filtering of sensory input into these regions [64]. Excitatory projections from this basolateral (BLA) complex target the central nucleus of the amygdala (CeA) either directly or via a series of GABAergic interneurons known as intercalated (ITC) cells located between the BLA and CeA [69], providing an effective means of gating CeA activity and output through a combination of direct excitation and feed-forward inhibition [64,70,71]. The CeA itself, principally the medial sector, sends GABAergic projections to brainstem, hypothalamic and basal forebrain regions

that control expression of autonomic, hormonal and behavioral responses to emotive situations 72,73]. It should also be noted that in addition to activating the CeA, the BLA projects to the adjacent bed nucleus of the stria terminalis (BNST), which in turn targets many of the same regions as the CeA to produce similar behavioral and physiological responses [73]. The MeA is also able to regulate these responses not only via its influence on hypothalamic nuclei and brainstem targets, but by modulating activity in the BNST and CeA [61,64].

The functional connectivity between the BLA, MeA and CeA ensures that sensory and contextual information associated with emotional situations, such as fearful or anxiogenic circumstances, is channeled to effector regions to produce appropriate responses necessary for survival. The BLA and CeA, unlike the MeA, do not appear necessary for expression of unconditioned fear responses to olfactory stimuli in rodents, e.g., to novel presentation of predator odor [74-76], although the BLA does appear to play a role in responses to other types of unconditioned stimuli [77,78]. However, the functional arrangement of the BLA and CeA with other regions facilitates learning about the situation, such that appropriate reactions are maintained if cues associated with initial exposure are experienced again. The BLA in particular appears to play a crucial role in encoding positive or negative salience to relevant stimuli for future reference, as indicated by numerous studies showing that the BLA is required for fear learning and acquisition of conditioned fear responses [see 56,60]. Once fear conditioning is acquired, the CeA is necessary for expression of the conditioned response [56,60], the magnitude of which will be influenced by BLA gating of CeA activity and output. Similarly, the BLA is needed for acquisition and expression of fear extinction [79,80], which requires a subject to learn that expression of a previously conditioned fear response is no longer necessary when the conditioned stimulus no longer predicts an aversive event [57,81]. To achieve this, the BLA must integrate new sensory information (absence of the unconditioned aversive stimulus) that will result in a dampening of CeA excitation. This may result from increased BLA excitation of ITC cells during fear extinction acquisition to enhance feed-forward inhibition of the CeA [79,82,83], followed by structural remodeling within the BLA during consolidation of the extinction memory to inhibit later BLA output [79]. However, while the roles of the BLA and CeA in fear behaviors are well established, their contribution to anxiety is less clear, especially for the CeA. Animal studies suggest that changes in BLA and CeA activity can alter state anxiety [9; also see Section 3.2.]. However, most investigations have focused on the BLA with the exact role of the CeA remaining ill-defined [for example, see 84,85], although it appears that BLA to CeA circuitry can directly regulate anxiety-like behavior as measured on the elevated plus maze [EPM, 86]. This direct control is thought to result from BLA excitation of GABAergic neurons in the lateral CeA to induce feed-forward inhibition of output from the medial CeA [86], similar to that induced by BLA excitation of ITC cells during fear extinction. Thus, suppression of CeA output may be equally important for mediating expression of both fear and anxiety. Alternatively, some studies have suggested that it is BLA activation of the BNST, not of the CeA, that is responsible for mediating anxiety-like behavior as measured using light-potentiated startle responses in rodents [56,87,88]. Startle responses are also potentiated by corticotropin releasing factor (CRF) infused into the BNST [56]. This effect is presumed to result through facilitation of glutamate release from BLA afferents by CRF neurons that

originate in the lateral CeA [88,89], implying that even if BNST is the principal output center for certain types of anxiety-like behaviors, the CeA may still play some modulatory role. Furthermore, the MeA has been strongly implicated in animal models of state anxiety [for example, see 90-93 and see Section 3.2], but whether its effects involve modulation of CeA activity is unknown. To direct translational research into the neurological underpinning of anxiety disorders more effectively, animal studies employing as wide a range of state anxiety paradigms as possible, along with animal models that generate trait anxiety, are required to establish the exact nature of CeA involvement and of amygdala subregion interplay in mediating anxiety-like behavior.

It is important to remember that while the amygdala can mediate fear and anxiety-like behavior, other brain regions play a major role in expression of these states, presumably by influencing activity in particular amygdalar subregions to alter the balance of output from the CeA. For example, input from the ventral hippocampus to the B/AB nuclei within the BLA is required for expression of conditioned fear responses to contextual cues in rodents and humans [60,94,95], and so receipt of this information presumably increases BLA activity, to in turn enhance CeA output in the aversive context. In rodents, the ventromedial prefrontal cortex (vmPFC) also appears to be crucial in regulating amygdalar activity, especially during fearful experiences [79]. The prelimbic (PL) subregion of the vmPFC can enhance conditioned fear expression via excitatory projections to the BLA and CeA [96-98]. In contrast, expression of conditioned fear appears to be decreased by activation of the infralimbic (IL) subregion of the vmPFC [99, but see 100]. The IL cortex is also required for effective consolidation and recall of fear extinction memories [79,98]. Both decreased conditioned fear responding and fear extinction require suppression of CeA output, which is thought to result in part via IL cortex stimulation of the series of inhibitory ITC cells that project to the CeA [71,79,96,101]. The bidirectional roles of the PL and IL cortices in regulating conditioned fear through opposing influences on CeA activity and output imply that imbalance in the influence of either cortical structure could contribute to amygdala hyperactivity seen in anxiety disorders characterized by excessive and inappropriate fear (see Table 1). This is supported by fMRI studies investigating neural correlates of impaired fear extinction in PTSD patients, who compared to healthy subjects show hyperactivity of the amygdala during extinction learning [102]. This enhanced amygdala function in PTSD patients is accompanied by greater activation of the dorsal anterior cingulate cortex (dACC, functionally equivalent to the rodent PL cortex, [3,57], which is also present during recall of the extinction memory [102]. This is in line with rodent studies demonstrating potentiated fear conditioning upon PL cortex activation [98]. However, PTSD individuals exhibit hypoactivation of the ventral portion of the vmPFC (equivalent to rodent IL cortex, [3,57]) during extinction learning and recall [102,103]. Human imaging studies also suggest that impaired regulation of amygdala activity by the ventral vmPFC may contribute to anxiety disorders characterized by hypervigilance in the absence of conditioned stimuli, such as in GAD. Specifically, the strength of the connection between the vmPFC and the amygdala, as measured using diffusion tensor imaging, predicts levels of self-reported trait anxiety, such that weaker connections are seen in more anxious individuals [104]. As mentioned earlier (Section 2.2), participants with GAD exhibited a failure of the vmPFC to exert negative top-

down control of amygdala activity during a task that involved emotional conflict [44]. Further, resting state fMRI revealed that in anxious individuals, vmPFC activity was negatively correlated with amygdala activity, while a positive relationship was observed for low anxious subjects [105]. The combination of animal and human studies strongly indicates that inadequate suppression by the ventral portion of the vmPFC, most likely of the CeA, is a key factor in amygdala hyperactivity underlying the emergence of excessive fear and anxiety states.

3.2. Monoaminergic neurotransmission in the amygdala: Relation to fear and anxiety

The monoamine neurotransmitters (serotonin, dopamine and norepinephrine) have long been associated with fear and anxiety, and drugs that alter monoaminergic function are often effective across the range of anxiety disorders [8, 9, 52, 55]. Animal studies suggest a variety of anxiogenic stressors or fearful stimuli increase monoamine levels in the amygdala. To illustrate, increased serotonin (5-HT) release or increased activity of 5-HT neurons in the amygdala have been observed in response to restraint or footshock, or in association with expression of conditioned fear behavior [106-110]. Similarly, dopamine (DA) and norepinephrine (NE) levels in the amygdala are increased following restraint, handling stress, footshock or during the expression of conditioned fear behavior [107,111-118]. The source of monoamines to the amygdala arise from monoaminergic cell body regions in the brainstem. Specifically, the dorsal raphe nucleus (dRN) provides 5-HT innervation to the amygdala, while NE and DA innervation of the amygdala arise from the locus coeruleus (LC) and ventral tegmental area (VTA) respectively [55,119,120]. Regulation of monoaminergic activity in the amygdala thus can occur at the level of these brainstem cell body regions, or within the terminal regions of the amygdala.

One of the important mediators of amygdala monoaminergic activity in response to anxiogenic or fearful stimuli is CRF. A strong body of evidence implicates central CRF in mediating fear and anxiety [12,121-128], and recent clinical studies suggest an important role for CRF in anxiety disorders [129]. Like anxiogenic and fearful stimuli, central infusion of CRF or CRF receptor agonists increases 5-HT, NE and DA levels in the amygdala [130-133], and stress-induced increases in monoamine levels in the amygdala are prevented by CRF receptor antagonists [108,111]. It is thought that CRF regulation of monoaminergic activity in the amygdala occurs at the level of the monoaminergic cell bodies. The monoaminergic cell body regions receive CRF innervation from the CeA and BNST, and CRF type 1 and 2 (CRF_1 and CRF_2) receptors are localized to the dRN, LC and VTA [134-140]. Direct infusion of CRF or CRF receptor agonists into the dRN stimulates 5-HT release in the CeA or BLA [131-133]. Interestingly, CRF-induced 5-HT release in the amygdala appears to be dependent on CRF_2 receptor activation in the dRN [131,133], and CRF_2 receptors are known to increase 5-HT neuronal firing rates in the dRN [141]. Importantly, increased neuronal surface expression of CRF_2 receptors occurs in the dRN as a result of stress [142], and increased expression of CRF_2 receptors in the dRN has been observed in rat models of high anxiety [11,128,137,143]. Furthermore, CRF_2 receptor antagonists infused

directly into the dRN reduce heightened anxiety-like behavior in rat models of amphetamine withdrawal or early life stress [12,128]. Combined, these findings suggest that CRF_2 receptor modulation of 5-HT activity in the amygdala may play an important role in heightened anxiety. While similar studies have not been performed to elucidate the role of CRF receptors in the LC and VTA in mediating NE and DA activity in the amygdala and anxiety states, some indirect evidence suggests an important role for CRF receptors in the LC and VTA stress responses [136,138,144]. Overall, it is clear that further investigations are needed to ascertain the role of CRF receptors in mediating NE and DA activity in the amygdala and how CRF modulation of this activity could relate to fear or anxiety.

Studies demonstrating increased monoamine activity in the amygdala in response to anxiogenic or fearful stimuli, and CRF modulation of these responses (as described above) do not allow conclusions to be made about the specific role of each monoamine in mediating anxiety or fear. Direct manipulation of monoaminergic activity within the amygdala or specific amygdala subregions, and the measurement of resultant anxiety-like or fear-related behaviors, have gone some way to providing a picture of how monoamine function in the amygdala might translate to anxiety or fear. Table 2 summarizes such studies directly manipulating 5-HT levels or 5-HT receptor activity in the amygdala. When 5-HT or 5-HT activity is decreased in the entire amygdala [145,146], a consistent increase in anxiety-like behavior is observed (Table 2). This would suggest that increased 5-HT activity in the amygdala would thus be associated with decreased anxiety, implying an anxiolytic role of 5-HT. However, this does not appear to be supported by experiments that directly manipulate 5-HT receptor activity in the amygdala with 5-HT receptor ligands (Table 2). For example, activation of postsynaptic excitatory $5\text{-}HT_2$ or $5\text{-}HT_3$ receptors in the amygdala decreases social interaction and increases anxiety-like behavior, whereas antagonism of $5\text{-}HT_3$ receptors in particular increases social interaction and decreases anxiety-like behaviors, suggesting that 5-HT actions on postsynaptic receptors is anxiogenic (Table 2), although, see [147] for an exception to this pattern. Similarly, activation of excitatory $5HT_2$ receptors in the BLA generally increases anxiety-like behavior (Table 2), suggesting an anxiogenic role for postsynaptic 5-HT receptors in the BLA (although an exception to this is observed, [148]). In contrast, inhibitors of $5\text{-}HT_2$ receptors in the MeA increase anxiety-like behavior while activation of these receptors increases social interaction and decreases anxiety behavior (Table 2). Thus like the some findings from the amygdala as a whole (Table 2), 5-HT activity in the MeA appears to play an anxiolytic role. The role of 5-HT or 5-HT receptors has not been well studied in the CeA. However, rats undergoing amphetamine withdrawal that exhibit greater anxiety-like behavior have greater 5-HT release in the CeA [12,133], suggesting a similar anxiogenic relationship between 5-HT and anxiety as for the BLA. Future work should determine whether 5-HT in the CeA reduces anxiety-like behaviors as is suggestive for the MeA, or in contrast, increases anxiety-like behaviors as appears to be the case for the BLA. Overall, the findings summarized in Table 2 suggest a dichotomy in the potential role of 5-HT in the amygdala in mediating anxiety depending on whether the entire amygdala or a specific subregion is targeted. Potential confounds in comparing the studies listed in Table 2 could be the different paradigms used to measure anxiety-like

behaviors and the relative selectivity of 5-HT receptor ligands across different experiments. Future studies directly comparing the effects of 5-HT manipulations within the different amygdala subregions across several well-validated tests of anxiety-like behaviors will better elucidate the role of amygdala 5-HT in mediating anxiety.

Amygdala Subregion	Monoamine or Receptor Involvement	Behavioral Outcome	Citation
Anxiety-like Behavior			
Amygdala	Decreased 5-HT (induced by MDMA)	Increased anxiety behavior	Faria et al. [145]
Amygdala	Decreased 5-HIAA (induced by stress)	Increased anxiety behavior	Niwa et al. [146]
Amygdala	5-HT_{1A} agonist	No change in anxiety behavior	Zangrossi and Graeff [149]
Amygdala	5-$HT_{2B/2C}$ agonist	Increased anxiety behavior	Cornelio and Nunes-De-Souza [150]
Amygdala	5-HT_3 agonist	Decreased social interaction	Higgans et al. [151]
Amygdala	5-HT_3 agonist	Decreased anxiety behavior	Costall et al. [147]
Amygdala	5-HT_3 antagonist	Increased social interaction	Higgans et al. [151]
Amygdala	5-HT_3 antagonist	Decreased anxiety behavior	Costall et al. [147]
Amygdala	5-HT_3 antagonist	Decreased anxiety behavior	Tomkins et al. [152]
BLA	5-HT_{1A} agonist	Decreased social interaction	Gonzalez et al. [153]
BLA	5-HT_{1A} agonist	No change in anxiety behavior	Gonzalez et al. [153]
BLA	5-HT_{2A} agonist	Increased anxiety behavior	Zangrossi and Graeff [149]
BLA	5-$HT_{2A/2C}$ agonist	No change in anxiety behavior	Cruz et al [148]
BLA	5-HT_{2C} agonist	Increased anxiety behavior	Vincente et al. [154]
MeA	5-HT_{2A} antagonist	Increased anxiety behavior	Zangrossi and Graeff [149]
MeA	5-HT_2 agonist	No change in anxiety behavior	Duxon et al. [155]
MeA	5-HT_{2B} agonist	Increased social interaction	Duxon et al. [156]
MeA	5-HT_{2B} agonist	Decreased anxiety behavior	Duxon et al. [155]

Amygdala Subregion	Monoamine or Receptor Involvement	Behavioral Outcome	Citation
MeA	5-HT$_{2B/2C}$ agonist	No change in anxiety behavior	Duxon et al. [155]
Fear-related Behavior			
CeA	Increased 5-HT	Increased unconditioned freezing	Forster et al. [132]
BLA	Increased 5-HT	Decreased conditioned freezing	Inoue et al. [157]
BLA	Increased 5-HT	Decreased unconditioned tonic immobility	Leite-Panissi et al. [158]
BLA	5-HT$_{1A}$ agonist	Decreased conditioned freezing	Li et al. [159]
BLA	5-HT$_{1A}$ agonist	Decreased acquisition and expression of conditioned defeat	Morrison et al. [160]
BLA	5-HT$_{1A/2}$ agonist	Decreased unconditioned tonic immobility	Leite-Panissi et al. [158]

Abbreviations: 5-HIAA = 5-Hydroxyindoleacetic acid (5-HT metabolite); 5-HT = serotonin; BLA = basolateral amygdala; CeA = central nucleus of the amygdala; MDMA = 3,4-methylenedioxy-N-methylamphetamine; MeA = medial amygdala.

Table 2. The Role of Serotonin in Anxiety-Like and Fear-Related Behaviors

Determining the role of amygdala 5-HT in fear-related behavior has mainly utilized studies of freezing or immobility responses in rodents, and of 5-HT manipulation in the BLA (Table 2). From these studies, it seems clear that 5-HT in the BLA decreases the expression of unconditioned and conditioned fear responses, likely via activation of the inhibitory postsynaptic 5-HT$_{1A}$ receptor (Table 2). Thus, it has been suggested that 5-HT in the BLA/amygdala ameliorates fear [8]. This conclusion is in contrast to the apparent role for BLA 5-HT in enhancing anxiety (Table 2), suggesting a fear versus anxiety dissociation for the role of 5-HT in the BLA. This dissociation, if upheld by more in-depth future work, could prove important information for the development of treatment strategies for the various anxiety disorders that differ in the degree of anxiety-like and fear-like symptomology (as discussed in Section 1.1).

A role for amygdala DA in anxiety has not been as well explored as for 5-HT. However, a summary of studies that have manipulated DA function in the amygdala provides a consistent picture of the role of amygdala DA in mediating anxiety in animal models (Table 3). Indirect evidence suggests that decreased DA in the amygdala leads to increased anxiety, and this is supported by direct manipulation of the CeA (Table 3). For example, decreased DA or DA receptor antagonism within the CeA all increase anxiety-like behavior (Table 3),

suggesting that DA activity in the CeA is anxiolytic. This role for DA in the CeA is in direct contrast to the BLA, where converging evidence suggests that decreased DA function in the BLA decreases anxiety-like behaviors while increased DA receptor activity in the BLA increases anxiety (Table 3). Thus, DA activity in the BLA is anxiogenic, revealing an opposite role for DA activity in the CeA and BLA in mediating anxiety-like behaviors in animal models.

Amygdala Subregion	Monoamine or Receptor Involvement	Behavioral Outcome	Citation
Anxiety-like Behavior			
Amygdala	Decreased DA	Decreased rearing in open field indicative of increased anxiety behavior	Summavielle et al. [163]
CeA	Decreased DA	Decreased voluntary activity indicative of increased anxiety behavior	Izumo et al. [164]
CeA	D_1 antagonist	Increased anxiety behavior	Rezayof et al. [165]
CeA	$D_{2/3}$ antagonist	Increased anxiety behavior	de la Mora et al. [166]
BLA	DA depletion	Decreased anxiety in males but not females	Sullivan et al. [167]
BLA	D_1 agonist	Increased anxiety behavior	Banaej et al. [168]
BLA	D_2 agonist	Increased anxiety behavior	Banaej et al. [168]
BLA	D_1 antagonist	Decreased anxiety behavior	Banaej et al. [168]
BLA	D_1 antagonist	Decreased anxiety behavior	de la Mora et al. [169]
BLA	D_2 antagonist	Decreased anxiety behavior	Banaej et al. [168]
Fear-related Behavior			
Amygdala	D_2 antagonist	Decreased acquisition and retention of fear conditioning	Greba et al. [170]
CeA	D_1 agonist	Increased conditioned fear behavior	Guarraci et al. [171]
CeA	D_1 antagonist	Inhibited conditioned fear behavior	Guarraci et al. [171]
CeA	D_2 antagonist	Decreased	Guarraci et al. [172]

Amygdala Subregion	Monoamine or Receptor Involvement	Behavioral Outcome	Citation
		conditioned fear behavior	
BLA	DA depletion	Decreased fear conditioning	Seldon et al. [173]
BLA	D_1 antagonist	Inhibited acquisition of fear conditioning	Greba and Kokkinidis [174]
BLA	D_2 antagonist	Inhibited fear potentiated startle	De Oliveira et al. [175]

Abbreviations: BLA = basolateral amygdala; CeA = central nucleus of the amygdala; DA = dopamine.

Table 3. The Role of Dopamine in Anxiety-Like and Fear-Related Behaviors

In contrast, the role of DA in mediating fear-related behaviors does not appear to differ based on amygdala subregion (Table 3). Reducing DA function in the amygdala reduces or inhibits processes associated with fear conditioning, while increasing DA receptor activity increases conditioned fear (Table 3). Thus, DA in the amygdala is required for fear conditioning, and enhanced DA levels in the amygdala as elicited by fearful stimuli and conditioned cues [107,112] would thus facilitate fear conditioning. It should be noted that the studies summarized by Table 3 indicate a role for both excitatory D_1 receptors and inhibitory D_2 receptors. Dopamine D_2 receptors are localized both pre- and post-synaptically, with pre-synaptic D_2 autoreceptors limiting DA neuronal activity and DA release [161,162]. Thus, antagonism of presynaptic D_2 receptors would actually increase DA within the amygdala. Since the effects of D_2 receptor antagonism on fear-related behaviors is characteristic of reduced, not enhanced, DA function in the amygdala, it may be concluded that the results of D_2 receptor antagonism summarized by Table 3 are due to postsynaptic D_2 receptor effects. However, this conclusion requires direct testing.

Very few studies have examined the role of amygdala NE in mediating anxiety-like behavior in animal models, surprising given that anxiogenic stimuli increase NE in this region [for example, see 111,115,116] and drugs that alter NE neurotransmission are used to treat anxiety disorders [8]. There appears to be little role for NE receptors in the CeA in mediating anxiety-like behavior, although infusion of a α_1 antagonist can increase social interaction following an anxiogenic stimulus [restraint; 176; Table 4]. It is clear that more experiments are required to delineate the role of amygdala NE in mediating anxiety.

Studies determining the role of NE in fear-related behaviors have concentrated on the BLA, due to the importance of this amygdala subregion in conditioned fear responses (see Section 3.1.). The major focus of the studies summarized by Table 4 has been on the role of NE in fear conditioning and reconsolidation of fear memories in conditioned fear paradigms. Taken as a whole, findings suggest that NE in the BLA facilitates fear conditioning and fear memory, via activation of adrenergic β receptors (Table 4). Recent evidence suggests a role for α_1 receptors in the BLA in mediating fear memory, in this case, activation of α_1 receptors by NE would appear to decrease fear memory (Table 4). Thus, it is possible that NE in the

BLA could have opposing effects on reconsolidation of fear memory based on the balance of α_1 versus β receptor activity – a hypothesis that requires direct testing. The role of NE in the BLA (and β receptors in particular) in fear memory has generated interest in targeting this NE system for the treatment of anxiety disorders where enhancement in fear memory is apparent, such as PTSD [for example, see 177]. Whether NE within the BLA plays a role in other aspects of fear processing (e.g. unconditioned fear responses to non-olfactory based stimuli) or NE within other amygdala subregions mediate fear should be subjects of future investigations to fully elucidate the role of amygdala NE in fear.

Amygdala Subregion	Monoamine or Receptor Involvement	Behavioral Outcome	Citation
Anxiety-like Behavior			
CeA	α_1 antagonist	Increased social interaction	Cecchi et al. [176]
CeA	α_1 antagonist	No effect on anxiety behavior	Cecchi et al. [176]
CeA	$\beta_{1/2}$ antagonist	No effect on social interaction	Cecchi et al. [176]
CeA	$\beta_{1/2}$ antagonist	No effect on anxiety behavior	Cecchi et al. [176]
Fear-related Behavior			
BLA	Increased NE	Increased memory and retention of fear conditioning	LaLumiere et al. [178]
BLA	Decreased NE	Impaired fear conditioning	Seldon et al. [173]
BLA	Decreased NE	Impaired fear memory	Debiec and LeDoux [177]
BLA	α_1 antagonist	Increased fear memory	Lazzaro et al. [179]
BLA	$\beta_{1/2}$ antagonist	Impaired of fear memory	Debiec and LeDoux [180]
BLA	β_1 antagonist	Impaired fear memory (as enhanced by glucocorticoids)	Roozendaal et al. [181]

Abbreviations: BLA = basolateral amygdala; CeA = central nucleus of the amygdala; NE = norepinephrine.

Table 4. The Role of Norepinephrine in Anxiety-Like and Fear-Related Behaviors

In summary, it is clear that more work is required to fully understand the role of amygdala monoamines in mediating fear and anxiety. However, several patterns of interest emerge from the current literature, namely that there are distinct subregion differences in the role

each monoamine plays in mediating anxiety and fear, with the one monoamine possibly playing opposing roles depending on subregion or depending on whether anxiety or fear measures are employed. Therefore, these findings suggest neurochemical dissociations between amygdala subregions and monoamines in mediating fear or anxiety.

4. The amygdala as a potential site of anxiolytic drug action

Psychopharmacological management of anxiety disorders includes the benzodiazepines, antidepressants, 5-HT_{1A} agonists and various "off-label" drugs such as β-blockers, mood stabilizers and antipsychotics. The mechanism by which these drugs produce anti-anxiety effects has yet to be definitively established and represents a frequently updated field of research. Because these drugs bind to target receptors throughout the brain, it is unlikely that their efficacy can be attributed to action in one particular region. However, given the role that the amygdala plays in fear and anxiety, modification of amygdala function by pharmacological agents represents a likely mechanism of action as well as a target to guide future drug development. The evidence for amygdala involvement in anxiolytic action comes from both human imaging studies as well as work in animal models.

4.1. Human imaging studies: Effects of anxiolytics on amygdala activity and emotion

Given the highly complex and subjective nature of anxiolytic drug response in humans, neuroimaging represents an invaluable tool for drug evaluation and discovery.

Benzodiazepines: Benzodiazepines exert their anxiolytic action through binding to $GABA_A$ receptors, which leads to enhanced GABA activity and a subsequent increase in inhibitory tone. Despite the long history and current prevalence of benzodiazepine use for anxiety disorders [182,183], there is a paucity of human neuroimaging studies utilizing this class of drug, especially compared to those using antidepressants. This may have to do with eclipse of benzodiazepines by antidepressants as first line agents for many anxiety disorders [182].

Various studies have utilized healthy volunteers undergoing experimental challenges in an attempt to elucidate the neurobiology underlying the anxiolytic effect of benzodiazepines. These studies have found that benzodiazepines have the ability to impair functions related to amygdala activity including fear conditioning [184-186], recognition of fearful emotional faces [187], and memory for emotional stimuli relative to neutral stimuli [188,189].

Neuroimaging work appears to support a role for the amygdala in benzodiazepine action, although this may be dependent upon the nature of the accompanying neuropsychological challenge. Specifically, lorazepam was found to decrease amygdala activation during an emotional face assessment task without modifying baseline levels of anxiety or task recognition [190]. A similar finding was found with diazepam, which decreased amygdala response to fearful faces, and also impaired fearful face recognition [191]. However, during anticipation of aversive electrical stimulation, lorazepam failed to produce changes in amygdala activity [192]. Thus, while there is support for benzodiazepine induced

modulation of the amygdala during processing of threatening/emotional stimuli, further studies are needed to clarify the neural correlates of benzodiazepine-induced anxiolysis.

β-Blockers: The β-blocker propranolol has a substantial history of being utilized to reduce somatic symptoms of fear and anxiety in situations such as stage fright [193] and acute panic [194-195]. More recently, research on the role of amygdala NE and β-receptors in facilitating emotional memory formation (see Table 4 and associated text) has caused much excitement and controversy about the use of propranolol to prevent PTSD [196-198]. Thus far, initial trials have demonstrated limited efficacy [199,200]. Despite lack of success in the application of propranolol to PTSD, neuroimaging studies in healthy human subjects have confirmed the ability of propranolol to modulate amygdala activation to emotional stimuli. Propranolol was found to decrease amygdala activation to emotional faces irrespective of emotional valence [201]. Furthermore, supporting a role for the amygdala NE in the encoding and consolidation of emotional stimuli, a separate study found that propranolol was able to decrease amygdala reactivity to emotional pictures of high valence as well as decrease the subject's memory for them [202].

Selective Serotonin Re-uptake Inhibitors: Antidepressant drugs, and selective serotonin re-uptake inhibitors (SSRIs) in particular, have become first line drugs for many of the anxiety disorders [182,203]. As such, there has been comparatively more work investigating these drugs in humans using advanced imaging techniques.

Most antidepressants are unique from benzodiazepines and β-blockers in that a time lag exists between initial treatment and onset of anxiolytic effects. In line with a potential anxiogenic role of serotonin in the amygdala (see Table 2 and associated text), some patients have reported an initial exacerbation of anxiety upon acute dosing of SSRIs [203]. In studies on healthy subjects, acute dosing of the SSRI citalopram can enhance recognition of fearful faces as well as increase emotion-potentiated startle response [204-206]. These effects are reversed when citalopram treatment is continued for 7 days [207,208].

Attempts to correlate the acute versus sub-chronic effects of SSRIs with neural activation have resulted in unexpected findings. On one hand, sub-chronic citalopram treatment was found to decrease amygdala activation to unconscious fearful stimuli [209], suggesting a relationship between repeated SSRI treatment, changes in emotional processing, and decreased amygdala activity. However, acute doses of citalopram have also been found to decrease amygdala activation to fearful faces [208,210,211]. Divergent effects of acute versus sub-chronic citalopram on emotional recognition but similar effects on amygdala response could suggest that the amygdala does not play a core role in acute SSRI-induced anxiety or chronic SSRI-induced anxiolysis. However, it has been emphasized that the effects of serotonergic challenge on fear recognition and amygdala activation appear to be dependent upon the individual's baseline sensitivity to threat [212], gender [213] and genotype [214]. Thus differences in subject profiles both between and within studies could have confounded results.

Overall, it appears that pharmacotheraputics commonly used to treat anxiety disorders may modulate amygdala function. In particular, it appears that anxiolytics can reduce amygdala

reactivity to highly emotive or fearful stimuli. Given that amygdala hyper-reactivity to similar stimuli is the most common finding across all anxiety disorders (with the exception of adult GAD – see Section 2.2), it is possible that the anxiolytic effects of these drugs may be in part, mediated by dampening amygdala function.

4.2. Evidence delineating effects of anxiolytic drugs on amygdala function in animal models of anxiety states

Benzodiazepines: While benzodiazepine receptors exist throughout the brain, there is a particularly high density in amygdala regions [215,216]. There is much evidence from animal models to suggest that it is the action of benzodiazepines in the amygdala that mediates their anxiolytic effect. For example, early evidence demonstrated that local amygdala infusion of benzodiazepines produces anxiolytic-like effects in conflict models of anxiety [217-220]. These effects can be reversed by systemic [217,219] or direct amygdala administration of benzodiazepine antagonists [220]. Anti-conflict effects are most apparent when the benzodiazepines are injected into the BLA, and are absent when injected into the CeA [219,220]. While anti-conflict effects of benzodiazepines have been observed in the CeA, these were with substantially higher doses [221]. Further studies suggest that the BLA and not the CeA is essential for the anxiolytic effects of benzodiazepines in the EPM [149,222,223]. However, with regards to the shock probe burying test, it appears that the CeA is responsible for benzodiazepine-induced impairment of passive avoidance [223]. Although contradictory results exist on the role of benzodiazepines in the BLA versus CeA, particularly when animals are tested on the EPM [9,84,224] have suggested that distinct benzodiazepine receptor subtypes located within subregions of the amygdala may differentially alter avoidance responses to "potential threat" (EPM and BLA) versus "discrete, unambiguous threat" (shock probe burying and CeA).

As discussed in the human studies in Section 4.1 above [184,188,189], a key aspect of benzodiazepine action may be the ability to modulate emotional memory. Here the BLA once again appears to be a main site of benzodiazepine action. Lesions of the BLA, but not the CeA, block the benzodiazepine induced deficits in inhibitory avoidance memory [225,226]. Similar impairments were seen by direct injection of benzodiazepine into the BLA and not the CeA [227]. Enhancement of memory consolidation could be induced by BLA infusion of a benzodiazepine antagonist [228]. Given that individuals with anxiety disorders may be hypervigilant to cues associated with threatening stimuli and biased to form memories regarding such stimuli [229,230], the pro-amnestic effects of benzodiazepines in the BLA may represent a putative mechanism of action.

β-Blockers: The evaluation of β-blockers (with propranolol being the prototypical agent) in animal models has revolved mainly around their utility in models of memory and fear conditioning. Within the BLA, stress hormone elicited increases in norepinephrine have been found to enhance the consolidation of emotionally relevant memories [231,232]. This appears to be particularly true with contextual fear conditioning [178] and reconsolidation of fear memory following extinction [180,197,233; Table 4]. In particular, local infusions of

propranolol are able to block reconsolidation of fear [180,233]. Recently, it has been demonstrated that β-adrenoreceptor activation within the BLA decreases surface expression of $GABA_A$ receptors, and this phenomenon is necessary for the reinstatement of fear following extinction [234]. It is proposed that propranolol, through blocking the decrease in $GABA_A$ receptor surface expression, prevents fear reinstatement by maintaining feed forward inhibition from BLA interneurons and thus dampening activity of BLA projections [234]. This finding is noteworthy as it suggests that hyperactive noradrenergic activity in PTSD [235,236] may lead to reduced $GABA_A$ availability, explaining a potential mechanism for the relative ineffectiveness of benzodiazepines in PTSD populations [237,238].

Despite the action of β-blockers within the amygdala to modulate fear conditioning (see Table 4), attempts at testing propranolol in other animal models of PTSD have met with mixed results, echoing the mixed efficacy seen thus far in humans [199,200,239,240]. One such model is exposure to predator odor in rodents, which produces long lasting increases in anxiety like behavior [241-243]. The increases in anxiety like behavior following exposure to predator odor is influenced by a long lasting potentiation in BLA activity [243], supporting the role of the amygdala in mediating the consequences of fear and trauma. Propranolol administered 1 minute following exposure to predator odor to rats blocks the development of anxiogenesis in various tests, including the EPM, one week later [241]. However, when propranolol administration is delayed to 1 hour following predator odor exposure, no effects are seen when rats are subsequently tested on the EPM 30 days later [242]. These results highlight once again a potential key role of timing if propranolol is to be effectively implemented in clinical patients. Similarly, findings that propranolol seems most effective in blocking the *re*consolidation of fearful memories [233, 180, 197] (also see Table 4) suggests that future work should be aimed at establishing protocols for the integration of propranolol during exposure therapy, in which extinction and reconsolidation processes are most active. Specifically, it would seem important that propranolol not be administered shortly after exposure therapy, as this might interfere reconsolidation processes within the amygdala. On the other hand, propranolol would likely have utility when PTSD patients encounter aversive stimuli outside the context of therapy which could potentially undermine the therapeutic process and lead to reinstatement.

Selective Serotonin Re-uptake Inhibitors: Similar to human studies, animal models of anxiety-like behavior demonstrate divergent behavioral effects of acute versus chronic SSRI administration. Increased anxiety-like behavior with acute treatment of SSRIs and its reversal with chronic treatment has been found in novelty-suppressed feeding [244], EPM testing [245,246], and the social interaction test [247]. While a large percentage of studies reveal acute anxiogenic effects and chronic anxiolytic effect, there are exceptions (for review, see [248]).

Much evidence suggests that enhanced activity at $5\text{-}HT_{2C}$ within the BLA by SSRIs produces acute anxiogenic effects, while the eventual downregulation of these receptors by chronic treatment leads to eventual anxiolysis. For example, amygdala or BLA $5\text{-}HT_{2C}$ receptors have been found to produce anxiety-like responses in a variety of tests [249,250] (see Table 2). Blockade of $5\text{-}HT_{2C}$ receptors within the BLA prevents the acute anxiogenic effect of the

SSRI fluoxetine on the Vogel conflict test [251]. Systemic 5-HT_{2C} antagonism also prevents the increase in fear conditioning [252], decrease in social interaction [247,253], and escape response to airjet [254] following acute SSRI treatment. Following chronic treatment with SSRIs, 5-HT_{2C} agonists have attenuated anxiogenic effects on the exacerbation of OCD symptoms in humans [255,256], on social interaction [257] and hyperlocomotion [258], suggesting down-regulation of the ability to 5-HT_{2C} receptors in the amygdala to produce anxiogenic responses following chronic SSRI treatment. Thus, the amygdala (BLA in particular) may be an important locus of action for the long-term effects of SSRIs on anxiety.

4.3. Future potential anxiolytic targets

The literature reviewed above suggests that in part, the effects of anxiolytic drugs may be mediated by altering amygdala function – either global dampening of the amygdala by benzodiazepines, or specific actions on 5-HT and NE receptors within particular amygdala subregions. However, to improve therapeutic efficacy and reduce relapse, several aspects of amygdala pharmacology discussed above might provide useful potential anxiolytic targets in the future.

Findings suggesting down-regulation of anxiogenic 5-HT_{2C} receptors in the amygdala following chronic SSRI treatment (Section 4.2.) present a potential strategy of reducing onset latency of SSRIs as well as enhancing their effects. Specifically, blocking 5-HT_{2C} receptors at the initiation of SSRI treatment would be expected to produce a faster onset of anxiolytic action. Currently, there are no selective 5-HT_{2C} antagonists available for human use. However, atypical antipsychotics [259] as well as atypical antidepressants such as mirtazapine [260] possess 5-HT_{2C} antagonist activity. While there is evidence that antipsychotic augmentation of SSRIs may improve anxiolytic efficacy, their use has been limited by poor tolerability [for review see 261]. Although research is lacking, mirtazapine and the melatonin receptor agonist/5-HT_{2C} receptor antagonist agomelatine [262] may provide the advantage of targeting anxiogenic 5-HT_{2C} in the amygdala with less side effects.

Furthermore, the recent observation that β-adrenoreceptor activation within the BLA results in decreased of $GABA_A$ receptor surface expression necessary for fear reinstatement [234] (and see Section 4.2.) suggests that the combination of propranolol and a benzodiazepine may have unique benefit for PTSD. By blocking β-adrenoreceptors with propranolol, one might be able to enhance benzodiazepine receptor availability, and increase benzodiazepine-induced inhibition of fear circuits within the amygdala. While currently speculative, the use of propranolol to enhance benzodiazepine action in the amygdala may represent a potential creative treatment strategy in a population that is traditionally refractory to benzodiazepine treatment.

While current pharmacotherapeutic strategies for the treatment of anxiety disorders target monoamine function, this has predominantly been related to altering 5-HT or NE levels or receptor activity [8]. However, Table 3 clearly shows a role for DA in the amygdala in mediating both fear and anxiety, and the role for DA and both D_1 and D_2 receptors in acquisition and retention of conditioned fear in particular appears quite robust. Thus,

reducing DA function might serve as means by which to treat anxiety disorders in which fear plays a major component. The obvious disadvantage of dopaminergic-based pharmacotheraputics is potential for major cognitive and motoric side-effects, limiting the treatment options with the currently available dopaminergic agents. Atypical antipsychotic drugs incorporate DA receptor blocking activity while avoiding many of the motoric and cognitive issues of traditional agents. There is evidence that atypical agents possess anxiolytic activity [261], but metabolic side effects make them poorly tolerated. Furthermore, because atypical antipsychotics also have high affinity for 5-HT receptors, the contribution of DA modulation to their anxiolytic effects in humans is currently unknown. One potential strategy may be the use of partial agonists to reduce DA activity in the amygdala via activation of inhibitory presynaptic D_2 autoreceptors. While non-selective for DA, the D_2 partial agonist aripiprazole has demonstrated anxiolytic efficacy similar to other atypical antipsychotic drugs [263]. In the future, more selective DA partial agonists may have additional benefit without unwanted side-effects.

Finally, CRF has been identified as an important neuropeptide in the regulation of monoaminergic activity in the amygdala in response to anxiogenic or fearful stimuli (Section 3.2). Furthermore, CRF and its receptors (CRF_1 and CRF_2) are implicated in fear and anxiety within animal models and in the development of anxiety disorders [12,121-129]. Upon the development of non-peptide CRF_1 receptor antagonists that cross the blood-brain barrier, there was great interest in the use of CRF_1 receptor antagonist in the treatment of anxiety disorders. To date, there have been limited phase II clinical trials published regarding the use of CRF_1 receptor antagonists in anxiety disorders [264]. Of those, preliminary findings suggest the CRF_1 receptor antagonist-treated groups did not differ from placebo-treated groups in anxiety symptomology in both social anxiety disorder and GAD [264]. However, it has been suggested that efficacious concentrations have not been established for the various CRF_1 receptor antagonists, and it is clear that further clinical trials are necessary. One potential promising area in the treatment of anxiety disorders may actually lie in CRF_2 receptor antagonists. As outlined in Section 3.2, CRF_2 receptors mediate 5-HT activity in the amygdala, are up-regulated in animal models of anxiety, and an antagonist of this receptor reduces heightened anxiety in rats [11,12,127,128,131,132,137). The challenge lies in developing non-peptide CRF_2 receptor antagonists that cross the blood-brain barrier, so that the efficacy of such ligands can be determined for anxiety disorders.

5. Conclusion

Human imaging studies in non-patient populations suggest amygdala activation in response to fearful stimuli, and that the magnitude of this response is positively correlated with trait anxiety. Furthermore, individuals suffering from an anxiety disorder (with the possible exception of adult GAD) show exaggerated amygdala responses to fearful or emotive stimuli, which again is positively correlated with the severity of symptoms. Moreover, reactivity of the amygdala to fearful stimuli is reduced by anxiolytic drugs in healthy subjects, and long-term pharmacotherapy or CBT reduces amygdala hyper-reactivity in anxiety disorders. Animal studies corroborate an important role for the

amygdala in fear and anxiety, with specific subregions mediating acquisition and expression of fear, fear memories and anxiety, and the monoamines within each of these regions often playing a very specific role in facilitating or attenuating fear or anxiety. Both human and animal studies suggest dysfunction of the amygdala might arise in part, from inadequate top-down control by regions such as the medial prefrontal cortex, and in part, from altered neuropeptide regulation of amygdala monoaminergic systems. Overall, the amygdala plays a critical role in anxiety disorders, and understanding the function of this region in fear and anxiety states and how dysfunction of the amygdala results in anxiety disorders is critical to improving long-term treatment outcomes.

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